MOLECULAR BIOLOGY OF NEURONAL SIGNAL TRANSDUCTION

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Developmental Biology (Joint)

PZ 001 THE ROLE OF PAX GENES IN MAMMALIAN DEVELOPMENT, Peter Gruss, Max Planck Institute of Biophysical Chemistry, Am Faßberg, D-3400 Göttingen/Germany.

In order to study molecular mechanisms underlying the development of the nervous system we have utilized mouse paired box containing genes (Pax genes) which were cloned on the basis of their homology to the *Drosophila* segmentation gene "paired". Seven members of this family are expressed in a spatial and temporal specific manner in the developing and adult nervous system. In particular, *Pax-3, 6, and 7* are active in cells of the ventricular zone of the developing neural tube. They respond to a notochord-floorplate induction cascade in a concentration or position-dependent manner. Therefore, since the pattern is set-up by a notochord-floorplate effect in cells of the ventricular zone which subsequently generate the terminally differentiated cells in a ventral to dorsal progressive manner these genes are good candidates which could control a part of this genetic program. In order to study the role of these genes we have examined loss-of-function mutations. *Pax-3* was correlated with a pre-existing mutant named "*splotch*" (*Sp*). This mutant shows exencephalus, spina bifida and partial lack of spinal ganglia. One of the advantages of working with the mouse as a model system is the relative ease to find homologous genes in the human system. For this particular gene a human homologue was identified in individuals with "Waardenburg Syndrom I". Several mutations have been discovered in individual cell imposing the DNA-binding of the mutated Pax-3 gene. Mutants with mutations in these genes in conjunction with mutational analyses will allow us to gain insight into molecular mechanisms underlying the development of the nervous system.

Our current research is directed towards establishing the network of control events. Experiments which shed light on the molecular function of these genes will be reported.

PZ 002 A COMMUNITY EFFECT IN MUSCLE DEVELOPMENT, J.B. Gurdon, E. Tiller and K. Kato, Wellcome CRC Institute, Cambridge, U.K.

In amphibia, and probably in most other animals, muscle is first formed as a result of a mesodermal induction. In Xenopus, vegetal blastula cells are believed to release growth factor-like substances which redirect animal hemisphere cells from an ectodermal into a mesodermal pathway of differentiation. We have used single cell transplantation of mid-gastrula mesoderm cells to determine the time in development when these cells are fully committed to a muscle fate. We find that single muscle precursor cells are not fully committed until the late gastrula to early neurula stage, that is long after the time (early gastrula) when cells can no longer emit or receive the mesoderm inducing factors. We conclude that a second type of cell interaction takes place during middle and late gastrulation and that this is required for cells to complete the process of commitment to muscle differentiation. Evidence for an interaction between cells of like type, that is among those taken from the dorso-lateral region of a gastrula, comes from experiments in which cells are transplanted singly or in aggregates into ectodermal sandwiches. We find that the muscle differentiation marker XMyoD is expressed only when cells are surrounded by like neighbours, and we have termed this a community effect. This type of cell interaction among cells from the same region is different from the mesoderm-forming induction and takes place during gastrulation. We will describe a range of cell transplantation and reaggregation experiments which characterise this new type of cell interaction in muscle development.

PZ 003 SPONTANEOUS ACTION POTENTIAL ACTIVITY AND THE PATTERNING OF CONNECTIONS DURING CNS DEVELOPMENT, C.J. Shatz, Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, CA.

Neural connections in the adult central nervous system are highly organized. In the visual system for example, retinal ganglion cells (RGCs) send their axons to target neurons in the LGN of the thalamus in such a way that axons originating from the two eyes terminate in adjacent but non-overlapping eye-specific layers. During early development, however, inputs from the two eyes are intermixed, and the adult pattern emerges gradually as axons from the two eyes sort out to form the layers. How do these layers form? Evidence does not favor the existence of specific molecular cues that designate left and right eye LGN zones. Rather, experiments suggest that the sorting out process, even though it occurs before vision, requires specific patterns of action potential activity and involves synaptic competition between axons from the two eyes for LGN neurons.

Experiments indicate that the machinery necessary for an activity-dependent competition is present during the relevant developmental times. RGC axons form synapses even prior to the onset of layer formation; some of these synapses, later eliminated, are initially located in territory that will ultimately belong exclusively to the other eye. Physiological studies demonstrate that about 90% of LGN neurons initially recieve convergent excitation from both optic nerves, whereas after the layers have formed, only about 10% do (1). Moreover, experiments have demonstrated that RCGs can generate action potentials spontaneously even in utero (2). To investigate whether activity is necessary for layer formation, we blocked it by infusing tetrodotoxin. Layers did not form, and in fact, RGC axons were highly abnormal in shape (3): axons formed terminal arbors across the entire LGN without regard for their normal laminar boundaries. These observations suggest that the segregation of RGC axons from the 2 eyes to form the set of eye-specific LGN layers requires the formation and selective elimination of functioning synaptic connections between RGC and LGN neurons.

Activity per se is not sufficient to promote the formation of eye-specific layers in the LGN; correlations in the firing of neighboring retinal ganglion cells are required (4). Using a multielectrode array, physiological recordings were made from up to 100 ganglion cells simultaneously in fetal cat or neonatal ferret retinas in vitro (5). Results showed that cells can fire spontaneously generated action potentials that are synchronized with each other in time and space: the pattern of RGC firing is wavelike, with nearest neighbors firing in near synchrony. Waves of activity sweep across the retina at a velocity of about 100 μm/sec. These waves are of the appropriate pattern and at the appropriate developmental times to be useful in providing essential information to postsynaptic LGN neurons concerning the location and eye of origin of the presynaptic ganglion cell axons. However, LGN neurons must be able to detect these correlations and strengthen or weaken these synaptic inputs accordingly. To study such synaptic mechanisms, we (6) made whole cell recordings from slices of neonatal ferret LGN in vitro. Tetanic stimulation of RGC axons produced marked and maintained (up to 2 hours) enhancement of synaptic transmission, indicating that retinogeniculate synapses can indeed undergo activity-dependent changes in strength. Thus, such changes may underlie the process of synaptic rearrangement occurring during the formation of layers within the LGN. Since spontaneously generated activity is present elsewhere in the developing CNS, these observations suggest a general role for neural activity in promoting the formation of precise connections. Supported by NSF IBN 9212640, NIHMH 48108, and The March of Dimes.

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Neurodevelopment

PZ 004 REGULATION OF CNS DEVELOPMENT BY A FAMILY OF CELL SIGNALLING MOLECULES RELATED TO THE PROTO-ONCOGENE *WNT*-1, Andrew McMahon, Brian Parr, Mary Dickinson, Shinji Takada, Kevin Stark, Galya Vassileva, Jill McMahon, Roche Institute of Molecular Biology, Nutley, New Jersey 07110

A number of experiments indicate that cell-cell signalling plays a key role in all stages of CNS organization. We have focused our attention on a family of signalling molecules related to the proto-oncogene *Wnt*-1, which, like *Wnt*-1 itself, are developmentally regulated in the early CNS of vertebrates. Studies in the mouse and chick have established that at least seven members of the *Wnt*-family are expressed in restricted patterns in the CNS within 24 to 36 hours of induction of the neural plate. Patterns of expression implicate *Wnt*-genes in the regulation of anterior-posterior and dorsal-ventral aspects of CNS development. To probe the function of individual family members, we have used two complimentary approaches to manipulate gene expression. By targetting the normal gene by homologous recombination in ES cells, we have introduced germ line mutation into *Wnt*-loci. Secondly, by using gain of function strategies to ectopically express *Wnt*-1, we have begun to examine the affects on CNS development of perturbation of the normal spatial distribution of *Wnt*-1 transcription. Together, these studies point to the importance of the normal distribution of *Wnt*-1 for correct development of the midbrain and spinal cord. These studies are being extended to incorporate additional family members to elucidate their unique and combinatorial roles in the CNS.

PZ 005 ANALYSIS OF NEURODEVELOPMENT USING TRANSGENIC MICE, James I. Morgan, Department of Neuroscience, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The development of structural and functional elements of the nervous system has been analysed using mice that carry lacZ-fusion transgenes that are expressed in specific neuronal populations at different times in development. This strategy has provided information at the cellular level of how functional compartments are organized during development, and at the genetic level of the DNA elements that underlie this process. The former evidence is derived either from analysis of promoter truncations of the reporter transgenes or from crossing the transgenic mice onto genetic backgrounds that have aberrant development. Precise genetic control elements are determined by site-directed mutation of the reporter transgenes combined with biochemical analysis of the promoters of the cognate genes. The results show that complex spatial (anatomical) maps can be represented in the promoters of certain genes. Within the cerebellum these maps appear to be related to functional units, inasmuch as they appear to reflect specific projection pathways. The data also implicate proteins of the homeo- and POU-domain type in this type of spatial organization of gene expression in the mammalian cerebellum. The details of these various interactions are reminiscent of the cascade of genes involved in segmentation in Drosophila and suggest further strategies to identify the transcription factors that orchestrate vertebrate neurodevelopment.

PZ 006 MECHANISMS OF AXON GUIDANCE IN THE DEVELOPING VERTEBRATE SPINAL CORD, Marc Tessier-Lavigne, University of California, San Francisco.

To understand how the specific neural connections that underlie the functioning of the nervous system are generated during development, we have focused on the development of the vertebrate spinal cord, asking how spinal axons are guided to their target fields. This talk will focus on one mechanism of axon guidance, axonal chemotropism. Although chemotactic mechanisms are central to the directed motion of a variety of cell types, and have long been suggested to guide axons to their targets, there has been little direct evidence for axonal chemotropism in vivo. Evidence for chemotropic axon guidance has, however, been obtained in embryological experiments in which explanted neurons confronted with their targets in vitro have been shown to grow toward those targets in a directed manner. We have, in this way, obtained evidence for the operation of chemotropic guidance mechanisms in the spinal cord. One set of spinal neurons, commissural neurons, extend axons to the brain via an intermediate target called the floor plate[1], located at the ventral midline of the spinal cord. We have shown that the floor plate secretes a diffusible factor that can selectively influence the direction of growth of commissural axons in vitro[2,3]. Consistent with a role in guidance in vivo, the factor is secreted only by floor plate cells and is an effective chemoattractant: it can diffuse considerable distances through the neural epithelium, setting up a concentration gradient capable of reorienting all responsive axons within its range of action. I will discuss our current efforts to identify the floor plate-derived chemoattractant, to determine how axons orient in gradients of diffusible factors, and to determine the contribution of chemoattractants to axon guidance during development *in vivo*.

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Cellular Interactions

PZ 007 THE AGRIN PROTEIN FAMILY: STRUCTURE, FUNCTION AND DISTRIBUTION, U.J. McMahan, Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305.

Several lines of evidence indicate that agrin mediates the motor neuron-induced aggregation of AChRs and other proteins comprising the muscle fiber's postsynaptic apparatus at neuromuscular junctions. Of the several alternatively spliced isoforms of agrin that have been detected in nervous tissue those highly active in inducing cultured myotubes to aggregate the postsynaptic proteins appear to have splicing inserts at two positions, designated positions A and B, near their C-terminus. The inserts at A (4 amino acids) and B(8, 11 and 19 amino acids) are required for the activity. I will present evidence based on PCR and in situ hybridizations that motor neurons throughout vertebrates express agrin having both A and B inserts, although the length of B insert can vary. I will also provide evidence that 1) many neurons other than motor neurons express agrin isoforms having both A and B inserts, and 2) although many non-neural cells thoughout the body express agrin, isoforms having both A and B inserts are specific to neurons. Altogether the findings support the hypothesis that motor neuron-released agrin induces the formation of the postsynaptic apparatus at the neuromuscular junction and they are consistent with the possibility that agrin also plays a role in the formation of the postsynaptic apparatus at neuron-neuron synapses throughout the nervous system.

PZ 008 ADHESIVE INTERACTIONS THAT REGULATE AXON GROWTH IN THE EMBRYONIC VISUAL SYSTEM.

Louis F. Reichardt, Blaise Bossy, Ivan de Curtis, Michael DeFreitas, Karla M. Neugebauer, Barbara Varnum-Finney, Kristine Venstrom, and David Sretavan. Department of Physiology and Howard Hughes Medical Institute, UCSF School of Medicine, U426, San Francisco CA 94143-0724

In past work, this laboratory has identified several cell adhesion molecules and extracellular matrix constituents recognized by neurons in the retina, using cell adhesion and neurite outgrowth assays. Cell surface molecules of potential importance in mediating interactions with astroglia include the cell adhesion molecules L1, NCAM, and N-cadherin. Extracellular matrix proteins include laminin, thrombospondin, tenascin, and vitronectin. Integrin-class receptors have been identified for each of the extracellular matrix constituents. These include the first β_1-class receptors identified for tenascin and thrombospondin.

In functional studies, different classes of neurons have been shown in many instances to utilize different integrins to interact with individual extracellular matrix constituents. In the neuroretina, a major receptor for laminin has been shown to be the integrin $\alpha_6\beta_1$. Two isoforms of the α subunit of this receptor, derived by alternative splicing, are present with distinct localization patterns in the embryonic retina. During development, there are dramatic changes in the interactions of subpopulations of retinal neurons with laminin. These appear to be caused by both changes in expression of the integrin α_6 gene in retinal ganglion cells and by changes in the functional activity of the $\alpha_6\beta_1$ integrin on the surface of other classes of retinal neurons.

In order to examine axonal outgrowth within the native embryonic cellular environment, the movements of growth cones or retinal ganglion cells in the optic stalk, optic chiasm, and optic tract have been imaged using time-lapse videomicroscopy. Several integrins, cell adhesion molecules and extracellular matrix proteins are strongly expressed by either cellular substrates or axons in this pathway. Molecular perturbation experiments examining growth cones in vivo indicate that at least some of the molecules important in mediating growth cone movements in vitro are also important in vivo.

Imaging experiments also suggest that interactions between retinal ganglion cell growth cones and cells in the optic chiasm determine whether individual growth cones progress into the ipsilateral or contralateral optic tracts. The optic chiasm before the arrival of retinal axons includes a population of antigenically recognizable cells that appear to be an early differentiating population of neurons. Ablations of these early chiasm cells result in dramatic perturbations in axonal guidance, suggesting that interactions between embryonic retinal axons and these chiasm cells are required for normal development of the primary visual pathways.

Polypeptide Factors

PZ 009 NEUROTROPHINS AND THEIR RECEPTORS ON NEURONS, Yves-Alain Barde[1], Georg Dechant[1], Roland Kolbeck[1] and Alfredo Rodríguez-Tébar[2], [1]Max-Planck Institute for Psychiatry, Department of Neurobiochemistry Martinsried, FRG, [2]Cajal Institute of Neurobiology Madrid, Spain.

The neurotrophins comprise a group of structurally related proteins able to prevent the death of embryonic vertebrate neurons. Some of these small and basic proteins have been demonstrated to have a clear neuronal specificity. Thus, nerve growth factor (NGF) is the neurotrophin supporting the survival of sympathetic neurons in the peripheral nervous system, and brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), as well as NGF, all act on (to a large extent) distinct sub-populations of sensory neurons. Likewise in the central nervous system, BDNF (but not NGF) is able to support the survival of spinal cord motoneurons or of dopaminergic neurons in cultures of dissociated rodent mesencephalon. All neurotrophins are not only strongly related at the level of their primary structures, but they probably all exert their biological activities as homodimers. In fact, it is unclear whether or not BDNF or NT-3 monomers (which can be readily isolated) display any biological activity. At the surface of the neurons, it appears that high affinity receptors for NGF, BDNF and NT-3 represent the entities able to clearly discriminate between these 3 neurotrophins and to confer their specificity of action, as demonstrated by performing binding studies with one (radioactively tagged) neurotrophin in the presence of another neurotrophin. In addition to the dissociation constants, the property of discrimination between 2 ligands in binding studies performed at equilibrium distinguishes the high affinity receptors from the low affinity ones, also demonstrated to exist for NGF, BDNF and NT-3 on neurons.

While the presence of high affinity receptors for neurotrophins on embryonic neurons can usually be correlated with a survival response, one clear-cut exception has been recently observed. Sympathetic neurons isolated from E11 chick embryos display significant numbers of high affinity NT-3 receptors, comparable to those of high affinity NGF receptors. Yet when these receptors are saturated, no survival response is observed with NT-3. In contrast, the majority of these neurons can be supported by the addition of low concentrations of NGF. A possible explanation for these results is that E11 sympathetic neurons express truncated or non-transducing forms of trkC, previously identified as the NT-3 receptor (Lamballe et al. Cell 66 967-979, 1992). In binding studies and functional assays, it was also observed that NT-3 is a relatively efficient agonist of the high affinity NGF receptors. This is in line with results demonstrating that NT-3 can bind to Trk, thought to be primarily the NGF receptor (Cordon Caro et al, Cell 66 173-183). We speculate that non-transducing, trans-membrane protein receptors like TrkC expressed by cells such as sympathetic neurons might represent a selective way of locally removing (by internalization) neurotrophins like NT-3 when their actions on sets of nerve terminals are not (or no longer) required. This mechanism (unlike cessation of receptor expression) would prevent the extracellular accumulation of ligands, which might otherwise lead to the activation of heterologous receptors such as the NGF receptors.

PZ 010 TOWARD UNDERSTANDING *TRK*/NEUROTROPHIN RECEPTOR FUNCTION: Dan Soppet; Pantelis Tsoulfas; Lino Tessarollo; Susan Reid; Janet Blair; Marie Mazzulla; and Luis F. Parada. Molecular Embryology Section ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland.

At least three related vertebrate genes are known to encode receptor tyrosine kinases (RTKs) that bind the NGF-related neurotrophins. We have taken four interrelated approaches toward assessing the biological significance of the interrelationships between the various *Trk*-family receptors ($p140^{protoTrkC}$; $p145^{TrkB}$; 150^{TrkC}; various isoforms) and their respective ligands: 1) transient and stable expression of the *Trk* receptors in PC12 and *nnr* cells where neurite outgrowth can be assayed 2) *in situ* hybridization studies aimed at comparing expression of each gene; 3) transgenic expression of dominant negative receptors; 4) gene knock out experiments in ES cells. Each of these approaches has provided information that enhances our understanding of *Trk* receptor function. Our current progress will be discussed. Research sponsored by the National Cancer Institute, DHHS, under contract no. NO1-CO-74101 with ABL.

PZ 011 CONTROL OF CELL PROLIFERATION AND SURVIVAL IN THE OLIGODENDROCYTE CELL LINEAGE, M.C. Raff, M. Jacobson and B. Barres, MRC Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, U.K.

We have studied the control of cell survival and proliferation in the oligodendrocyte cell lineage. We have shown that oligodendrocytes and their precursors require signals from other cells in order to avoid undergoing programmed cell death in culture, and we have identified a number of signalling molecules that promote the survival of these cells *in vitro*, including IGF-1, PDGF, CNTF and NT-3. Whereas PDGF is mitogenic for oligodendrocyte precursor cells, IGF-1, CNTF and NT-3 are not. As in the case of many types of hemopoietic cells and some other cell types, the over-expression of the proto-oncogene *bcl-2* suppresses the death of survival-factor-deprived cells in the oligodendrocyte cell lineage. We have also shown that 50% of newly-formed oligodendrocytes in the rat optic nerve die during normal development and that most of this normal cell death can be suppressed if the levels of PDGF or CNTF are experimentally increased in the developing CNS, suggesting that this death may reflect a competition for limiting amounts of survival factors present in the normal developing optic nerve. Thus, oligodendrocyte development seems to work much like neuronal development, in that the cells are over-produced and are then culled, presumably to match the number of oligodendrocytes to the number of axons that they have to myelinate.

Consistent with the view that axons regulate the number of oligodendrocytes that survive, transection of the developing optic nerve greatly increases the number of oligodendrocytes that die in the nerve. This increase in death can be prevented by experimentally increasing the amount of CNTF in the cut nerve. Axons also seem to regulate the proliferation of oligodendrocyte precursor cells in the developing optic nerve, as transection of the nerve greatly decreases the proliferation of these cells. This axonal regulation of cell proliferation seems to depend on electrical activity in the axons: if the axons are silenced by an intra-ocular injection of tetrodotoxin, the proliferation of the precursor cells is inhibited; the inhibition can be overcome by increasing the concentration of PDGF in the electrically silenced nerve.

Receptors

PZ 012 NEUROTRANSMITTER TRANSPORTERS: FAMILIES OF TRANSPORT PROTEINS INVOLVED IN SYNAPTIC SIGNAL TERMINATION. Susan G. Amara[1], Jeffrey Arriza[1], Kari Buck[1], Janet Clark[2], Gwynn Daniels[1], Wendy Fairman[1], John Kilty[1] & Dominique Lorang[1], [1]The Vollum Institute, Oregon Health Sciences University, Portland, OR, [2]Yale University, New Haven, CT.

For most classical neurotransmitters, the action of released transmitter is terminated by specific reuptake systems located on pre-synaptic terminals and surrounding glia. Neurotransmitter transport activities have generally been characterized on the basis of their substrate specificities, pharmacological sensitivities and ionic dependencies. Inhibition of reuptake can have a major impact on synaptic signalling. In particular, the monoamine carriers are the site of action for a wide range of clinically important drugs, including amphetamines, cocaine, and various tricyclic antidepressants. The best characterized class of neurotransmitter transporters all link substrate influx to the cotransport of Na^+ and Cl^- ions across the plasma membrane. However, excitatory amino acid transport appears mechanistically distinct, as glutamate influx is coupled to the cotransport of Na^+ and the countertransport of K^+ with no apparent dependence on Cl^-. Recent insights into the nature of these reuptake systems have been provided by the molecular biologic description of an extensive family of Na^+/Cl^--dependent transporters including the carriers for each of the biogenic amines, choline, proline and taurine, as well as multiple carrier subtypes for GABA and glycine. This family is characterized by twelve transmembrane domains and several regions of highly conserved sequence. In contrast, the molecular cloning of several glutamate carriers demonstrates that they have no significant sequence similarity to the Na^+/Cl^- cotransporters and are members of a different family of transport proteins, more closely related to the bacterial proton-coupled glutamate transporters. Hydrophobicity analyses predict a unique structural organization with eight to ten potential membrane spanning domains.

This talk will cover recent progress in our laboratory on the study of the expression, structure-function relationships and diversity of these two neurotransmitter transporter gene families. These studies begin to address the contribution of reuptake systems in determining net synaptic transmission and form a basis for future investigations of the physiologic consequences of regulatory processes, pathologic changes and pharmacologic blockade.

Ion Channels (Joint)

PZ 013 SODIUM CHANNEL DEFECTS IN HUMAN NEUROMUSCULAR DISORDERS. Stephen C. Cannon, Department of Neurology and Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114.

The electrical excitability of muscle and nerve is mediated by voltage-gated ion channels. Several inherited neuromuscular disorders are caused by altered excitability of the sarcolemmal membrane and the underlying ion channel defects are now being elucidated at the molecular and functional levels. Hyperkalemic periodic paralysis (HPP) is inherited autosomal dominantly and is characterized by recurrent episodes of flaccid paralysis in association with raised serum K (5.5 to 7.5 mM range; normal 3.5 to 4.5). Attacks last minutes to hours and are not associated with sensory symptoms or alterations in consciousness. During an attack affected muscle fibers are depolarized and refractory to firing action potentials. Voltage-clamp studies of biopsied fibers showed that a non-inactivating TTX-sensitive current causes the depolarization. A related disorder, *paramyotonia congenita* (PC), presents as cold-induced stiffness due to electrical hyperexcitability of the sarcolemma with repetitive action potentials and impaired relaxation (myotonia). In some families, patients with HPP also have myotonia.

In all families tested to date, both HPP and PC are linked to the gene encoding the α-subunit of the skeletal muscle sodium channel (SCN4A) on 17q. Two mutations in SCN4A have been identified in families with HPP and 6 others have been found in association with PC. In all cases the mutation is a transversion of a single nucleotide which causes a substitution at one amino acid residue. Most of the substitutions interchange one neutral residue for another and most are located at the cytoplasmic face of the membrane, in transmembrane segments adjacent to the putative pore-forming region.

We have investigated the functional consequence of the HPP mutations. Sodium currents were measured in human myotubes cultured from a patient with HPP. The functional defect is a K-induced disruption of inactivation. Single-channel conductance and activation are not affected. At low [K] normal and mutant channels are indistinguishable. In 10 mM [K] a small proportion of mutant channels have prolonged open times and open repetitively throughout the depolarization. The steady-state open probability is only about 0.02 to 0.05, but this is 10 to 50 times larger than that observed for normal Na channels. These HPP myotubes contained the Met1592→Val mutation. We showed that this mutant and the Thr704 → Met mutation are sufficient to disrupt inactivation by using site-directed mutagenesis in the normal rat Na channel and expression in HEK cells. A toxin-based animal model and computer simulation were used to show that a small proportion of non-inactivating sodium current (Popen of 0.015 to 0.05) is sufficient to produce the myotonic and paralytic phenotypes.

The physiology explains the mechanism of dominant inheritance. As serum [K] increases, from exercise or ingestion, Popen approaches 0.01 to 0.02. The steady inward current slightly depolarizes the cell towards threshold and repetitive action potentials occur. With higher [K], Popen increases to 0.02 to 0.05 and the persistent current now causes a large depolarization that inactivates the normal Na channels. This renders the fiber refractory from firing action potentials and causes paralysis.

PZ 014 MOLECULAR CHARACTERIZATION OF CALCIUM RELEASE CHANNELS: GENE REGULATION, STRUCTURE AND FUNCTION, Andrew R. Marks, Molecular Medicine Program, Department of Medicine, Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029

Intracellular calcium release signals numerous biologic processes including muscle contraction, neurotransmitter release, cell differentiation and growth. A family of intracellular calcium release channels (ICRCs) have been described. Two types of ICRCs are ryanodine receptors (RyRs) predominantly on the sarcoplasmic reticulum of cardiac and skeletal muscles, and the inositol 1,4,5-trisphosphate receptor receptors (IP_3Rs) on the endoplasmic reticulum of most cell types. The RyRs and IP_3Rs share limited homology in terms of primary structure but have similar ultrastructural properties characterized by four-fold symmetry. The RyRs are comprised of four identical 565,000 MW subunits, and the IP_3Rs contain four 313,000 MW subunits. The RyR genes are developmentally regulated in muscle, exhibiting increased expression at birth. The RyR gene is downregulated by fibroblast growth factor in a myogenic cell line (skeletal), during end-stage heart failure in humans (cardiac), by doxorubicin treatment in rats (cardiac), and by phenylephrine in cultured cardiocytes (cardiac). The IP_3R gene is up-regulated in cardiac myocytes during end-stage heart failure suggesting that a hormonally-sensitive pathway may become important in regulating cardiac contractility and/or hypertrophy in disease states. We have characterized ICRC expression in cardiac myocytes and in skeletal muscle demonstrating distinct patterns of expression for both the RyR and IP_3R. IP_3R expression in some muscle types may provide an alternative pathway for modulating cytoplasmic calcium levels (as opposed to membrane depolarization which activates ryanodine receptors via the voltage-gated calcium channels on the plasmamembrane). The cloned expressed skeletal RyR yields a caffeine-sensitive ICRC in *Xenopus* oocytes. We have described an association between the RyR and FKBP-12, the binding protein for the immunosuppressant FK506 and rapamycin. Rapamycin, but not FK506, blocks calcium dependent proliferation in a myogenic cell line. We have also detected RyRs in endothelial cells and in vascular smooth muscle where their role in modulating cytoplasmic calcium concentration remains to be elucidated. The interaction between RyR and IP_3R may provide a means for positive and/or negative feedback between the two divergent pathways for intracellular calcium release in cells in which both types of channels are expressed.

Signal Transduction

PZ 015 REGULATION OF NEUROTRANSMITTER RECEPTORS BY SERINE AND TYROSINE PROTEIN PHOSPHORYLATION. Richard L. Huganir, HHMI, Neuroscience Department, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Neurotransmitter receptors mediate signal transduction at the postsynaptic membrane of synaptic connections between cells in both the central and peripheral nervous systems. This pivotal role in the mechanism of synaptic transmission suggests that neurotransmitter receptors may be potential targets at which synaptic plasticity could occur. Modulation of the function, expression, or density of neurotransmitter receptors in the postsynaptic membrane could have profound effects on the efficacy of synaptic transmission. A variety of data now suggest that protein phosphorylation of neurotransmitter receptors is a primary mechanism for the regulation of neurotransmitter receptor function. We have used the nicotinic acetylcholine receptor (AChR) as a model system to study the role of protein phosphorylation in the regulation of neurotransmitter receptor and ion channel function. The AChR is a pentameric complex of four types of subunits (α, β, γ and δ) in the stoichiometry of $\alpha_2\beta\gamma\delta$. The AChR is multiply phosphorylated in vitro and in vivo by cAMP-dependent protein kinase (PKA), protein kinase C (PKC), and a protein tyrosine kinase (PTK). The phosphorylation of the AChR subunits occurs on the major intracellular loop of each subunit between the third and fourth transmembrane domains. Phosphorylation of the receptor by each of these protein kinases appears to regulate the rate of desensitization of the receptor. The phosphorylation of the AChR in muscle is under the control of a variety of nerve derived factors including acetylcholine itself and the neuropeptide calcitonin gene-related peptide. Recent studies have also shown that tyrosine phosphorylation of the AChR is regulated in vivo and in vitro by neuronal innervation of muscle. Experiments to characterize the components of nerve involved in the regulation of tyrosine phosphorylation have demonstrated that agrin, a neuronal extracellular matrix protein, regulates tyrosine phosphorylation in a manner similar to innervation. Agrin is thought to be released from the neuron and to mediate the nerve-induced clustering of the AChR at the neuromuscular junction. These results suggest that tyrosine phosphorylation of the AChR may be involved in the regulation of agrin- and nerve-induced clustering of the receptor at synapses. In recent studies we have begun to examine the role of protein phosphorylation in the regulation of the major inhibitory and excitatory neurotransmitter receptors in the nervous system, the $GABA_A$ receptors and the glutamate receptors. These receptors are also phosphorylated by a variety of protein kinases including PKA, PKC and PTKs. The phosphorylation of these receptors also occurs on the major intracellular loop of the receptor subunits. Phosphorylation of $GABA_A$ receptors consisting of the α_1 and β_1 or the α_1, β_1 and γ_2 subunits by PKA or PKC inhibits the peak response and modulates the desensitization kinetics of these receptors. In contrast, PKA phosphorylation of glutamate receptors consisting of the GluR6 subunit by PKA potentiates the peak response of the GluR6 protein. These studies suggest that protein phosphorylation of neurotransmitter receptors is a major mechanism for the modulation of their function and thus may play an important role in the regulation of synaptic plasticity.

Plasticity

PZ 016 FORMATION AND LONG-TERM MODULATION OF AN IDENTIFIED SYNAPTIC CONNECTION IN VITRO: POSSIBLE ROLES OF CELL ADHESION MOLECULES, Samuel Schacher, Columbia University College of Physicians and Surgeons, New York.

The mature nervous system retains the capacity to undergo long-lasting alterations in connectivity in response to injury, to abnormal patterns of activity, or to stimuli leading to changes in behavior. The cellular and molecular signals required for the initiation and maintenance of these changes are poorly understood. Both invertebrate and vertebrate model systems, examined either in vivo or in vitro, are now used to explore these mechanisms. In the marine mollusc *Aplysia*, some non-associative and associative forms of learning involve alteration in the efficacy of connections between identified presynaptic cells and their follower cells. The changes in behavior and the accompanying changes at the synapses can last from minutes to hours (short-term) to days and weeks (long-term). Although studies indicate that the short-term and long-term plasticity share certain pathways, they differ in at least two features. The expression of the long-term involves a) changes in macromolecular synthesis and b) changes in the neuritic arbor and the number of synaptic contacts. These two changes specific for the long-term process may converge. The expression and distribution of a group of molecules, isoforms of the *Aplysia* homologue of NCAM called apCAM, and the selective modulation of these molecules on the surface of presynaptic or postsynaptic cells by applications of specific neuromodulators capable of evoking long-term functional and structural changes at established synaptic connections, might be important not only in synapse formation during development, but also in affecting the structural changes underlying long-term synaptic plasticity in the mature CNS.

The ability to reconstitute the connection between mechanosensory cells and the gill and siphon motor cell L7 in cell culture has been an important tool for studying the cellular and molecular events of long-term synaptic plasticity. During the first four days in culture the amplitude of the EPSPs and the number of sensory branches and varicosities contacting the neurites of the motor cell increases to a plateau value. One factor influencing the number of sensory branches is the defasciculation of sensory neurite bundles upon contact with the motor cell. Both the EPSP and the neuritic arbor of the sensory cell change little after four days in the absence of any manipulation. Repeated applications of the neurotransmitter 5-HT or the neuropeptide FMRFamide result in long-lasting changes (increases and decreases, respectively) in both the amplitude of the EPSP and in the number of sensory cell branches and varicosities. Both the long-term functional and structural changes are blocked by inhibitors of protein or RNA synthesis. Moreover, the long-term changes in the structure of the sensory cell requires the presence of the target motor cell. These results coupled with the recent findings that 5-HT and FMRFamide down regulates the level of apCAMs on the surface of sensory cells and motor cell L7, respectively, by a cyclic AMP-mediated increase in endocytosis, raise the possibility that selective modulation of apCAM on presynaptic versus postsynaptic cell could selectivley affect the degree of sensory-sensory and sensory-motor interactions. Thus, decreasing apCAMs on the surface of sensory cells with 5-HT could decrease sensory-sensory contacts and increase interaction between sensory and motor neurites. Conversely, down regulation of apCAMs on the surface of motor cell L7 with FMRFamide might reduce sensory-motor interaction and contribute to retraction of sensory neurites.

A number of experimental observations are consistent with this hypothesis. Treatments with neurotranmsitters that down regulate apCAM levels result in defasciculation of neurites from homologous cells (sensory-sensory with 5-HT and motor-motor with FMRFamide). The pattern of neurite extension and varicosity formation by sensory cells on a motor cell correlate with the distribution of apCAM on the motor cell. For example, selective blocking of apCAM on the motor cell by treating the motor cell with high level of anti-apCAM antibodies interferes with sensory-motor interaction, varicosity formation, and the formation of chemical connections. New sensory cell varicosities following treatments with 5-HT tend to form at sites on the motor neurites with relatively higher levels of apCAM compared to surrounding areas. Thus, the modulation of apCAM on the surface of the cells along with other changes initiated by the neurotranmitters and orchestrated by their respective second messengers evoke a sequence of events leading to the changes in connectivity. How these changes are orchestrated and the other molecules involved remain to be elucidated.

PZ 017 ANALYSIS OF α CAMKII AND NMDA RECEPTOR MUTANT MICE, Susumu Tonegawa, Alcino J. Silva, and Yuqing Li.

We have been applying the ES cell-gene targeting technique to several genes whose products are suspected to be involved in synaptic plasticity in hippocampus and/or other parts of CNS. We have shown that α CAMKII mutant mice are deficient in LTP in the hippocampal CA1 region and are defective in spatial learning as revealed by Morris water task. Some of these exhibit status epilepticus on the first or second stimulation to the amygdala, while no kindling can be established in the remainder. We are currently examining the cause of these dual phenotypes (a collaboration with James McNamara's lab). Mice with a mutation in the NMDA receptor R1 subunit have also been made. $R1^-$ heterozygotes grow apparently normally, but their NMDA current and the strength of LTP in the hippocampal CA1 region are about half of those in wild-type mice (a collaboration with Chuck Stevens' laboratory). $R1^-$ homozygotes die within 48 hours after birth. LTP in the CA1 region as well as anatomical features of these mutant mice are being analyzed (in collaboration with Carla J. Shatze's laboratory).

Animal Models

PZ 018 NON-RECEPTOR TYROSINE KINASES IN THE HIPPOCAMPUS: DISRUPTED NEURONAL ARCHITECTURE, LONG-TERM POTENTIATION AND SPATIAL LEARNING IN *FYN* MUTANT MICE, Seth G.N. Grant[1], Thomas J. O'Dell[1,2], Kevin A. Karl[1,2], Paul L. Stein[3], Philippe Soriano[3,4], and Eric R. Kandel[1,2], [1]Center for Neurobiology and Behavior, [2]Howard Hughes Medical Institute, College of Physicians and Surgeons of Columbia University, New York 10032, [3]Baylor College of Medicine, [4]Howard Hughes Medical Institute, Houston, Texas 77030.

We have used a genetic approach to identify molecules involved with the synaptic plasticity that underlies vertebrate learning and memory. Long-term potentiation (LTP) is the prolonged enhancement of synaptic strength resulting from a brief period of increased neuronal activity. The induction of LTP requires the activation of the NMDA-subtype glutamate receptor, calcium influx and activation of both serine/threonine and tyrosine kinases. Because the tyrosine kinase inhibitors that block LTP (O'Dell et al., 1991) do not discriminate between tyrosine kinases, we sought to identify specific kinases involved with LTP by testing mice harboring mutations in non-receptor tyrosine kinase genes. We examined mice carrying homozygous null-mutations in *fyn*, *src*, *yes* and *abl*, which are expressed in the hippocampus (Grant et al., 1992).

There was a blunting of LTP in mice lacking *fyn* but not *src*, *yes* or *abl* tyrosine kinase expression. This defect was selective for LTP, since synaptic transmission and other forms of synaptic plasticity were intact. Since the hippocampus is required for spatial learning, and LTP is a cellular model of learning, we tested the ability of *fyn*$^-$ mice to learn a spatial task. The *fyn*$^-$ mice showed impaired spatial learning in the Morris water maze, supporting a functional link between LTP and learning.

The *fyn* gene is also necessary for the normal development of the hippocampus, since there are anatomical abnormalities in the CA1 and CA3 regions and the dentate gyrus. The cell body layer of the CA3 and dentate gyrus undulates due to an increase in cell number, and the apical dendrites of the CA1 region appear disorganized. Despite these morphological changes, the CA3 to CA1 synapses show normal synaptic transmission and simple forms of plasticity, including paired-pulse facilitation and post-tetanic potentiation. Consistent with the expression of *fyn* at synapses, this data suggests that *fyn* plays a role in regulating synaptic plasticity both in the developing and mature synapses.

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Late Abstracts

GENE EXPRESSION AND LEARNING IN DROSOPHILA. Gert Bolwig[1], Carrie Chromey[1], Jill Crittenden[1], Brigitte Dauwalder[1], Jim DeZazzo[1], Kyung-An Han[1], Pyung-Lim Han[2], Alan Nighorn[1], Yuhong Qiu[2], Efthimios Skoulakis[1], Robert West[1], Kwok Wu[1], and Ronald L. Davis[1], [1]Center for Learning and Memory, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724, [2]Baylor College of Medicine, Houston. TX, 77030.

We have conducted a large enhancer detector screen of about 6000 lines by serial sectioning of adult heads with subsequent staining for *lacZ*. Diverse patterns of *lacZ* expression were detected among the lines, many of which followed known neuroanatomical boundaries. For example, numerous lines showed specific staining in the primary, 1st order, or 2nd order processing stations of the visual system (photoreceptors, lamina and medulla, respectively). Others were specific to the olfactory system with staining of the antennal lobes. Some showed staining of the central brain at the exclusion of the visual system. Still others displayed staining at dispersed locations, with no obvious relationship to neuroanatomical boundaries. To search for genes involved in olfactory learning and memory, we focused upon lines showing specific or preferential expression in mushroom bodies, putative neuroanatomical substrates for insect learning. Many of the lines showing preferential mushroom body expression have been tested in an olfactory, classical learning situation and shown to be defective. Several classes of defects have been observed, including lines showing defective learning/memory immediately after training and others with normal immediate learning/memory but with a labile memory at later stages. Molecular and genetic analyses have shown that one line had the enhancer detector element inserted into the dunce locus, the prototypic learning gene in Drosophila and the structural gene for cAMP phosphodiesterase. Seven lines were found to have the enhancer detector element inserted into the rutabaga locus, the structural gene for adenylyl cyclase. One line marked the structural gene for the catalytic subunit of protein kinase A. Several other lines marking new genes involved in learning and memory are currently under study.

TRANSCRIPTIONAL CONTROL IN SCHWANN CELL DIFFERENTIATION & MYELINATION, Edwin S. Monuki, Rainer Kuhn, and Greg Lemke, Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

We have been studying transcriptional regulation by SCIP, a POU domain transcription factor expressed by a subset of glial and neuronal progenitors in the peripheral and central nervous systems. These cells include the proliferative blast cells that give rise to Schwann cells in the PNS, and to oligodendrocytes and a subset of neurons in the CNS. In such cells, SCIP often apppears to function as a general transcriptional repressor of end-stage differentiation-specific genes that are expressed at low levels in dividing progenitors and at high levels in mature, differentiated cells. We have examined SCIP regulation of one such gene expressed by differentiating Schwann cells - the gene encoding P_0, the major structural protein of peripheral myelin. SCIP represses transcription of this gene by the joint action of two modular domains - the POU domain, which mediates specific DNA binding, and an amino terminal regulatory domain. The POU domain subserves two roles required for full repression. The first of these is DNA binding; mutations that destroy DNA binding compromise the ability of SCIP to repress by ~10-fold. The second function of the POU domain is to mediate protein-protein interactions; replacement of the POU domain with a heterologous DNA binding domain yields a hybrid protein that now functions as a *transactivator* of a P_0 promoter that contains binding sites for the domain. These SCIP POU domain activities are "generic" to the extent that they can be replaced by the POU domain of Oct-2. In contrast, the SCIP amino terminal domain acts cell-specifically in two assays. SCIP mutants that lack the amino terminal domain are dead as repressors of the P_0 gene, even though they retain full DNA binding activity. The SCIP amino terminal domain cannot be replaced by the regulatory domains of other POU domain transcription factors. When linked to a heterologous (Gal4) DNA binding domain, the SCIP domain acts as an exceptionally strong transactivator of Gal4 targets in Schwann cells, but is inactive in three hererologous ($SCIP^-$) cell types. These results suggest a model in which the SCIP amino terminal domain acts cell-specifically by interacting with a Schwann cell-specific transcriptional adaptor, and that this adaptor is normally required for the expression of differentiation-specific genes. They further suggest that cell-specific transactivation or repression mediated by transcription factor regulatory domains may be a general feature of regulators that, like SCIP, are normally expressed in restricted sets of cells.

Molecular Neurobiology I

PZ 100 TRANSCRIPTIONAL REGULATION BY MURINE HOX PROTEINS: ISOLATION OF POTENTIAL TARGET GENES. Cory Abate, Katrina Catron, Nancy Iler, Zhigang Shang, Isabelle Pellerin, Veronica Ebu-Issac. Center for Advanced Biotechnology and Medicine, Piscataway, NJ 08854.

The homeobox encodes a DNA binding motif (the homeodomain) that is conserved among numerous genes whose protein products regulate transcription during embryogenesis. The *hox* gene family, the most prevalent class of murine homeobox genes, contains at least twenty members that are located within four chromosome clusters. These genes are expressed throughout murine embryogenesis in overlapping spatial and temporal patterns and play a fundamental role in directing developmental processes. Although the *hox* genes encode putative transcriptional regulators, their function as such has not been well characterized. In particular, target genes regulated by Hox proteins during development have not been identified and this is due, in part, to the limited biochemical information available regarding their DNA binding and transcriptional properties. Therefore, to facilitate isolation of potential target genes, we have characterized the biochemical properties of several Hox polypeptides, including Hox 1.5, 2.6, and 2.9 using purified proteins that we obtained by expression in *E. coli.* We identified optimal DNA binding sites for these proteins using an oligonucleotide selection strategy and showed that they bind to a common consensus motif (C/GTAATTG). The specific nucleotides flanking the conserved TAAT core distinguish the binding specificities among the related polypeptides. The Hox proteins bound with high affinity to their consensus sites *in vitro* and activated transcription through these sites *in vivo* as determined using transient transfection assays. The Hox binding sites were also present in the genomic control regions of potential target genes as detected using an immunoprecipitation assay. Therefore, these biochemical studies have lead to the identification of potential target genes regulated by Hox proteins during development, and this is an initial step toward understanding their function as transcriptional regulators.

PZ 101 **THE ANALYSIS OF *PKC-γ* MUTANT MICE.** **Asa Abeliovich and Susumu Tonegawa, HHMI at the Center for Cancer Research, MIT, Cambridge, MA 02139.**

A role for Protein Kinase C (PKC) has been implicated in synaptic plasticity in the hippocampus[1] and the cerebellum[2] of the rat, in the modulation of neuronal excitability[3], and in complex behavioral phenomena such as spatial learning[4] and classical conditioning[5]. PKC-γ is a Ca^{++}, diacylglycerol, and phospholipid activated isotype of PKC expressed primarily in the cerebral cortex, hippocampus, amygdala, cerebellum, and to a lesser extent elsewhere in the CNS of adult rats[6]. This unique pattern of expression suggested to us and to others that the PKC-γ isotype may serve a CNS-specific function. To investigate this hypothesis, we have generated mice that harbor a deletion in *PKC-γ* by homologous recombination in embryonic stem cells. Homozygous *PKC-γ* mutant mice lack PKC-γ activity, as expected, and are viable. We are currently investigating the role of PKC-γ in long term potentiation (LTP) in the hippocampus, in long term depression (LTD) in the cerebellum, and in the modulation of NMDA receptors and K^+ channels. We are also investigating the effect this mutation has on spatial learning in the Morris water maze task and on classical conditioning in the nictitating membrane/eyelid response.

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2 Linden and Connor, *Science* **254**, 1656.
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4 Wehner *et al., Brain Res.* **523**, 181.
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6 Nishizuka, *Nature* **334**, 661.

PZ 102 MELANOCORTIN RECEPTORS AND IMMEDIATE EARLY GENES MEDIATING THE EFFECT OF MELANOTROPIC PEPTIDES ON NERVE REGENERATION, Roger A.H. Adan, L.C. Plantinga, J.P.H. Burbach and W.H. Gispen, Rudolf Magnus Institute, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands.

Melanocortins accelerate the regeneration of damaged neural tissue such as the rat sciatic nerve. Regeneration of both sensory and motorneurons is enhanced by melanocortins after a nerve crush. Studies were initiated to understand the signal transduction pathway mediating the effect of melanocortins on nerve regeneration. Therefore mRNA levels of the immediate early genes, c-Fos, c-Jun, junB and JunD were measured in putative target tissues (dorsal root ganglia, spinal cord and sciatic nerve) after a rat sciatic nerve crush and following melanocortin treatment in order to reveal the target tissues for melanocortins. From these tissues cDNAs were amplified by the PCR in order to identify G-protein coupled receptors expressed in the damaged nerve. Putative G-protein coupled receptors mediating the effect of melanotropins on nerve regeneration will be expressed in neural derived cell lines in order to study the signal transduction pathways coupled to the receptor.

PZ 103 MOLECULAR CHARACTERIZATION OF A FIBRINOGEN-LIKE SIGNAL IN THE DROSOPHILA EYE, Nicholas E. Baker, E-Chiang Lee and Xiaoxi Hu, Department of Molecular Genetics, Albert Einstein College of Medicine, New York NY 10461.

It appears that every cell in the *Drosophila* retina becomes determined through a series of cell-cell interactions, mediated by molecules such as sevenless, the EGF receptor homologue, and Notch.

Cells of the R8 photoreceptor class are the first to differentiate, and are probably then important in the determination of many other cell types. The *scabrous* gene is required in the R8 photoreceptor cells to initiate the proper pattern of R8 cells, indicating that *scabrous* is part of a signal regulating R8 determination.

The *scabrous* gene sequence predicts a 774 amino-acid protein with an N-terminal signal sequence. The N-terminal portion is probably alpha-helical. A C-terminal domain of about 200 amino acids is related to the beta- and gamma-fibrinogen chains. The scabrous protein enters the secretory pathway, and has an apparent molecular weight of 120 kD, due to posttranslational modifications.

About 30 mutations of *scabrous* have been isolated genetically as lacking activity in vivo. Almost all block passage of the protein through the secretory pathway. Only two mutations affect *scabrous* activity without affecting processing. In one case, loss of *scabrous* function results from mutation of a conserved Ca-binding loop in the fibrinogen-like domain. In the other, a dominant-negative mutation results from deletion of the fibrinogen-like domain and expression of the N-terminal, alpha-helical segment.

The phenotypes of *scabrous* mutations resemble those of mutations of *Notch* and *Delta*, two EGF-related transmembrane proteins, and mutations of these three loci show a number of genetic interactions. We are investigating whether either *Notch* or *Delta* encodes a scabrous-binding protein.

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PZ 104 THE BINDING DOMAIN OF $p75^{NGFR}$: SITE-DIRECTED MUTAGENESIS OF THE SECOND CYSTEINE REPEAT, Anne N. Baldwin and Eric M. Shooter, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.
The p75 nerve growth factor receptor contains an NGF binding domain composed of four repeats of a six-cysteine pattern. We have previously shown that no significant binding activity is retained if any one of the four repeats is deleted, although protein lacking the fourth repeat is capable of recognizing monoclonal antibody MC192, and protein lacking the first repeat still recognizes MAb 217c (1). Protein lacking the second repeat was not stably expressed. In order to investigate further the role of this repeat, we have introduced N-glycosylation sites at three locations within this repeat, in each case by converting to asparagine a serine two residues upstream from a second serine or threonine. In all cases, NGF binding was considerably reduced, but small amounts of binding could be seen. An increase in apparent molecular weight suggests that glycosylation has occurred. At two of these sites, Ser42 and Ser 66, replacement of serine with alanine resulted in fully active protein. In contrast, replacement of Ser 50 with either Ala or Thr resulted in a protein with greatly reduced binding activity. The mutant proteins S50N, S50A, and S50T also showed reduced reactivity toward MC192, whereas S42N, S42A, S66N, and S66A did not. This indicates that Ser50 probably plays a structural role in p75 itself, rather than being directly involved in NGF binding. It is not known whether the extra glycosylation at residues 42 and 66 blocks NGF binding by disrupting the tertiary structure of the cysteine-rich domain of p75, or by blocking NGF binding directly.

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PZ 105 ROLE OF INTRACELLULAR CALCIUM IN GROWTH CONE COLLAPSE EVOKED BY MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS. Christine E. Bandtlow, Marc F. Schmidt*, Tim Hassinger*, Stanley Kater* and Martin E. Schwab. Brain Research Institute, Univ. Zurich, Switzerland and *Colorado State University, Fort Collins, CO.
The myelin-associated protein NI-35 from the central nervous system is known to inhibit regeneration of lesioned neuronal fiber tracts in vivo and growth of neurites in vitro. Growth cones of cultured rat dorsal root ganglion neurons arrested their growth and collapsed when exposed to liposomes containing NI-35. Direct measurements of the concentration of free intracellular calcium levels, $[Ca^{2+}]i$, revealed a rapid and large increase in $[Ca^{2+}]i$ within growth cones exposed to NI-35-containing liposomes, that preceded the morphological changes. Neither an increase in $[Ca^{2+}]i$ nor growth cone collapse was detected in the presence of antibodies that block the inhibitory activity of NI-35. Dantrolene, a blocker of caffeine-sensitive intracellular calcium stores, protected growth cones from NI-35-evoked collapse. Depletion of these caffeine-sensitive intracellular calcium stores prevented the NI-35-evoked increase in $[Ca^{2+}]i$. These results suggest that NI-35 to exerts its neurite growth inhibitory effect through a sequence of events including interaction with a membrane receptor followed by a series of yet undefined processes that lead to a release of Ca^{2+} from intracellular stores.

PZ 106 EXPRESSION OF TYROSINEKINASE ENCODING PROTOONCOGENES DURING NEURONAL DIFFERENTIATION
Angelika Barnekow, Edith Ossendorf and Mechthild Rosing, Dept. Exp. Tumorbiology, University Muenster, Badestr. 9, W-4400 Muenster, Germany
Using the murine embryonal teratocarcinoma cell line P19, which can be induced to differentiate into neuron-like cells in vitro by addition of $5x10^{-7}$M retinoic acid to the culture medium, we investigated the differentiation-dependent expression of tyrosinekinase encoding protooncogenes e.g. c-src and c-yes on the levels of transcription and translation. Surprisingly, both genes displayed a differential expression pattern with a transcriptional and translational increase in c-src gene expression and a differentiation-dependent decrease in c-yes expression. Experiments are in progress to identify specific target proteins of these kinases. Characterization of the tyrosinekinases and their physiological target proteins like synaptophysin[1,2], which is specifically phosphorylated by $pp60^{c\text{-}src}$, but not by the highly related kinases $pp62^{c\text{-}yes}$ or $pp59^{fyn}$, will help to clarify the role of tyrosine kinases in neuronal signal transduction pathways and/or axonal transport mechanisms.
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[2]Barnekow et al.: NeuroProtocols in press (1993).

PZ 107 ACTIN ASSEMBLY IN SYNAPSES OF CULTURED NEURONS, Barbara W. Bernstein and James R. Bamburg, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523
Investigation of the role that the regulation of actin assembly may play in terminal functioning has been extended from a synaptosomal model system to a cultured neuronal system. Neurons, dissociated from sympathetic ganglia of 10-11 d chick embryos, were plated on poly-D-lysine- and laminin-coated glass coverslips in 50µl, parafin-sealed glass chambers. After 2-3 d in culture, an extensive network of interconnecting processes exists. The terminals of these processes were visualized with DIC microscopy and labeled with a styryl dye (FM1-43, Molecular Probes, Eugene, OR), thus allowing monitoring of exocytosis in real time. FM1-43 preferentially labels vesicle membranes during activated endocytosis that follows sustained exocytosis. Stimulating parameters were selected to induce reversible increases in cytoplasmic free Ca^{++} from approximately 10^{-7} to 10^{-6} M. Identified active terminals were fixed and double stained with rhodamine phalloidin and fluorescein DNase to locate sites of filamentous and monomeric actin, respectively. Laser scanning confocal fluorescence microscopy of the stained, identified active terminals indicated in preliminary experiments that depolarization induces a reversible disassembly of actin during the time course observed for exocytosis (2-3 min). We have stabilized filaments in the terminals of live neurons by loading the terminals of live neurons with phalloidin. The phalloidin stabilization reduces the rate and extent of exocytosis observed. These data are consistent with our model based on synaptosomal studies: actin filament disassembly is necessary for maximal transmitter release, and reassembly is necessary for modulating release during sustained depolarization. Supported in part by grants GM 35126, NS 28338, NS 28323, and RR 07127 from NIH.

PZ 108 FUNCTION AND EXPRESSION OF PLATELET-ACTIVATING FACTOR (PAF) RECEPTOR IN RAT CENTRAL NERVOUS SYSTEM, Haruhiko Bito, Zen-ichiro Honda, Hiroyuki Mutoh, and Takao Shimizu, Department of Biochemistry, University of Tokyo Faculty of Medicine, Tokyo 113, Japan.

Platelet-activating factor (PAF), an alkylether phospholipid, is a physiological ligand that elicits a variety of biological effects in different tissues through a G-protein-linked membrane receptor. Its production has been shown to occur in the CNS upon dopamine or acetylcholine application or electrical convulsant stimuli. Blockade of long-term potentiation in rat hippocampus by a competitive PAF antagonist has also been reported. We have recently demonstrated the presence of functional PAF receptor in the rat brain (H. Bito et al. (1992) Neuron, 9, 285-294): 1) Xenopus oocytes injected with brain poly(A)$^+$ RNA expressed PAF receptors; 2) binding assays using a stable antagonist of PAF (WEB 2086) and RNA blot analyses were carried out to confirm this observation, and a ubiquitous distribution of PAF receptor was shown in the rat CNS. Moreover, we found that PAF elicits intracellular Ca^{2+} mobilization in primary cultured hippocampal cells. Some NMDA receptors colocalized with PAF receptors, suggesting that PAF receptor might play a role in the metabotropic modulation of the postsynaptical $[Ca^{2+}]_i$.
Cloning of rat PAF receptor cDNA revealed the presence of two forms of transcripts that possess two different 5'-noncoding regions which are alternatively spliced to a common exon containing the full open reading frame. We also observed a similar finding in human PAF receptor gene and cDNAs, and have shown that the expression of PAF receptor mRNA is regulated under the control of two different promotors in a tissue-specific manner. Data on the specific expression of each transcript in different CNS areas will be presented.

PZ 109 PROTO-TRK MEDIATES TERMINAL DIFFERENTIATION IN NEUROBLASTOMA *IN VITRO* AND *IN VIVO*, Emil Bogenmann, *Hiroshi Matsushima, Division of Hematology-Oncology, Childrens Hospital Los Angeles, Los Angeles, CA 90027; University of Southern California, School of Medicine, Los Angeles, CA 90033. *Department of Pediatrics, Jikei University School of Medicine, Minato-ku, Tokyo, 105, Japan.

The role of the low ($gp75^{NGFR}$) and high affinity ($gp140^{proto\text{-}trk}$) Nerve Growth Factor (NGF) receptor genes in neuronal growth and differentiation is not entirely resolved. Neuroblastoma (NB) is a common childhood neoplasm of neural crest origin which shows various degrees of differentiation. Spontaneous regression occurs in Stage IVS disease. We hypothesize that the NGF signal cascade is a mechanism regulating NB cell differentiation. To study the biological properties of $gp140^{proto\text{-}trk}$ we transfected the human proto-trk cDNA into NB cells (HTLA230) lacking expression of $gp75^{NGFR}$. Stably transfected cells express high levels of proto-trk mRNA in Northern blots, and surface bound receptors as determined by FACS analysis. Treatment of these transfected cells with NGF elicits activation of immediate early genes (i.e., c-*fos*, c-*jun*) and inhibits cell proliferation, which is paralleled by down regulation of the highly expressed *myc*N gene. NGF responsive cells are morphologically differentiated and express genes associated with neuronal differentiation (i.e., SCG-10, NF-L, GAP43, ret-proto-oncogene). Transfectants remain tumorigenic despite the presence of a functional NGF cascade, however, NGF treatment of tumor bearing mice induces growth arrest and NB differentiation. Thus, terminal differentiation of human NB *in vitro* and *in vivo* is induced by NGF mediated by the $gp140^{proto\text{-}trk}$ in the absence of $gp75^{NGFR}$ expression. (Supported by grant from NIH/NS25795)

PZ 110 SINGLE-CELL ANALYSIS OF SCIP PROTEIN EXPRESSION IN THE OLIGODENDROCYTE LINEAGE, Oliver Bögler^*, Rainer Kuhn^, Alan Entwistle*, Mark Noble* and Greg Lemke^, ^Molecular Neurobiology Lab., Salk Institute, 100010 North Torrey Pines Rd., La Jolla, CA 92037 and *Ludwig Institute, 91 Riding House Street, London W1P8BT, UK.

The POU transcription factor SCIP is expressed in the Schwann cell and oligodendrocyte lineages. Studies at the population level show that differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitors into oligodendrocytes is associated with rapid reductions in SCIP mRNA and protein.

More detailed data was obtained using a novel method for analysing relative protein levels in single cells, employing a laser scanning confocal microscope to quantitate fluorescent emission from immunocytochemically labeled cells (LSCM analsysis). Controls demonstrate a linear relationship between emission and fluorophore concentration, as well as the specificity and sensitivity of the antibodies. SCIP mRNA levels measured by Northern blot correlate well with SCIP protein levels measured by LSCM analysis. SCIP expression in individual O-2A progenitors and oligodendrocytes from parallel cultures was analysed. Cells that were grown in PDGF, bFGF or PDGF plus bFGF, conditions which elicit markedly different cellular behaviours, expressed similar levels of SCIP. Also, even though O-2A progenitors typically expressed more SCIP than oligodendrocytes, there was a degree of overlap between the two populations. LSCM analysis allowed the study of SCIP expression in $O\text{-}2A^{adult}$ progenitors, cells found too rarely to allow biochemical analysis. SCIP expression in O-2A progenitors and oligodendrocytes acutley isolated by tissue printing was shown to be similar to their *in vitro* counterparts.

In summary, the more detailed analysis made possible by LSCM revealed that the relationship between SCIP protein levels and differentiation state is more complex than was anticipated by biochemical data alone.

PZ 111 PHARMACOLOGICAL CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF THE CLONED RAT SSTR4 SOMATOSTATIN RECEPTOR. J.F. Bruno, Y.XU, J. Song and M. Berelowitz Division of Endocrinology, SUNY at Stony Brook, NY 11794.

Somatostatin (SS14) is an important regulator of endocrine and brain function exerting its action after binding to high affinity membrane receptor subtypes. Its diverse physiological activities include inhibition of hormone secretion from pituitary, pancreas and gut. In the CNS, SS14 acting as a neurotransmitter/neuromodulator exerts inhibitory effects on neural function. Recently, three SS14 receptor genes, SSTR1, 2 & 3, have been cloned and characterized. We recently cloned and characterized a novel fourth member of this gene family from a rat genomic library, SSTR4, which is expressed predominantly in neural tissue. When stably expressed in CHO-K1 cells, SSTR4 binds SS14, SS28 and $DTrp^8SS14$ with high affinity; however, the SS14 analogs SMS201-995 and MK678 failed to displace specific binding. High affinity agonist binding was diminished by prior exposure to both GTPγS and pertussis toxin (PTX) but was not effected following agonist pretreatment indicating SSTR4 is coupled to a PTX-sensitive G protein but does not desensitize. SSTR4 expressed in CHO cells is coupled by a PTX-sensitive G protein to inhibition of adenylyl cyclase since treatment of transfected cells with SS14 resulted in the inhibition of forskolin-stimulated cAMP accumulation, an effect that was abolished by PTX treatment. The cloning of four SS14 receptor subtypes provide molecular probes for structure-function studies and for identifying those responsible for mediating the diverse physiological action of SS14.

PZ 112 DEVELOPMENTAL EXPRESSION OF THE ONCONEURAL PROTEIN Ri, Buckanovich R.J., Manley G., Darnell R.B., Rockefeller University, New York, NY. 10021.

Paraneoplastic neurologic diseases (PND) are thought to arise when an immune response is generated against neuronal proteins that are being ectopically expressed in tumor cells. Genes encoding such onconeural proteins have been cloned by screening cDNA expression libraries with serum from PND patients, identifying in several cases proteins known or suspected to be involved in neuronal signal transduction. Utilizing this technique we have cloned a novel gene (Ri) encoding an onconeural antigen associated with paraneoplastic *opsoclonus-myoclonus*, a neurologic disorder affecting midline cerebellar and brainstem motor systems. Northern blot analysis of human polyA+ mRNA reveals that the Ri gene is specifically expressed in the brain. Using bacterially expressed Ri fusion protein to affinity purify Ri antisera, we have studied Ri expression at various stages of mouse development . In the adult brain all neurons express Ri antigen, in the developing CNS Ri expression is (1) limited to the neurons in the anterior vs. the posterior portion of the spinal cord, and (2) in a gradient with greatest expression seen in the caudal spinal cord and expression diminishing by the level of the midbrain. These studies identify a neuronal protein associated with spinal cord and brainstem motor dysfunction that is preferentially expressed in these same areas of the CNS during mammalian development.

PZ 113 DIFFERENT ELECTRICAL ACTIVITY PATTERNS SELECTIVELY REGULATE GENE EXPRESSION

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We have analyzed how depolarization patterns of skeletal muscle by motoneuron-elicited electrical activity either selectively stimulate or repress gene expression. The contractile properties of muscle, as well as the distribution of receptors along a myofiber, are regulated by motoneuron-induced depolarization. Denervation of adult rat muscles was found to cause a dramatic decrease of transcripts coding for the fast and slow troponin I isoforms after 2 days. However, if the rat soleus (composed of slow-twitch myofibers) was denervated and immediately stimulated with a "slow" muscle pattern (20 Hz,10s every 30s) using extracellular electrodes, expression of the slow troponin mRNAs was maintained at the same levels of innervated muscle. The response to the activity pattern was selective, because stimulation with a "fast" muscle pattern (100 Hz,1s every 100 sec) could not prevent the down-regulation of the slow troponin gene after denervation. Interestingly, the fast pattern activated transcription of the fast troponin gene in this slow muscle. In order to identify the elements underlying regulation of the slow-troponin gene, it was cloned, and sequences required for its transcriptional regulation were studied in transfected cells and transgenic mice. We found that an upstream sequence of the gene confines transcription specifically to skeletal muscle and preferentially to the slow-twitch fibers. Although members of the MyoD family were found to regulate transcription of this gene during myogenesis, we believe it is unlikely that they underlie the selective responses to stimulation frequency. In contrast to the troponin genes, myogenin and MyoD mRNA levels increased by 50- and 25-fold after denervation (**PNAS** 88, 1349), and this accumulation was repressed by either fast or slow frequency stimulation. In transgenic mice the myogenin gene upstream sequences conferred specific skeletal muscle expression, as well as, directed increased transcription after denervation. In conclusion, we have found that nerve-induced electrical stimuli are transduced to signals that either repress or stimulate transcription of muscle genes, and that stimuli frequency selectively activate different programs of gene expression that underlie the plasticity of muscle phenotype.

PZ 114 ISOLATION OF A NEW 5´-SEQUENCE OF THE cDNA FOR RAT MINERALOCORTICOID RECEPTOR AND CHARACTERIZATION OF THE CORRESPONDING GENOMIC SEQUENCE, Maija Castrén, Florian Holsboer and Klaus Damm, Dept. of Neuroendocrinology, Max-Planck-Institute of Psychiatry, Clinical Institute, Munich, Germany.

The mineralocorticoid receptor (MR) is a nuclear receptor for adrenal steroids. Depending on the corticosteroid which binds to the receptor the MR can serve different functions in different tissues. To elucidate the molecular mechanisms that are involved in the tissue-specific regulation of MR, we have isolated hippocampal cDNAs for rat MR and characterized a promoter for the gene. Two types of rat MR cDNAs have been isolated from rat hippocampus. The protein coding sequences of the isoforms are identical but they differ in their 5'-untranslated sequences. A novel rat cDNA isoform has a 5´-untranslated exon highly homologous (90%) to the human kidney cDNA suggesting that it has some important and specific function(s) which has been evolutionarily preserved. We have isolated a rat genomic clone containing the corresponding genomic sequences and characterized the sequence upstream of the exon, which is separated from the first coding exon by an intron of 5.2 kb. The sequence is very G+C-rich and lacks TATA or CAAT elements. The putative promoter region contains several short sequences that are similar to known cis-acting enhancers or transcription factor binding sites. The DNA sequence surrounding the transcriptional initiation site closely resembles the consensus sequence 5´-CTCANTCT-3´ thought to be important core sequence for transcription initiation found in RNA polymerase B(II) transcribed promoters devoid of TATA element. In addition, three GC boxes, putative binding sites for transcription factor Sp1, were identified. In preliminary transient transfection experiments we have demonstrated that the genomic sequence of 635 bp has promoter activity in kidney (CV1) and neuroblastoma (SK-N-MC) cell lines.

PZ 115 **SELECTIVE REQUIREMENT FOR Raf-1 KINASE WITHIN THE SAME CELL IN ACTIVATION OF MAP KINASE BY DIFFERENT GROWTH FACTORS.**

Tsung-Shu Oliver Chao*, David A. Foster#, Ulf Rapp@, and Marsha R. Rosner* *Ben May Institute and Dept. of Pharmacology and Physiology, University of Chicago, Chicago, IL 60637, # Institute for Biomolecular Structure and Function and the Dept. of Biological Sciences, Hunter College, City University of New York, New York, NY 10021, and @ Laboratory of Viral Carcinogenesis, NIH/NCI, Frederick, MD 21702-1021.

Although Raf-1 kinase has been implicated as a potential upstream activator of mitogen-activated protein (MAP) kinase in certain cell lines, the precise role of raf-1 in MAP kinase activation has not been elucidated. We therefore determined whether 1) raf-1 is absolutely required for activation of MAP kinase in cells responsive to raf-1; and 2) stimulation of raf-1 is the only mechanism for activation of MAP kinase in these cells. Using a murine Balb/c 3T3 derivative stably transfected with a dominant-negative allele of raf-1 (LA-R1), we found that MAP kinase activation by insulin-like growth factor-I (IGF-I) was essentially abolished in the absence of functional raf-1. In contrast, epidermal growth factor (EGF) remained as a potent stimulator of MAP kinase. A similar pattern was observed with induction of fos, an event further downstream in the signalling pathway. IGF-I stimulation of fos was significantly inhibited by loss of active raf-1, whereas EGF stimulation of fos was effectively unchanged These results indicate that raf-1 is required for maximal activation of MAP kinase or fos by certain agents, such as IGF-I, but represents only one of the multiple pathways by which EGF activates the MAP kinase.

PZ 116 ONTOGENY OF 5HT$_2$ RECEPTOR GENE EXPRESSION IN DEVELOPING RAT BRAIN. Roland D. Ciaranello, Steven J. Garlow and David A. Morilak. Nancy Pritzker Laboratory of Developmental and Molecular Neurobiology, Department of Psychiatry, Stanford University School of Medicine, Stanford, CA. 94305-5485

Previous work from our laboratory has shown that whole brain 5HT$_2$ receptor mRNA appears around embryonic day 17 (E17) in the rat. Messenger RNA levels increase slowly to postnatal day 4 (P4), then undergo a burst in transcription from P4 until P12, when mRNA levels have increased by 13-fold. Message levels then decline by about 25-50% between P12-P27, when they attain adult levels. Quantitative radioligand binding exhibits the same profile as receptor mRNA. The changes taking place in 5HT$_2$ mRNA over this period correspond to a time when the cerebral cortex is undergoing serotonergic hyperinnervation followed by axonal pruning. To explore further the developmental control of 5HT$_2$ receptor gene expression, we prepared an antibody to the receptor. This antibody was used to map the developmental profile of receptor protein. Receptor mRNA was visualized during development using a 230 bp Alu I fragment from the receptor cDNA in *in situ* hybridization mapping studies. In contrast to serotonergic innervation, which makes its appearance early in brain development, 5HT$_2$ receptors appear considerably later, and proceeds in a caudal-to-rostral pattern. 5HT$_2$-expressing cells in the pontine dorsal tegmental nucleus appear as early as E18, and attain adult numbers and morphology by P4. In the caudate nucleus, some receptor labelling can be seen at P0, and the adult pattern is seen at P12. In the hippocampus, no receptor labelling is seen until P4, and a functional adult pattern of cell morphology and number is not seen until sometime after P12. Finally, in the cerebral cortex, receptor labelling begins to appear around P7, and reaches a peak of complexity at P12. Cortical 5HT$_2$-bearing neurons exhibit an extensive and complex arborization, but only a few cells actually show immunopositivity. Between P12 and P27, there is a decline in the extent of cellular arborization, consistent with the previously observed decrease in receptor binding and mRNA. Among the principal conclusions from this study are that a surprisingly small number of neurons in the brain express 5HT$_2$ receptors, although in most brain regions these all exhibit considerable dendritic arborization. In most cases, 5HT$_2$ receptor expression appears to take place on either cholinergic or GABA-ergic interneurons. Despite the extensive ramification of the 5HT2-bearing neurons, our results suggest that disease processes which effect the absolute number of 5HT$_2$-expressing neurons could have a significant impact on the total brain concentration of these receptors.

PZ 117 NERVE GROWTH FACTOR (NGF)-RESPONSIVE ELEMENTS IN THE RAT STROMELYSIN-1 (ST-1) GENE, Gary Ciment[†], Cynthia Machida[†] and Janice Lochner[‡], [†]Dept Cell Biology & Anatomy, Oregon Health Sciences Univ., Portland, OR 97201; [‡]Dept. Chemistry, Lewis and Clark College, Portland, Oregon 97201

In previous work, we found that NGF induced ST-1 mRNA and protein expression in rat PC12 pheochromocytoma cells. Levels of the ST-1 mRNA were found to increase at least 1000-fold, and this induction was found to be due, at least in part, to *de novo* transcription. Since ST-1 is a secreted metalloproteinase known to be involved in the degradation of various extracellular matrix proteins associated with basal laminae, it has been suggested that the regulated expression of this gene product in PC12 cells may reflect on the invasive properties of neuronal growth cones through basal laminae..

Here, we characterize a 750 base pair region in the 5'-nontranscribed region of the ST-1 gene known to confer NGF-responsiveness to the chloramphenicol acetyl transferase (CAT) reporter gene in transient transfection assays. Deletion studies from the 5'-end of this promoter reveal a region between bases -247 and -315 (with respect to the transcription start site) which are necessary for NGF-responsiveness, but which do not contain identifiable cis-acting elements. DNA footprint analysis is currently underway to determine whether one or another protein binds to particular sequences within this region Site-directed mutagenesis studies indicate that an AP1 sequence (5'-TGAGTCA-3') near the 3'-end of the ST-1 promoter is also necessary for NGF-responsiveness.

In sum, these studies show that multiple cis-acting elements in the 5'-untranscribed region of the ST-1 gene, including one potentially novel site, are necessary for NGF-responsiveness. Identification of the transcriptional proteins binding to these sites may provide important additional information concerning the NGF-signalling pathway in various neuronal cell types.

PZ 118 *Abstract Withdrawn*

PZ 119 THE PARANEOPLASTIC ANTIGEN CDR2, A PURKINJE NEURON-SPECIFIC LEUCINE ZIPPER PROTEIN, HAS A NOVEL STRUCTURE IN TUMOR CELLS, John P. Corradi and Robert B. Darnell, Laboratory of Molecular Neuro-Oncology, The Rockefeller University, New York, NY 10021

Paraneoplastic cerebellar degeneration (PCD) is a neurologic syndrome in which the expression of Purkinje neuronal proteins by tumor cells elicits an autoimmune response, resulting in Purkinje cell destruction. PCD is associated almost exclusively with gynecological tumors, suggesting that these antigens provide a selected function in tumor cells, and raising interest as to their normal role in Purkinje neuronal function. Serum from individuals with PCD reacts specifically with Purkinje cell cytoplasm in immunohistochemical studies, and identifies a major antigen of 52kD (CDR2) in neuronal extracts analyzed by Western blot. Partial cDNAs encoding this antigen had been reported previously from cerebellar and HeLa cell cDNA libraries, which identify a novel protein with an extended leucine zipper motif. More complete cDNAs have now been cloned from brain and HeLa cell libraries. A complete brain cDNA sequence is highly homologous in human and mouse, diverging significantly only in the 5' UTR. Interestingly, the HeLa CDR2 sequence has an extended open reading frame at the 5' end as compared to the brain sequence. Thus CDR2 is a Purkinje neuron specific leucine zipper protein with structural and perhaps functional differences in neurons and tumors.

PZ 120 NOVEL OPIOID BINDING SITES ASSOCIATED WITH THE NUCLEI OF NG108-15 NEUROHYBRID CELLS.
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Opioid binding sites have been discovered in nuclei of NG108-15 cells. Marker enzyme analyses and electron microscopy studies attested to the purity of nuclear preparations. Immunohistochemical staining of cryostat sections of NG108-15 cells with an anti-opioid receptor *antibody corroborated* a nuclear localization of these sites. Homologous and heterologous competition binding assays, kinetic and stereospecificity data, satisfied criteria for the presence of δ opioid sites in purified nuclear preparations. Opioid peptide agonists bind with high affinity to nuclear membranes and with lower affinity to chromatin and nuclear matrix preparations. High affinity sites of the partial agonist diprenorphine were localized in chromatin and nuclear matrix preparations, while low affinity binding was found in nuclear membranes. Gpp(NH)p sensitivity of agonist binding was detected in nuclear membranes but neither in chromatin nor matrix. Thus, NG108-15 cells may contain newly synthesized G protein-coupled δ receptors in nuclear membranes and G protein-*uncoupled opioid* sites in chromatin and/or matrix. Binding sites for neurotrophins have been reported to undergo retrograde transport to the nucleus, possibly explaining their ability to alter gene expression. β-Endorphin (IC_{50} 0.7 nM) displays transient, anti-mitogenic activity in developing brain cells. These findings permit the speculation that the chromatin/matrix δ-site may play a role in a putative genetic regulation of cell proliferation.

PZ 121 FUNCTIONAL TESTING OF HUMAN DOPAMINE D_1 RECEPTOR EXPRESSED IN A STABLE cAMP-RESPONSIVE LUCIFERASE REPORTER CELL LINE.
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Bender + Co. GmbH., Dr. Boehringer-Gasse 5-11, A-1121 Vienna, Austria.

A large number of G-protein coupled receptors are known to modulate adenylyl cyclase activity. Most compounds showing agonist or antagonist activity on specific receptor subtypes have been identified originally by their ability to bind or compete for binding with radioligands to cell surface receptors. In order to find new compounds modulating the activity of specific G-protein coupled receptors we have developed a cellular screening system that measures the biological activity of receptors coupled to the cAMP signal transduction pathway via the transcriptional activation of a reporter gene. A chinese hamster ovary (CHO) cell line was stably transformed after transfection with a reporter plasmid containing the *Photinus pyralis* luciferase gene under the transcriptional control of a minimal promoter sequence and six cAMP responsive elements (CRE). This CRE reporter cell line showed 20 to 30-fold increase in luciferase activity after stimulation of adenylyl cyclase with forskolin, but did not respond to dopamine agonists or activators of the inositol phosphate-diacylglycerol pathway. A stable test cell line for dopamine D_1 receptor was developed by transfecting the reporter cell line with an expression plasmid containing the human dopamine D_1 receptor gene. Treatment of this test cell line with dopamine receptor agonists increased the bioluminescence in a dose-dependent manner. Simultaneous treatment of the test cell line with dopamine receptor antagonists inhibited the luciferase induction. The rank of potency of various dopamine receptor agonists and antagonists seen on the D_1 receptor test cell line was in good agreement with reported data obtained from binding studies. Due to the high sensitivity of the luciferase reporter system, this non-isotopic assay can be performed in microtiter plate format and is far less work intensive than the determination of adenylyl cyclase by direct cAMP measurement.

PZ 122 INHIBITION OF RETINOIC ACID AND THYROID HORMONE RESPONSE BY DOMINANT NEGATIVE RECEPTOR MUTANTS, Klaus Damm, Dept. of Neuroendocrinology, Max-Planck-Institute of Psychiatry, Munich, Germany.

The two closely related proto-oncogenes erbAα and erbAβ encode thyroid hormone receptors that act as hormone-inducible trans-acting factors similar to other members of the nuclear hormone receptor superfamily. A novel property of the TR/c-ErbA proteins is their ability to repress the basal transcription level of promoters containing thyroid hormone responsive elements in the absence of hormone. Binding of thyroid hormone relieves the repression activity of the DNA bound receptor and reveals or induces the transcriptional activation function. The biological significance of the repressor function is illustrated in the v-erbA oncogene product, a mutated and oncogenic derivative of the TR/c-ErbA, which has lost the ability to bind and respond to hormone and acts as a dominant negative inhibitor of the TR. This inhibition of endogenous receptor activity by the expression of dominant negative receptor derivatives repesents one approach to study the distinct effects of the hormones in vivo and in vitro. The constitutive negative phenotype of v-ErbA is due to mutations in the C-terminal stretch of the ligand binding domain, a region where v-ErbA, TRs and the closely related retinoic acid receptors (RARs) exhibit a 53% amino acid similarity. Using site directed mutagenesis both isoforms of thyroid hormone receptors (TRα and TRβ) as well as the three isoforms of RARs (RARα, RARβ and RARγ) were converted into potent negative transcriptional regulators that no longer respond to the cognate hormone. The mutant receptors block wildtype receptor function and actively repress the basal transcription level of normally hormone responsive promoters. Additional mutagenesis experiments identify regions of the proteins required for dimerization, transcriptional activation and repression as well as dominant negative inhibition.

PZ 123 PRIMARY STRUCTURE AND LOCALIZATION OF A PUTATIVE CYCLIC NUCLEOTIDE-GATED CHANNEL IN DROSOPHILA MELANOGASTER, Janine A. Davis and Randall R. Reed, Dept. of Molecular Biology and Genetics, Johns Hopkins University, Howard Hughes Medical Institute, Baltimore, MD 21205

Vertebrate visual and olfactory signal transduction pathways employ distinct, but related cyclic nucleotide-activated cation channels to regulate the flow of ions across the photoreceptor and olfactory neuronal cell membranes. To determine whether a homologous channel is operative in invertebrates, an adult Drosophila melanogaster (Dm) head cDNA library was screened using the vertebrate channels as a probe. Two cDNA clones, DC 1 and DC 2, predict identical proteins except for a 271 amino acid insertion in DC 1. The putative proteins encoded by DC 1 and 2 share sequence homology with the vertebrate cyclic nucleotide-gated channels (40% amino acid identity with the rat olfactory channel), retain a membrane topography similar to the vertebrate channels, and preserve a cyclic nucleotide-binding domain common to cyclic nucleotide-binding proteins. A third cDNA (DC 3) predicts a protein which is identical to DC 1, but then diverges within, and is terminated shortly after, the cyclic nucleotide-binding domain. Whether the sequence variation observed in the Dm channels, which is most likely due to alternative RNA splicing, reflects functionally diverse channels remains to be demonstrated. To date, we have been unable to detect cyclic nucleotide-dependent current activity across membrane patches from cells expressing individual, putative channel proteins.

To examine the tissue distribution of the putative channel in Dm embryos and adult flys, we have generated an anti-peptide antibody to the carboxy-terminus predicted by DC 1& 2. Preliminary results demonstrate that it specifically recognizes proteins of the anticipated size in heterologous cell extracts transiently expressing DC 1 or 2. A second approach, utilizing in situ hybridization with a probe common to DC 1,2, & 3, has demonstrated intense hybridization throughout the entire central nervous system of the Dm embryo. In situ hybridization will allow us to discern the distribution of each of the forms of the putative channel in both the embryo and adult fly.

PZ 124 REGULATION OF NERVE GROWTH FACTOR (NGF) SIGNALLING THROUGH MODULATION OF gp140^{trk} EXPRESSION IN BRAIN AND PC12 CELLS. R.E. Davis, T. Hepburn, H. LeVine, K. Spiegel, R.J. Wyborski and B.D. Shivers, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48106

NGF influences neuronal function through action at two cell surface binding proteins, identified by their relative molecular masses as p75^{NGFR} and gp140^{trk} binding proteins. While an understanding is emerging of the short-term (minutes to hours) events involved in gp140^{trk}-mediated signal transduction, very little is known concerning the regulation of gp140^{trk} expression and its relationship to cellular signalling. We have studied this relationship in PC12 cells undergoing NGF-mediated differentiation and after direct intrastriatal NGF injections in rat brain.

Upon treatment with NGF, PC12 cells undergo striking morphological and functional changes developing neuron-like characteristics. In concert with these changes we found that both p75^{NGFR} and gp140^{trk} expression are induced along a similar time course when PC12 cells are exposed to NGF (100 ng/ml) for up to 7 days in culture. Increased expression of these two binding proteins is apparent at the messenger RNA and protein levels after the first day of growth factor treatment. These changes are accompanied by increases in choline acetyltransferase activity (ChAT) but a decrease in NGF-mediated stimulation of MAP kinase activity (ERK 1 and 2). These findings suggest that as PC12 cells develop a neuronal phenotype, NGF-mediated intracellular signalling may be diminished.

In the adult rodent brain, we have found that the corpus striatum expresses only gp140^{trk} at detectable levels. Continuous intracerebroventricular infusion of NGF increases ChAT activity but does not alter the content of gp140^{trk} in the striatum. Continuous intrastriatal infusion also increases ChAT activity but at high doses NGF (1μg/day) decreases gp140^{trk} levels in this brain region. Thus, in the adult rat NGF can alter the functional capacity of cholinergic neurons in absence of detectable p75^{NGFR} suggesting that this binding protein is not essential for some of the central actions of NGF. In contrast to PC12 cells, exposure to NGF down-regulates gp140^{trk} levels in the adult striatum. At present, we do not know the long-term consequences of down-regulation of gp140^{trk} but these data suggest that overdosing with NGF may have deleterious effects on a subset of central cholinergic neurons.

PZ 125 M2 MUSCARINIC RECEPTORS INTERACT WITH $G_{\alpha i2}$ AND $G_{\alpha i3}$ TO STIMULATE PHOSPOLIPASE C AND INHIBIT ADENYLYL CYCLASE: SPECIFICITY OF G-PROTEIN COUPLING DETERMINED BY SITES IN THE THIRD CYTOPLASMIC LOOP, Mark L. Dell'Acqua, Reed C. Carroll, and Ernest G. Peralta, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

Previous studies using transfected CHO cells have shown that the m3 muscarinic acetylcholine receptor subtype regulates the stimulation of phospholipase C (PLC) through PTX-insensitive G proteins while the m2 receptor subtype regulates both the stimulation of PLC and the inhibition of adenylyl cyclase (AC) through PTX-sensitive G proteins. In this study, we used cholera toxin (CTX) to ADP-ribosylate PTX-sensitive G_{α} subunits as part of the ternary complex formed by heterotrimeric G proteins and agonist-bound receptors to characterize the interactions between transfected muscarinic receptors and the endogenous G proteins in CHO cells. In membranes derived from cells expressing the m2, but not the m3 receptor the cholinergic agonist carbachol stimulated CTX modification of both $G_{\alpha i2}$ and $G_{\alpha i3}$. Importantly, similar carbachol dose-dependancies and PTX dose-sensitivities were observed for m2 receptor-mediated PLC signalling and both PTX and CTX modification of $G_{\alpha i2}$ and $G_{\alpha i3}$ suggesting that these G proteins couple to stimulation of PLC. However, agonist-induced desensitization processes which blocked m2 receptor interactions with $G_{\alpha i2}$ and $G_{\alpha i3}$ equally effected both PLC stimulation and AC inhibition suggesting the additional involvement of $G_{\alpha i2}$ and $G_{\alpha i3}$ in mediating inhibition of adenylyl cyclase. These suggestions were confirmed by the analysis of m2-m3 hybrid muscarinic receptors in which the entire putative-third cytoplasmic loops were reciprocally exchanged. Replacement of the third loop of m3 with that from m2 resulted in a receptor which when expressed in CHO cells was able to stimulate PLC, inhibit AC, and promote CTX-modification of $G_{\alpha i2}$ and $G_{\alpha i3}$ all in a PTX-sensitive manner. Correspondingly, replacement of the third loop of m2 with that from m3 resulted in a receptor able to stimulate PLC in a PTX-insensitive fashion, but unable to promote CTX-modification of $G_{\alpha i2}$ and $G_{\alpha i3}$ and unable to mediate inhibition of adenylyl cyclase. These results suggest that sites in the third cytoplasmic loop of the m2 receptor are responsible for specifying the interactions of this receptor subtype with PTX-sensitive G proteins coupled to both stimulation of PLC and inhibition of AC.

PZ 126 NEUROGENIC EXPRESSION OF *PAX-2* IN NORMAL AND *Sd* MICE Gregory R. Dressler, Dawn Phelps, National Institute of Child Health and Human Development, Bethesda, MD.

The mammalian Pax genes encode DNA binding transcription factors that regulate specifc morphogenic processes during development and have been associated with a variety of mutations in mouse and man. The pattern of Pax-2 protein expression during the development of the mouse was examined with specific antibodies. Beginning at embryonic day 8 (E8), Pax-2 proteins are detected in the neural ectoderm of the head fold at the prospectrive midbrain-hindbrain region with some staining near the optic placode. By E9, the boundary between midbrain and hindbrain is clearly demarcated by Pax-2 positive cells. The developing optic cup and otic vesicle are also expressing high levels of Pax-2 at E9. By E11, Pax-2 expression in the midbrain-hindbrain region is replaced by a second phase of expression specific for differentiating cells of the intermediate zone of the spinal cord. Two compartments immediately dorsal and ventral to the sulcus limitans express Pax-2 at a time consistent with neuron birth. Pax-2 is not expressed in the neuroepithelium, nor is Pax-2 expressed in the most ventral one-fourth of the spinal cord, were presumptive ventral motoneurons are being born. By E12, Pax-2 expressing cells have migrated laterally to settle in the prospective intermediate grey region of the spinal cord.

The pattern of *Pax-2* expression was also examined in Danforth's *short tail* (*Sd*) homozygous mice. Because these mice lack a notochord in caudal regions, the floor plate of the spinal cord is not induced and posterior mesoderm derived structures are also affected. The expression of *Pax-2* during neural differentiation in the spinal cord was normal in anterior sections, but ectopic expression in the ventral half of the basal plate was observed in regions lacking the floor plate. The data support the hypothesis that *Pax-2* expression domains are influenced by signals emanating from the floor plate and that *Pax-2* functions during the dorsal-ventral patterning of the spinal cord at a time when cells are differentiating from the neuroepithelium. In addition, the Danforth's *short tail* mutation is a useful model for the study of developmentally regulated genes that are under the influence of the notochord or floor plate.

PZ 127 COUPLING OF ALPHA$_2$-ADRENERGIC RECEPTOR (α_2-AR) SUBTYPES IN A CELL-TYPE SPECIFIC MANNER, E. Duzic and S.M. Lanier, Department of Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, SC 29425

α_2-AR subtypes may activate diverse signaling pathways depending on the particular G-proteins and effector molecules expressed in any given cell. To address this issue we stably expressed the α_{2B}- and α_{2D}-AR subtypes in three functionally distinct cell types (NIH-3T3 fibroblasts, DDT$_1$ MF-2 smooth muscle cells, and the pheochromocytoma cell line PC-12). Agonist mediated alteration of basal or forskolin-activated adenylylcyclase served as an indicator of receptor-effector coupling. In NIH-3T3 and DDT$_1$ MF-2 cells, receptor activation inhibited basal and forskolin-induced elevation of cellular cAMP. This inhibition is pertussis toxin sensitive. However, in PC-12 transfectants the same receptor subtype actually increased basal cAMP and augmented the effect of forskolin. This augmentation is insensitive to pertussis toxin and likely involves receptor coupling to a Ca^{2+}/calmodulin sensitive adenylylcyclase based on the following: 1) the augmentation is blocked by loading the cells with acetoxymethyl BAPTA which minimizes changes in $[Ca^{2+}]_i$ by calcium chelation, 2) functional analysis of adenylylcyclase activity indicates that PC-12, but not NIH-3T3 and DDT$_1$ MF-2 cells express a Ca^{2+}/calmodulin sensitive cyclase, and 3) mRNA blot analysis with type-selective and non-type-selective probes indicates the presence of a Ca/calmodulin sensitive type III adenylylcyclase in PC-12, but not NIH-3T3 cells. Although both the α_{2B}- and α_{2D}-AR subtypes appear to augmented forskolin-stimulated increases in cAMP by increasing $[Ca^{2+}]_i$, the two receptors my increase $[Ca^{2+}]_i$ by different mechanisms based on their differential sensitivity to chelation of extracellular Ca^{2+}. Whatever mechanism is involved, intracellular Ca may play a critical role in determining whether the receptor potentiates or inhibits adenylylcyclase activation. The ability of a single receptor to trigger different responses to agonist adds considerable flexibility to the cell-signaling system and has important *ramifications in developmental* biology and within the central nervous system.

PZ 128 **NITRIC OXIDE POTENTIATES THE EFFECT OF CALCIUM ON TRANSCRIPTION IN NEURONAL CELLS,** Grigori Enikolopov and Natalia Peunova, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Long lasting changes in synaptic plasticity correlate with changes in gene activity in neurons. We are interested in whether nitric oxide (NO), which might act as a retrograde messenger during certain long term phenomena in the brain, can stimulate gene activity. We have tested a series of reporter genes and found that although NO alone does not stimulate activation of transcription within a wide range of concentrations, it greatly potentiates the action of agents that elevate Ca^{2+} concentration in the cell. NO and Ca^{2+}-agents have to act within a very narrow time window for potentiation to occur, suggesting that in the nervous system this effect might be restricted to the recently active synapses. To identify NO/Ca^{2+}-activated signal transduction pathways that are involved in the induction of gene transcription, we have studied a series of simplified reporter genes in combination with specific recombinant protein kinase inhibitors. These studies suggest that at least three different protein kinases act through distinct transcription factors to activate gene transcription following NO-potentiated Ca^{2+} action.

PZ 129 THE HUMAN 5-HT1A RECEPTOR EXPRESSED IN HeLa CELLS DIRECTLY BUT DIFFERENTLY REGULATES THE ACTIVITY OF AC AND PLC *via* THE SAME G PROTEIN. Annick Fargin, Department of Pharmacology, University of Montreal, Montreal, Qc, H3C 3J7. We have previously reported the expression of the cloned human 5- HT1A receptor in HeLa cells (J. Biol.Chem., 264:14848-14852, 1989). We showed that 5-HT can elicit both an inhibition of cAMP formation and a stimulation of PI hydrolysis. Here, we demonstrate that the 5-HT1A receptor is able to directly modulate the activity of the two effectors AC and PLC. In both cases this capacity is equally altered by Pertussis Toxin. Morever, using anti-peptide antibodies recognizing the C-terminal part of the α subnit of Gs, Go, Gil-3 and preventing the coupling of the receptor to these G proteins, we could demonstrate that *via* the same Gi protein (mostly Gi3 in HeLa cells), the receptor can modulate the activity of both AC and PLC. To further explore and compare the two signal transduction pathways, we conducted a detailed quantitative study of 5-HT-induced effects. To do so, 1) we used a clonal cell line of tranfected HeLa cell highly expressing the receptor (5-7 pmoles/mg prot.), 2) progressively depleted the receptor concentration with the use of an alkylating antagonist (BIM, a derivative of pindolol), 3) for each receptor concentration, we followed 5 HT-induced cAMP and IPs formations. We could therefore quantify the maximal response (em) and the 5-HT concentration at which half of the maximal response is obtained (A50), 4) finally, these data were analysed using an operational model of agonism. It is noteworthy that for high receptor concentration (5-7 pmoles/mg prot.), the A50 for both responses are similar (10-30 nM). However, at low receptor concentration (0.2-0.5 pmoles/mg prot.), while em for cAMP formation is not altered, em for PI hydrolysis is dramatically decreased (90% inhibition of cAMP formation and 50% (*vs* 150%) stimulation of IPs synthesis). This is the first time that the human 5-HT1A receptor is shown to directly modulate the activity of two effectors (AC and PLC). Morever, the G-protein transducer is equivalent. Quantitive analysis of the two agonist-induced responses reveals that the receptor strongly and weakly regulates the activity of AC and PLC, respectively.

PZ 130 **CLONING OF AN N-TYPE CALCIUM CHANNEL EXPRESSED IN THE RAT HIPPOCAMPUS,** Rodrigo Franco, Michael Cuddy, Carolyn Hardy, David Shuey, Thomas J. Colatsky and Ling Sing Chen. Division of Cardiovascular and Metabolic Disorders, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543

The N-type voltage dependent calcium channel has been linked to release of neurotransmitter in various parts of the central and peripheral nervous system. In order to begin an analysis of the molecular mechanisms underlying the regulation of neurotransmitter release, we have cloned a cDNA from rat hippocampus that encodes an alpha-1 subunit of an N-type channel. The electromotor nucleus of the marine electric ray is a tissue known to be a rich source of binding protein for omega conotoxin, the only known specific blocker of N-type channels. Using a probe generated from a novel ion channel encoding cDNA isolated from the electromotor nucleus (kindly provided by Dr. Richard Tsien, Stanford), we screened a rat hippocampal cDNA library. Several overlapping cDNA clones have been isolated and sequenced and encode a protein with 95% homology to the recently identified human N-type channel . Definitive classification awaits further characterization of expressed channels. Southern analysis of rat genomic DNA is consistent with the existence of a single gene. Northern analysis reveals brain specific expression of multiple messages in the size range of 8 to 9.5 kb. Preliminary *in situ* hybridization experiments demonstrate expression limited to the dentate gyrus and CA fields of the hippocampus. Further analysis of the structure, function and expression of this channel is in progress.

PZ 131 A JUN-LIKE PROTEIN IN RAT STRIATUM IS REGULATED BY MORPHINE, Meredith Garcia and Richard Harlan, Dept. of Anat. and Prog. in Neurosci., Tulane Univ. Sch. of Med., New Orleans, LA 70112

Previous studies from this laboratory (Chang et al, 1988) have shown that c-Fos expression is regulated by morphine (MOR) in rat striatum. Because a heterodimer of c-Fos and a Jun protein is required for transcriptional activation at the AP-1 site, we studied the effect of MOR on Jun expression in rat brain, using immunocytochemistry (ICC). To test the effects of acute MOR, male Sprague-Dawley rats (n=3) were given vehicle, MOR (10 mg/kg) or naltrexone (NAL; 10 mg/kg) followed by MOR and sacrificed 3 hr later. To test the effects of chronic MOR, rats were implanted with a 75 mg MOR pellet, daily for 5 days (n=5); control animals were given vehicle pellets. ICC was performed on 60 um sections of perfusion-fixed brain, using methods standard in this lab. Four different antibodies (Abs) were used: two anti-peptide antibodies (anti-c-Jun Ab-1 and Ab-2, Oncogene Science) and two Abs against expressed Jun proteins (anti-JunB #725/3 and anti-JunD #783/3, a gift of R. Bravo). Comparison of immunoreactivity (ir) with mRNA distribution (from Mellstrom et al, 1991) showed good agreement in the case of the anti-c-Jun Ab-1, anti-JunB and anti-JunD. However, the pattern of ir seen with anti-c-Jun Ab-2 did not match the distribution of any of the mRNAs (Harlan and Garcia, *Abstr. Soc. Neurosci.*, 1992), with expression restricted to the nuclei of cells in striatum and associated structures. In control brains, it appeared within the "matrix" compartment of striatum, based on comparison with calbindin(CD)-ir in adjacent sections. In preliminary studies, acute MOR increased the number and optical density of cells labeled by the anti-c-Jun Ab-2 in the nucleus accumbens and the dorso-medial caudate-putamen (CPu). The effect was blocked by prior injection of NAL. In more extensive studies, chronic MOR increased markedly the number and optical densities of labeled cells in the nucleus accumbens and throughout the CPu, especially in the "patch" compartment, which is highly enriched in mu opiate receptors. These studies suggest that the anti-c-Jun Ab-2 antibody may recognize a Jun-like protein, different from the other known members of the Jun family. The restricted distribution of this Jun-like-ir in the striatum and related structures suggests a unique role in the functions of the forebrain. In a previous study (Garcia and Harlan, *Abstr. Soc. Neurosci.*, 1992) we found chronic MOR to increase CD-ir in striatal patches, possibly through increased corticostriatal glutamatergic activity. These findings suggest that regulation of Jun-like ir by morphine, especially within the "patch" compartment of the striatum, may occur through a similar mechanism. (Supported by DA-05411 to MMG and DA-06194 to REH)

PZ 132 K-252A AND STAUROSPORINE PROMOTE CHOLINE ACETYLTRANSFERASE ACTIVITY IN RAT SPINAL CORD CULTURES. Marcie A. Glicksman, J. Eric Prantner, M. Elizabeth Forbes, and Nicola Neff, Cephalon, Inc. 145 Brandywine Parkway, West Chester, PA 19380

The K-252a family of organic molecules has been shown to possess multiple activities. Neurotrophin-3 activity is potentiated by K-252b in several neuronal systems, and K-252a inhibits the phosphorylation of the high affinity NGF receptor, *trk*, in PC-12 cells. We have discovered a new neurotrophic activity of these K-252a compounds in spinal cord cultures. K-252a increased ChAT activity in rat embryonic spinal cord cultures in a dose-dependent manner (EC_{50} ≅100 nM), with maximum stimulatory activity at 300 nM resulting in a 3 to 4-fold increase. A single application of K-252a completely prevented the marked decline in ChAT activity occurring over a five day period following culture initiation. Out of 11 kinase inhibitors, only the structurally related inhibitor staurosporine also increased ChAT activity (EC_{50} ≅0.5 nM). Effective concentrations of K-252a were not cytotoxic or mitogenic, and did not alter the total protein content of treated cultures.

Insulin-like growth factor-I (IGF-I), basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (CDF/LIF) yielded dose-dependent increases in ChAT activity in spinal cord cultures. The combination of K-252a with IGF-I or bFGF increased ChAT activity up to 8-fold over untreated controls, which was greater than that observed with each compound alone. K-252a combined with CNTF or CDF/LIF demonstrated no additive or synergistic effects on ChAT activity. These results suggest that there are multiple mechanisms for the maintenance of cholinergic neurons in spinal cord cultures.

The enhancement of spinal cord ChAT activity by K-252a and staurosporine defines a new neurotrophic activity of these small organic molecules for cholinergic neurons and raises the possibility that they may activate some regulatory elements in common with the CNTF and CDF/LIF family of neurotrophic proteins.

PZ 133 DISTINCT PATHWAYS OF CONVERGENCE ONTO ERK/MAP KINASES FOR DEPOLARIZATION-, BRADYKININ-, AND PROTEIN-TYROSINE KINASE-INITIATED SIGNALING. Steven H. Green and Mengsheng Qiu, Department of Biological Sciences, University of Iowa, Iowa City, IA 52242.

Neurotransmitters and nerve membrane electrical activity are capable of eliciting some responses in common with those elicited by neurotrophic factors. Presumably, this is due to recruitment of overlapping sets of intracellular signal pathways by these diverse stimuli. To exemplify neurotransmitters, we used the neuropeptide bradykinin (BK) which activates Protein Kinase C (PKC) and causes a rise in cytosolic Ca^{2+}. To mimic nerve membrane electrical activity, we depolarized with 60 mM K^+. (The observed effects of 60 mM K^+ were entirely dependent on the presence of extracellular Ca^{2+}, indicating that depolarization-induced responses are, more directly, a consequence of elevated cytosolic Ca^{2+}.) PKC was also activated directly with phorbol ester (TPA) and cytosolic Ca^{2+} was elevated directly with Ca^{2+} ionophore A23187. Responses to all of these stimuli were compared with responses to NGF, FGF and EGF.

In the PC12 rat pheochromocytoma cell line, used here as a neuronal model, all of these stimuli activate the ERK (Extracellular signal-Regulated Kinase/MAP Kinase) family of protein-serine/threonine kinases. ERK activation by BK or by TPA was accompanied by stimulation of tyrosine-phosphorylation of several cellular proteins and by activation of $p21^{ras}$. All of these effects of BK or TPA were blocked by the protein-tyrosine kinase inhibitors genistein or herbimycin. Activation of ERKs by BK or by TPA was also blocked by expression of a dominant inhibitory *ras* mutant. These data imply that PKC activates $p21^{ras}$ and consequently ERKs by recruiting a protein-tyrosine kinase. (We are currently attempting to identify this protein-tyrosine kinase.) In contrast, depolarization with 60 mM K^+ or treatment with A23187, both of which elevate cytoplasmic Ca^{2+} without affecting PKC activity, activated ERKs without concomitant $p21^{ras}$ activation. This implies that there is a Ca^{2+}-stimulated $p21^{ras}$-independent pathway for ERK activation in addition to the $p21^{ras}$-dependent pathway.

ERK activation by all of these stimuli is transient, as is also the case for EGF. In contrast, ERK activation by the trophic factors NGF and NGF is greatly prolonged. Thus, to the extent that these stimuli elicit similar responses, such convergence can be explained, in part, by a shared ability to recruit ERKs. Differences in responses elicited by these stimuli are presumably due, in part, to recruitment of different sets of other, non-ERK, effectors. However, differences in responses may also derive from differences in the temporal characteristics of the induced intracellular signals.

PZ 134 **THE NEUROFIBROMATOSIS TYPE 1 (NF1) GENE PRODUCT, NEUROFIBROMIN, IS INVOLVED IN *RAS*-MEDIATED SCHWANN CELL DIFFERENTIATION.** **David H. Gutmann, Lynn Rutkowski, Jeffery L. Cole, Gihan I. Tennekoon and Francis S. Collins. Departments of Internal Medicine, Neurology and Pediatrics, The University of Michigan, Ann Arbor, MI 48109.**

The *NF1* gene product, neurofibromin, is a ~250 kDa cytoplasmic *ras*-GTPase-activating protein (GAP) expressed predominantly in the nervous system, both in neurons and myelin-producing cells (Schwann cells and oligodendrocytes). Previous work has demonstrated that Schwann cells differentiate in response to high dose cAMP analogs (1.0 mM) or forskolin (100-200 μM) stimulation and then express galactocerebroside (Galc) and the myelin P_0 protein. We found that the introduction of an activated *ras* protein likewise results in differentiation and Galc/P_0 expression. To understand the role of neurofibromin in rat Schwann cell differentiation, two approaches were taken. In the first approach, *NF1* mRNA and neurofibromin levels were measured during differentiation and were shown to be upregulated in response to high dose cAMP analogs or forskolin but not in response to low, mitogenic doses (0.1 mM cAMP or 1-10 μM forskolin). *NF1* mRNA upregulation was evident after 1 hour of stimulation, but increased neurofibromin expression was not seen until after 24 hours. An isoform of type 1 neurofibromin has been identified which arose from the insertion of 21 amino acids within the GAP domain (type 2 neurofibromin). In addition, only high dose stimulation resulted in predominantly type 2 *NF1* mRNA expression whereas no treatment or low dose stimulation resulted in the expression of predominantly type 1 *NF1* mRNA. The second approach involved generating Schwann cells overexpressing activated *ras* or antisense *NF1*. Both these transfected cell lines assumed a differentiated phenotype and expressed Galc and P_0. Taken together, these data suggest that in response to differentiative cues, neurofibromin expression is upregulated and the predominant isoform switches from type 1 to type 2, a form which is a poor *ras* regulator. The failure to downregulate *ras* either by activated *ras* overexpression, *NF1* antisense, or neurofibromin isoform switching culminates in Schwann cell differentiation.

PZ 135 CELLULAR LOCALIZATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND NEUROTROPHIN-3 mRNA EXPRESSION IN THE EARLY CHICKEN EMBRYO. Finn Hallböök, Carlos F. Ibáñez*, Ted Ebendal and Håkan Persson*. Dept. of Devl. Biology, Uppsala University, Uppsala, *Dept. of Physiol. Chemistry, Karolinska Institute, Stockholm, Sweden.

Degenerate primers from conserved regions in nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were used in the polymerase chain reaction to isolate DNA fragments from the chicken BDNF and NT-3 genes. A genomic clone coding for chicken NT-3 was isolated and the structure of the chicken NT-3 mature protein was deduced. Northern blot analyses of mRNA from chicken embryos from embryonic day 3.5 (E3.5), E4.5, E8, E12 and E18 showed that expression of both BDNF and NT-3 mRNA peaked at E4.5 and decreased at later stages of development. The cellular localization of BDNF and NT-3 mRNA in the E4 embryo was studied by *in situ* hybridization. Labeling for BDNF was seen over cells in restricted parts of the epithelium of the otic vesicle. Analysis of adjacent sections for the low-affinity NGF receptor mRNA showed that regions in the otic vesicle epithelium which labeled for BDNF mRNA also labeled for low-affinity NGF receptor mRNA. No labeling for NT-3 was detected in the otic vesicle. Labeling for BDNF mRNA was also found over mesenchyme dorsal to the wingbud, in the wingbud and in the splanchnopleural lining of the stomach. Labeling for NT-3 mRNA was found at E4 over the epidermis on the ventral side in the region of the branchial arches. The labeling extended the maxillary processes to Rathke's pouch. NT-3 mRNA was also detected in the mesenchyme surrounding the oesophagus and lung buds. The regional expression pattern is in agreement with the established role for BDNF and NT-3 as target-derived neurotrophic factors but the results also support the view that neurotrophic factors can play a role in differentiation of both neuronal and non-neuronal tissues.

PZ 136 HOW THE STRIATUM GETS ITS PATCHES. Richard Harlan, Lin Zhang, and David Song. Department of Anatomy, Tulane Medical School, New Orleans, LA 70112.

The striatum, a critical forebrain region controlling movement, is organized into two compartments: patches, which comprise 15% of total volume, surrounded by a matrix of the remaining 85%. Restricted primarily to the rostral half of the striatum, the neurons within the patch compartment differ from those in the matrix compartment in their connections, receptors, and regulation. During development, patch neurons also undergo final mitosis earlier than matrix neurons (E12-15 vs. E17-P0). In the mature striatum, neurons expressing the two most abundant neuropeptide genes, those which encode preproenkephalin (PPE) and preprotachykinin A (PPT-A), are nearly homogeneously distributed between patch and matrix. Using *in situ* hybridization, we have found that during ontogeny, neurons expressing PPE or PPT-A first appear within the ventrolateral striatum at E16. Those expressing PPE are found caudal to those expressing PPT-A. Over the next 4 days, neurons expressing these genes spread out in a ventrolateral to dorsomedial gradient. From E20 through P5, these neurons appear to migrate into the typical patchy distribution of the adult, presumably because matrix neurons which have not yet begun to express PPE and PPT-A migrate into the ventrolateral striatum and displace the patch neurons. As these later-developing matrix neurons begin to express these genes, the distribution of labeled neurons assumes the more homogeneous pattern of the adult striatum. Interestingly, expression of the PPT-B gene, which encodes another tachykinin peptide, neurokinin B, does not begin until P15 or later. Expression of this gene first occurs in the ventrolateral striatum, as well, suggesting that expression of neuropeptide genes occurs first in the ventrolateral region before developing in the remaining regions. In summary, striatal patches appear to form as clusters of early-born neurons which are displaced by the migration of later-born matrix neurons into the ventrolateral striatum. In this model, patch neurons which are not displaced become the "subcallosal streak." Studies employing bromodeoxyuridine labeling are in progress to test this model. (Supported by NS24148 to REH).

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PZ 200 THE RECOMBINANTLY EXPRESSED RAT CEREBELLAR $\alpha6\beta2\gamma2$ $GABA_A$ RECEPTOR EXHIBITS A DISTINCT PHARMACOLOGICAL AND PHYSIOLOGICAL BEHAVIOR IN THE PRESENCE OF NEUROSTEROIDS. [1]Charlotte A.E. Hauser, [2]Dominique Chesnoy-Marchais, [1]Paul Robel, [3]Peter H.Seeburg and [1]Etienne-Emile Baulieu. [1]INSERM U33, Lab. Hormones, 94275 Bicêtre Cedex, France, [2]Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75230 Paris Cedex, France, [3]Laboratory of Molecular Neuroendocrinology, Center for Molecular Biology, University of Heidelberg, 6900 Heidelberg, FRG

Neurosteroids have been shown to influence the $GABA_A$-Benzodiazepine (BZ) receptor ionophore. We have selected 3α-hydroxy-5α-pregnan-20-one (THPROG) and pregnenolone sulfate (PREGS), which have been reported to be positive and negative modulators of GABAergic transmission respectively, and the sulfate ester of THPROG which competes with PREGS for binding to synaptosomal membranes. We investigated the modulation of ^{3}H-muscimol and ^{3}H-Ro15-4513 binding and the electrophysiological properties of the recombinant $\alpha6\beta2\gamma2$ $GABA_A$ receptor by these natural steroids. The $\alpha6\beta2\gamma2$ subtype is characterized by a distinct BZ pharmacological profile, since it binds the inverse BZ agonist and ethanol antagonist Ro15-4513 with high affinity, whereas it has lower affinity for BZ agonists. It is exclusively localized in the cerebellum.

We now show that the $\alpha6\beta2\gamma2$ subtype also exhibits a distinct pharmacology in the presence of the three neurosteroids. The binding of 6nM muscimol or 6nM Ro15-4513 is enhanced in the presence of 10 nM steroid. The effects of the steroids decrease at higher steroid concentrations, in the 50nM-10μM range. A related profile of steroid action is found by electrophysiological recording. Currents evoked by 10μM GABA are potentiated at 10nM steroid concentration, whereas they are inhibited at 100nM. Even in the absence of GABA, THPROG by itself provokes inward currents with a positive dose-response relationship. In conclusion, the neurosteroids investigated display a biphasic pattern in the modulation of the $\alpha6\beta2\gamma2$ $GABA_A$ receptor at physiological concentrations.

PZ 201 IMMUNOCHEMICAL CHARACTERIZATION OF A P-TYPE, AN N-TYPE AND TWO DIFFERENT L-TYPE CALCIUM CHANNELS FROM RAT BRAIN. Johannes W. Hell[1], Ruth E.Westenbroek[1], Michael K. Ahlijanian[1], Michael E. Adams[2], Terry P. Snutch[3], and William A. Catterall[1], [1]Department of Pharmacology, University of Washington, Seattle, WA 98195, [2]Department of Entomology, University of California, Riverside, CA 92521, and [3]Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., V6T 1W5.

Calcium channels are involved in a variety of neuronal functions like neurotransmitter release or integration of electrical signals in dendrites. To characterize the calcium channels on a molecular level polyclonal antibodies were produced against synthetic peptides which correspond to sequences of α1 subunits of the P-type (CNA1), N-type (CNB1), class C L-type (CNC1), and class D L-type (CND1) calcium channels cloned from rat brain. CNA1 specifically precipitated the ω-Aga IVA-receptor (P-type channel), CNB1 the ω-CgTx GVIA-receptor (N-type channel), and CNC 1 and CND1 the PN200-110-receptor (L-type channel). Immunoblotting demonstrated the existence of two different size forms of the α1 subunits of the N-type channel (240 and 220 kD), the class C L-type channel (210 and 190 kD), and the class D L-type channel (200 and 180 kD). The N-type and the class C L-type channels were phosphorylated by cAMP dependent kinase and by protein kinase C *in vitro*. Immunohistochemical investigations revealed that the P-type as well as the N-type channels are preferentially found throughout the length of dendrites and in some nerve terminals. In contrast, both L-type channels are localized at the cell body and the proximal parts of dendrites. CNC1 and CND1 stained different, although partially overlapping, populations of neurons corroborating that CNC1 and CND1 recognize two different classes of L-type α1 subunits. The differential subcellular distribution of these calcium channels suggests their involvement in different cellular functions. The existence of two size forms of several calcium channel α1 subunits may explain observed differences of electrophysiological properties within a given class of calcium channels.

PZ 202 $G_{\alpha i3}$ MEDIATES PERTUSSIS TOXIN-SENSITIVE ACTIVATION OF PHOSPHOLIPASE C BY THE M2 MUSCARINIC ACETYLCHOLINE RECEPTOR IN CHO CELLS, Timothy W. Hunt, Reed C. Carroll, and Ernest G. Peralta, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The m2 muscarinic acetylcholine receptor, when transfected into CHO cells, couples to the inhibition of adenylyl cyclase and the stimulation of phospholipase C (PLC) through pertussis toxin (PTX) sensitive G proteins. Two PTX sensitive G proteins, $G_{\alpha i2}$ and $G_{\alpha i3}$, are expressed in CHO cells, and are presumably responsible for mediating the responses. To investigate the functional properties of specific G proteins in this system, we constructed a $G_{\alpha i3}$ mutant that is insensitive to PTX and transfected it into CHO cells expressing the m2 receptor. Thus, any PTX-insensitive responses mediated by the m2 receptors in the resulting cells can be directly attributed to $G_{\alpha i3}$. To assay receptor coupling, we used cholera toxin (CTX), which ADP-ribosylates PTX-sensitive G proteins complexed with agonist-bound receptors in the absence of guanine nucleotides. In the presence of PTX, carbachol stimulated CTX labeling of 40kDa proteins in membranes of cells expressing the mutant $G_{\alpha i3}$ but not in membranes of cells expressing wild type $G_{\alpha i3}$. Intracellular Ca^{2+} release generated by m2 receptor stimulation of PLC is greatly diminished in cells pretreated with PTX. However, in cells expressing the PTX-insensitive mutant $G_{\alpha i3}$, the m2-dependent Ca^{2+} response is largely resistant to PTX. Studies are in progress to determine the role of $G_{\alpha i3}$ in the inhibition of adenylyl cyclase.

PZ 203 *SHAB* SUBFAMILY K^+ CHANNELS: IMMUNOHISTOCHEMISTRY REVEALS UNIQUE CELLULAR AND SUBCELLULAR LOCALIZATIONS IN BRAIN AND PERIPHERAL TISSUES, Paul M. Hwang, Ann M. Cunningham and Solomon H. Snyder, Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Recently we described the molecular cloning of a novel mammalian member of the *Shab* subfamily designated CDRK (Hwang et al., Neuron 8:473, 1992) and contrasted its properties with the only mammalian *Shab* channel previously characterized, DRK1 (Frech et al., Nature 340:642, 1989). The two channels are closely related in structure with about 90% amino acid identity in the N-terminal and middle transmembrane portions, while there is no amino acid homology in the distal C-terminal cytoplasmic domain. Both CDRK and DRK1 display similar delayed rectifier properties, but we have observed different mRNA distributions for the two channels in rat brain and peripheral tissues by Northern and *in situ* hybridization studies.

To examine with higher resolution the localization differences between these K^+ channels, we have made antibodies against unique amino acid sequences of CDRK and DRK1 and have conducted immunohistochemical analyses. We now report striking differences in the cellular and subcellular dispositions of CDRK and DRK1 proteins in brain and peripheral tissues. Our observations begin to address important issues such as compartmentalization of K^+ channel proteins in cells expressing multiple members of the same subfamily, as well as aiding in experiments to determine regions of the protein responsible for subcellular targeting. Although the limited electrophysiologic characterization of CDRK and DRK1 has revealed no major differences, further investigations using our antibodies may uncover clues to functional distinctions.

PZ 204 **Neurotrophic Factors, their Receptors and the Signal Transduction Pathways they Activate**

Nancy Y. Ip, Samuel Davis, Trevor N. Stitt, David J. Glass, Teri G. Boulton, Neil Stahl, and George D. Yancopoulos Regeneron, Inc., 777 Old Saw Mill River Road, Tarrytown, NY

Neurotrophic factors were discovered for their ability to prevent neuronal cell death. We have found that two distinct classes of neurotrophic factors, those related to Nerve Growth Factor (including Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 and Neurotrophin-4/5) and those related to Ciliary Neurotrophic Factor (CNTF), utilize fundamentally different receptor systems. Each of these, however, are related to receptor systems which function outside of the nervous system. The Nerve Growth Factor (NGF) family of factors utilize receptor tyrosine kinases (known as the Trks) to mediate their effects on responding cells. We have been able to show that the Trks are very similar to receptor tyrosine kinases used by traditional growth factors such as Fibroblast Growth Factor (FGF) or Platelet-Derived Growth Factor (PDGF), in that they interact with many of the same intracellular signaling substrates and can elicit similar phenotypic effects when expressed in fibroblasts. The ultimate biological effects elicited by the neurotrophins, however, depend on the type of cell in which the Trk receptor is expressed. Our cloning of the receptor for CNTF, as well as our studies of the signaling pathways activated by this factor, have revealed that CNTF shares receptor components with those utilized by a particular subclass of cytokines (including Interleukin-6 and Leukemia Inhibitory Factor) that act on hemopoietic cells. However, the CNTF receptor system also displays a number of unique and unprecedented features. In addition to demonstrating that there are at least two fundamentally different pathways that can keep neurons alive, we have found that these signaling pathways can collaborate in remarkable ways during the developmental process that results in the birth of neurons.

PZ 205 VISUALIZATION OF MULTIPLE IMIDAZOLINE / GUANIDINIUM RECEPTIVE SITES. Biljana Ivkovic, V. Bakhtavachalam[1], J. Neumeyer[1] and S.M. Lanier. Dept.Pharmacol., Medical Univ. of South Carolina, Charleston, SC 29425, [1]Research Biochemicals Inc., Natick, MA 01760.

In addition to their action on monoamine receptors, various imidazoline and guanidinium compounds also interact with a distinct membrane protein termed the imidazoline/guandinium receptive site (IGRS) IGRS is defined by its high affinity for imidazolines, such as cirazoline and idazoxan, and compounds with a guanidinium moiety such as guanabenz and amiloride. The molecules recognized by IGRS elicit many effects including altered ion flux, stimulation of insulin secretion and regulation of blood pressure via sites in the central nervous system. Some of these responses are mimicked by an endogenous bioactive material known as clonidine displacing substance, which is also recognized by IGRS. Utilizing a high affinity photolabile adduct ^{125}I-AZIPI that selectively labels the ligand binding subunit of IGRS we identified a family of related proteins. In rat liver mitochondria ^{125}I-AZIPI photoincorporates into two major peptides (Mr= ~55,000, ~61,000). Photolabeling is inhibited by various ligands with a rank order of potency expected for an IGRS. However, the labeling of the two peptides is differentially sensitive to the guanidinium compound amiloride. Similar results were obtained in brain and kidney suggesting that the two species are distinct proteins. In contrast, ^{125}I-AZIPI covalently labels a major M_r = ~61,000 peptide in membranes prepared from PC-12 cells. The labeling of this species is blocked by amiloride and guanabenz but not idazoxan. These data indicate the existence of three different types of IGRS that are distinguished by their ligand recognition properties, their M_r or their tissue distribution. The presence of IGRS at both the plasma membrane and in intracellular membranous structures parallels observations with the inositol trisphosphate receptor and benzodiazapine receptors suggesting an additional level of signal processing by the cell.

PZ 206 A HOMEOBOX GENE, *unc-30*, CONTROLS THE TERMINAL DIFFERENTIATION STEP OF A CLASS OF GABAERGIC MOTOR NEURONS IN *C. ELEGANS*, Yishi Jin, Roger Hoskins and H. R. Horvitz, HHMI, Dept. Biology, MIT, Cambridge, MA 02139, • MRC, LMB, Cambridge, England.
The motor neurons in the ventral cord of *C.elegans* are divided into five classes based on their morphologies, neurotransmitters, and connectivities. The class-D neurons are GABAergic and appear to function as cross-inhibitors to the body wall muscles in generating the characteristic sinusoidal locomotory pattern of the animal. The *unc-30* gene controls several aspects of the terminal differentiation of the class-D neurons. In animals that lack of *unc-30* function, the D-neurons do not have the neurotransmitter GABA, and their axons, which normally occupy well-defined neighborhoods, wander at random in the ventral cord and occasionally make a few and improper synapses (S. McIntire, J. White et al. personal. communication.). To study how neuronal cells differentiate into their final fate and how they function, we have cloned *unc-30* gene by transformation rescue. We isolated cDNAs corresponding to the *unc-30* transcript and determined the *unc-30* sequence. The predicted Unc-30 protein shows sequence similarity to homeodomain proteins of the *bicoid* class. Two observations confirm that the homeodomain-containing protein is the *unc-30* gene product. First, three *unc-30* alleles show chromosome rearrangements within the rescuing fragment. Second, two other alleles have nucleotide changes in the homeodomain. Furthermore, using β-galactosidase as a reporter, we found that the *unc-30* gene is expressed mainly in the class D-neurons at the time when these neurons differentiate to function, consistent with its role in determining the identity of this group of neurons. We will present both the molecular and biochemical studies of *unc-30* gene.

PZ 207 REVERSE GENETICS OF NERVOUS SYSTEM SIGNAL TRANSDUCTION IN DROSOPHILA, Kim Kaiser, Stephen F. Goodwin and Audrey Duncanson, Institute of Genetics, University of Glasgow, Scotland.
Two *Drosophila* genes encoding components of the cAMP second messenger pathway (*dunce*, cAMP phosphodiesterase; *rutabaga*, an isoform of adenylate cyclase) are also implicated in associative learning. As part of a programme to study the involvement of other components of the cAMP pathway in learning, we have used the technique of 'site-selected' P-element mutagenesis to generate lesions in the gene for an RI regulatory sub-unit of cAMP-dependent protein kinase. Two homozygous-viable lines have been generated in which transposon insertion has occurred within a ≈ 30bp region containing multiple transcription start sites. In an olfactory conditioning paradigm, both lines display a significant learning decrement. Preliminary experiments show them also to be defective in their recovery from jump reflex habituation. We are also in the process of characterising site selected mutants of DC0, a gene encoding a CNS-predominant catalytic subunit of PKA, and of genes encoding two G protein alpha subunits.

PZ 208 THE GUANINE NUCLEOTIDE-BINDING PROTEIN $G_{i\alpha1}$ CONFERS CELL RESPONSIVENESS TO SERUM AND TRANSFORMING GROWTH FACTOR-β_1. R. Kataoka and S.M. Lanier, Department of Pharmacology, Medical University of South Carolina, Charleston, SC 29425, U.S.A.

Receptors for growth factors utilize a variety of intracellular signaling pathways. Some cell responses to a subgroup of such molecules may involve pertussis toxin-sensitive heterotrimeric guanine nucleotide-binding proteins ($G_{i\alpha1\text{-}3}$, $G_{o\alpha1,2}$) as signal transducers. We report that the responsiveness of NIH-3T3 fibroblasts to serum growth factors depends on the type of pertussis toxin-sensitive G_α subunit expressed. Normal NIH-3T3 cells, which express G_{i2} and G_{i3}, are minimally responsive to the cytokine transforming growth factor (TGF) -β_1. However, expression of $G_{i\alpha1}$ in these cells by stable transfection confers sensitivity to TGF-β_1 and the transfectants exhibit augumented responsiveness to serum growth factors as reflected by increased DNA synthesis. Furthermore, the addition of TGF-β_1 elicits a morphological transformation in each $G_{i\alpha1}$ transfectant. Following the addition of TGF-β_1, the cells lengthened and became thinner eventually forming concentric matrices. This effect of TGF-β_1 was reversible, not observed in control cells and was blocked by pertussis toxin pretreatment. Each of the $G_{i\alpha1}$ transfectants (4 independent clonal cell lines) exhibited a similar morphological response to TGF-β_1. Whereas $G_{i\alpha12,3}$ are widely expressed, $G_{i\alpha1}$ expression is more limited in terms of both cell type and the stage of cell development. The restricted expression of $G_{i\alpha1}$ may allow the cell to respond to TGF-β_1 and other such factors only at certain stages of differentiation or tissue development. It is also possible that aberrant expression of $G_{i\alpha1}$ may promote exaggerated cell growth or morphological transformations by virtue of the "unexpected" cell sensitivity to such factors.

PZ 209 CONTROL OF F-SPONDIN EXPRESSION IN THE DEVELOPING NERVOUS SYSTEM, Avihu Klar, Ariel Ruis i Altaba, Thomas M. Jessell. Howard Hughes Medical Institute, Columbia University, New York, NY 10032
The floor plate is a transient cell group implicated in the control of neural cell pattern and axon guidance in the developing vertebrate nervous system. We have previously isolated a novel floor plate gene, F-spondin, which encodes a secreted protein with 6 thrombospondin type I repeats. F-spondin promotes neural cell adhesion and neurite extension in vitro, suggesting that the protein has a role in axon growth and guidance in the developing spinal cord. comparison of the cloned rat, chick, frog and zebra fish F-spondin revealed that both the structure and distribution of F-spondin are conserved in vertebrate evolution.
To examine further the function of F-spondin in neural development, the domains of F-spondin responsible for the promotion of axonal growth are being examined using regions of the protein expressed in baculovirus. To begin to investigate the factors that control F-spondin expression we have isolated genomic clones of the 5' region of the mouse F-spondin gene. Expression of LacZ in transgenic mice, under the control of the 5' region of F-spondin, recapitulate the pattern of expression of F-spondin mRNA in the embryonic spinal cord, confine high level of LacZ expression in the floor plate. The F-spondin enhancer therefore, provides a vehicle with which to modify the expression of genes implicated in cell patterning and axon guidance. To provide further information about the factors that control F-spondin expression, we have examined the role of genes of the HNF3/*fork head* family of transcription factors that are expressed in the floor plate. Injection of RNA encoding the gene *pintallavis* into frog embryo induces ectopic expression of F-spondin in cells of the dorsal midline of the neural tube, suggesting that F-spondin expression is regulated by *pintallavis*. Preliminary in vitro results shows that member of the HNF3 gene family can transactivate reporter genes under the control of F-spondin enhancer.

PZ 210 REGULATION OF CHOLINERGIC NERVE TERMINALS BY NGF and BDNF

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The mechanisms how the two structurally unrelated receptor types, $p75^{NGFR}$ and the $TRK_{A,B,C}$ mediate the biological actions of neurotrophins are still elusive. NGF is supposed to establish the differentiated status of neurons after being retrogradly transported into the nucleus; in addition there is some evidence that NGF may also affect second messenger pathways. It is presently unclear if the dual mode of action is initiated by interaction of NGF with different receptor types. We have studied neurotrophin-induced second messenger generation in isolated nerve terminals and found that a cAMP- and IP_3-response is elicited by NGF and also by BDNF. A similar second messenger signal was also generated in PC12-cells, which contain $p75^{NGFR}$ and TRK_A but not TRK_B. In TRK deficient fibroblast cells, which express $P75^{NGFR}$, NGF as well as BDNF induced the formation of cAMP and IP_3, suggesting a functional role of $p75^{NGFR}$ in neurotrophin-induced second messenger signaling. We know from previous studies that the activity of cholinergic synapses is controlled by receptor activated, second messenger mediated kinase reactions, therefore the effects of NGF and BDNF on presynaptic cholinergic transport processes were studied. Upon application of NGF or BDNF the evoked release of acetylcholine was significantly enhanced; furthermore, the rate of choline uptake as well as the number of carriers in the membrane were considerably increased. On the other side muscarinic agonists enhance mRNA level and protein concentration of BDNF and NGF in hippocampus, indicating to a mechanism of enhancement of presynaptic strength via neurotrophins. We postulate a role of neurotrophins in fascilitation and potentiation phenomena via $p75^{NGFR}$-receptor induced second messenger cascades.

PZ 211 FUNCTIONAL DETERMINANTS IN TM1 AND TM2 OF KAINATE RECEPTOR CHANNELS: DIVERSITY BY RNA EDITING, Martin Köhler, Nail Burnashev*, Bernd Sommer, Rolf Sprengel, Bert Sakmann*, Peter H. Seeburg, Center for Molecular Biology (ZMBH), Univ. of Heidelberg, *Max-Planck Institut für Medizinische Forschung, Heidelberg, Germany

Glur-6, a subunit of high-affinity kainate receptor channels in mammalian CNS carries a glutamine (Q) or arginine (R) residue in a critical position (Q/R) site of the putative channel forming segment TM2. One form, GluR-6(Q), is encoded by the GluR-6 gene, the other, GluR-6(R), is generated by RNA editing. Further analysis of cloned GluR-6 cDNA revealed that two additional positions, located in TM1, are diversified by RNA editing, to generate either isoleucine (I) and valine (V) in one, and tyrosine (Y) or cysteine (C) in the other TM1 position. As recognized from the exonic GluR-6 TM2 sequence, a single TM1 exon encodes the I,Y form of TM1, indicating that the three configurations (V,C; I,C; V,Y) not encoded by this exon are similarly generated by RNA editing. In GluR-6 receptor channels, in contrast to AMPA receptor channels, the presence of Q in the TM2 Q/R site determines channels with low Ca^{2+} permeability, whereas an R determines a higher Ca^{2+} permeability if TM1 is fully edited. In the TM1 unedited form of GluR-6 Ca^{2+} permeability is less dependent on the presence of either Q or R in TM2. Thus, Ca^{2+} permeability of kainate receptor channels can vary, dependent on editing of both, TM1 and TM2.

PZ 212 MONOAMINE-ACTIVATED ALPHA-2-MACROGLOBULIN MAY INHIBIT NGF-PROMOTED NEURITE-OUTGROWTH BY COMBINING WITH NGF-RECEPTOR (trk) AND BLOCKING trk AUTOPHOSPHORYLATION AND SIGNAL TRANSDUCTION, Peter H. Koo and Wan-song Qiu, Department of Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Monoamine-activated alpha-2-macroglobulin (MA-alpha-2-M) has been shown to inhibit beta-nerve growth factor (NGF)-promoted neurite outgrowth and survival of sensory and CNS neurons, whereas normal alpha-2-M has very little or no such activities (P.H. Koo and D.J. Liebl, J. Neurosc. Res. 31:678 (1992); D.J. Liebl and P.H. Koo, J. Neurosc. Res., in press). The objective of this study is to determine the mechanism of inhibition of MA-alpha-2-M. MA-alpha-2-M also dose-dependently inhibited NGF-promoted neurite outgrowth by pheochromocytoma PC12 cells but had no effect on their viability. The binding of MA-alpha-2-M to the trk proto-oncogene product, which participates in the formation of high-affinity NGF binding sites, was studied with PC12 cells, PC12 (6-24) cells overexpressing trk, and NIH-3T3 fibroblasts expressing trk. The trk protein of each cell lysate was immunoprecipitated by protein-A-agarose coated with rabbit anti-trk (pan) antibodies, and the ability of MA-alpha-2-M versus normal alpha-2-M to combine with the immunoprecipitated trk was evaluated by Western blotting. MA-alpha-2-M formed stable complexes with trk, whereas normal alpha-2-M did not. The binding of MA-alpha-2-M to trk was dose-dependently blocked by NGF. MA-alpha-2-M could also prevent NGF-stimulated tyrosine phosphorylation of trk of all the three cell lines in vivo, whereas normal alpha-2-M could not. Neither MA-alpha-2-M nor normal alpha-2-M, however, blocked PDGF-stimulated tyrosine phosphorylation of the PDGF-receptor. We conclude that MA-alpha-2-M may block neurite outgrowth and neuronal survival by its specific binding to NGF-receptors (trk) and thus blocking the NGF-promoted activation of intracellular signal transduction. (PHK is in part supported by NIH NS-30698).

PZ 213 EXPRESSION CLONING OF INWARD RECTIFIER POTASSIM CHANNEL cDNA FROM A MOUSE J774 MACROPHAGE CELL LINE, Yoshihiro Kubo, Timothy J. Baldwin, Yuh Nung Jan and Lily Y. Jan, Howard Hughes Medical Institute, Departments of Physiology and Biochemistry, University of California, San Francisco, San Francisco, CA94143-0724

Inward rectifier K^+ channels are known to exist in skeletal muscle cells, cardiac muscle cells, and also in some types of oocytes, neurons, glial cells and blood cells, and are reported to play a significant role in maintaining resting potential. Inward rectifier K^+ channels are unique in the rectification property, and are expected to be quite different from other K^+ channels cloned so far. To uncover the structure and rectification mechanism of this channel at molecular level, we started cloning its cDNA by expression method in Xenopus oocytes. Poly A^+ RNA of J774 mouse macrophage cell line induced inward rectifying current, which was characterized to be authentic inward rectifier by the following criteria. (1) The activation potential was shifted in accordance with changes of E_K. (2) The activation kinetics was almost instantaneous. (3) It was very sensitive to external Ba^{2+}. Unidirectional cDNA library was constructed from a positive fraction (4.5Kb-5.5Kb) of poly A^+ RNA. We will report our recent progress.

PZ 214 m3 MUSCARINIC ACETYLCHOLINE RECEPTOR-G PROTEIN INTERACTIONS, Maya T. Kunkel and Ernest G. Peralta, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

There are five structurally related muscarinic receptor subtypes (m1-m5) which activate G proteins to *transduce* different cellular responses. The m1, m3 and m5 receptors are potent activators of PLC, while the m2 and m4 receptors are weak activators of PLC and efficient inhibitors of adenylyl cyclase. The structural characteristics of the receptor sequences required for G protein binding and activation are unknown. However, it has been hypothesized that receptor sequences within the third cytoplasmic domain may form a charged helical structure that is directly involved in G protein activation. Such sequences can be found at the amino and carboxy terminal domains of the third cytoplasmic loop of the m3 receptor. We have investigated the role of these receptor sequences in G protein activation through a substitution mutagenesis approach and have found that conversion of certain charged residues to hydrophobic residues results in a decrease in agonist response. To define the minimal receptor sequences that are required for G protein activation, we have also constructed and analyzed "minimal third loop mutants" in which the third cytoplasmic domain of the m3 receptor was drastically reduced from 240 to 22 amino acids. Interestingly, the minimal loop receptor generates responses comparable to the wild type receptor; this approach should define which residues are most important for G protein coupling and activation by the m3 receptor.

PZ 215 EXAMINATION OF N-CADHERIN AND ITS ASSOCIATED PROTEINS DURING NEURONAL DIFFERENTIATION. Robert M. Kypta, Cynthia Murphy-Erdosh and Louis F. Reichardt, HHMI and Department of Physiology, University of California San Francisco, CA 94143.

N-cadherin is a calcium-dependent cell adhesion molecule (CAM) which is present on neuronal growth cones and promotes neurite outgrowth by binding to the same molecule expressed by other cells or presented as a purified substrate (for a review see Takeichi, Science 251, 1451, 1991). N-cadherin is associated with cytoplasmic proteins named catenins which have been shown to be important in mediating the adhesive functions of other cadherins, notably E-cadherin. A role for catenins in mediating neurite outgrowth has not been established.

We have examined N-cadherin-associated proteins in PC12 cells, which differentiate into neuron-like cells when exposed to NGF or when grown on a monolayer of cells expressing N-cadherin. NGF induces the tyrosine phosphorylation of a ~95kd protein in N-cadherin immune precipitates. This protein is recognised by antibodies to beta catenin (a gift of Drs. P. McCrea and B. Gumbiner). We are determining whether N-cadherin also stimulates phosphorylation of beta catenin. The preliminary results suggest that NGF may stimulate PC12 cell differentiation, in part, by regulating CAM functions through phosphorylation.

PZ 216 THE ROLE OF THE *c-RET* PROTO-ONCOGENE IN THE DEVELOPMENT OF THE MAMMALIAN ENTERIC NERVOUS SYSTEM, Lena Larsson[1], Anita Schuchartd[2], Frank Costantini[2], and Vassilis Pachnis[1], [1] Laboratory of Gene Structure and Expression, NIMR, The Ridgeway, Mill Hill, Londond NW7 1AA, UK, [2] Department of Genetics and Development, Columbia University, New York, N.Y. 10032.

The *c-ret* proto-oncogene, a member of the receptor tyrosine kinase (RTK) superfamily, was originally identified by the 3T3 cell transformation assay. *In situ* hybridization experiments on sections from mouse embryos revealed that *c-ret* is expressed predominantly in the developing excretory and nervous system, including the enteric nervous system where *c-ret* mRNA has been detected in the migratory vagal neural crest and its neuronal derivatives. To test the importance of *c-ret* for the development of the nervous and excretory systems, we have used gene targeting techniques in embryonic stem cells to generate mice that carry a mutant allele of *c-ret*, *ret.k⁻*. *ret.k⁻* encodes a truncated receptor lacking kinase activity and therefore is defective in signal transduction. Among the defects observed in newborn homozygous mutant mice (which die within 24hrs after birth) is complete absence of all peristaltic movements of the gut. Histological analysis revealed that *ret.k⁻/ret.k⁻* mice are lacking the ganglia of the entire enteric nervous system (ENS). Immunocytochemistry on sections from the gut of newborn mice showed that both the neurons and the glia of the enteric ganglia are missing. As a first step towards a detailed characterization of the ENS defect in ret-deficient mice we have attempted to determine the stage at which the migration, differentiation or survival of the enteric neuronal lineage is affected. Antibodies specific for the migratory precursors and postmigratory neurons and glia of the enteric ganglia have been used on sections from mutant, heterozygous and wild-type mouse embryos from various stages of embryogenesis. Our results support the hypothesis that the RET signal transduction pathway is required for the migration and differentiation of the neural crest derived precursors of the enteric nervous system.

PZ 217 **STRUCTURAL ELEMENTS INVOLVED IN DISTINCT MODES OF ADENYLYL CYCLASE REGULATION**

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Various isoforms of adenylyl cyclase have been identified using molecular cloning techniques. Subsequent heterologous expression of individual isozymes has revealed distinct biochemical properties, perhaps explaining the various, discrete cellular responses to extracellular signals. Type I adenylyl cyclase (ACI) is activated by calmodulin in the presence of calcium, while G_s protein α subunit activation is inhibited by the addition of $\beta\gamma$ subunits. In contrast, the Type II enzyme (ACII) is unaffected by calmodulin, and $G_{s\alpha}$ activation is potentiated by $\beta\gamma$ subunits. Little is known of the structural determinants responsible for these biochemical differences. Inspection of ACI amino acid sequence does not reveal a consensus calmodulin binding domain, and G protein interaction sites on adenylyl cyclase have not been identified. Random chimeras between ACI and ACII were constructed by an *in vivo* mechanism which concentrates cross-over sites at the regions of highest homology. Chimeras were transiently expressed in HEK293 cells and tested for their responsiveness to Ca^{2+}/CaM or $\beta\gamma$ activation. This approach has allowed identification of regions of adenylyl cyclase responsible for the different modes of regulation.

PZ 218 A NOVEL NEUROFILAMENT KINASE DISPLAYS HIGH SEQUENCE HOMOLOGY TO P34^{cdc2}. John Lew, Robert J. Winkfein, Katherine Beaudette and Jerry H. Wang. MRC Group in Signal Transduction, Department of Medical Biochemistry, University of Calgary, Calgary T2N 4N1, CANADA.

The carboxyterminal regions of neurofilament high (NF-H) and middle (NF-M) molecular weight proteins have been suggested to be phosphorylated in vivo by a protein kinase which may be related to the cell cycle control protein kinase, p34^{cdc2}. This suggestion is based on: 1) the in vivo phosphorylation site motif which corresponds to that recognized by p34^{cdc2}, and 2) the demonstration in vitro of the phosphorylation of NF-H and NF-M by p34^{cdc2} kinase purified from Xenopus oocytes (Hisawaga, S.I., Kusubata, M., Okumura, E. and Kishimoto, T., J. Biol. Chem. 266:21798-21803, 1991). We have recently purified a novel protein kinase from bovine brain which displayed similar substrate sequence specificity to that of HeLa cell p34^{cdc2} (Lew et al., J. Biol. Chem. 267:13383-90) and which has been found to phosphorylate purified NF-H and NF-M at sites identical to those phosphorylated by Hela cell p34^{cdc2} kinase. During purification, it was found that this kinase represented the major cdc2-like kinase activity in brain. The brain kinase is composed of a 33 kDa and a 25 kDa subunit. The 33 kDa subunit was partially sequenced, and degenerate oligonucleotide primers corresponding to the amino acid sequence information were used to clone the subunit by polymerase chain reaction (PCR). Two overlapping PCR products obtained by 3' RACE and 5' RACE comprised a complete open reading frame of 292 amino acids. The 33 kDa subunit displays 58% and 59% amino acid identity to human p34^{cdc2} and human cdk2, respectively, the latter which is believed to also be involved in cell cycle control. These results suggest that the brain kinase may be the in vivo cdc2-like neurofilament kinase, and that some members of the cdc2 kinase family may have major functions unrelated to cell cycle control.

PZ 219 LEUKEMIA INHIBITORY FACTOR (LIF) AND CILIARY NEUROTROPHIC FACTOR (CNTF) ACTIVATE p21-RAS IN A NEUROBLASTOMA CELL LINE. Susan Lewis, Michael Schwarzschild, William Dauer, Lila Hamill, Aviva Symes, J. Stephen Fink and Steven Hyman, Molecular Neurobiology Lab., Massachusetts General Hospital, Charlestown, MA 02129.

The cytokines LIF and CNTF are able to regulate survival and differentiation of many types of neurons. Receptors for both LIF and CNTF have been cloned and both appear to associate with the same signal transducing subunit, gp130, however, little is known about the second messenger systems involved. The recent identification of a LIF and CNTF responsive neuroblastoma cell line, NBFL, has allowed examination of the signal transduction mechanisms activated by these factors. One putative intracellular effector, the product of the proto-oncogene ras, has been implicated in the signal transduction pathways of other growth factors and cytokines. We therefore investigated a role for Ras in LIF and CNTF responses in NBFL cells.

Ras activity was measured as the % GTP of total ^{32}P-labelled guanine nucleotide bound to immunoprecipitated Ras. In NBFL cells, LIF and CNTF increase levels of activated Ras in a rapid, transient, and dose-dependent fashion. In addition, both LIF and CNTF increase the tyrosine phosphorylation of several protiens in NBFLs. The tyrosine kinase inhibitor tryphostin blocked the tyrosine phosphorylation of these proteins and the increase in active Ras with a similar dose-response curve.

One effect of LIF and CNTF in the NBFL cells is induction of VIP mRNA. We have used active and dominant negative mutants of Ras to begin to examine a role for Ras in VIP gene regulation. Our preliminary data suggest that Ras may be involved in the regulation of VIP gene expression by LIF and CNTF in this cell line, but that Ras activation alone is probably not sufficient.

PZ 220 EXPRESSION OF GLIA-DERIVED NEXIN IN THE DEVELOPING AND TRANSGENIC MOUSE

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Glia-derived nexin (GDN), also known as protease nexin I (PN-I), is a serine protease inhibitor, that *in vitro* promotes neurite outgrowth from neuroblastoma cells, sympathetic and hippocampal neurons. *In vivo*, GDN is constitutively expressed in the olfactory system, where axonal degeneration and neurogenesis occur continuously throughout life. It is also upregulated following lesion of the rat sciatic nerve and following transient global ischemia in the gerbil hippocampus. Therefore GDN could influence both degenerative events and/or axonal regeneration *in vivo*. Spatial and temporal expression of GDN was examined by *in situ* hybridisation and immunohistochemistry methods. In the embryo, the major sites of GDN expression are cartilage, bone, lung and heart whereas it is detected in a few restricted areas of the mouse developing nervous system. Postnatally, GDN expression gradually increases in the brain and is widely expressed in the adult central nervous system. In addition, we generated several strains of transgenic mice carrying the chimeric Thy-1-GDN gene. This transgene directs expression of high levels of rat GDN in the brain. Transgenic mice of three different lines were extensively analysed at RNA and protein level and went through a whole series of behaviour tests. Transgenic GDN was shown to be biologically active by its capability to form SDS stable complexes with thrombin. Furthermore, the role of GDN was evaluated in CNS regeneration processes. The overexpression of GDN did not result in detectable developmental morphological or physiological abnormalities.

PZ 221 P21 RAS MEDIATES NEUROTROPHIC FACTOR-INDUCED SURVIVAL IN SENSORY, BUT NOT IN SYMPATHETIC CHICK EMBRYONIC NEURONS. A. Markus[1], G.D. Borasio[2], R.B. Nehring[1], F.J. Klinz[1], Y.-A. Barde[3] and R. Heumann[1]. [1]Abt. Molekulare Neurobiochemie, Ruhr-Universität Bochum, 4630 Bochum; [2]Neurologische Poliklinik der Universität München, 8000 München 70; [3]Abt. Neurobiochemie, Max-Planck-Institut für Psychiatrie, 8033 Martinsried; Germany

Little is known about the signal transduction mechanisms involved in the response to neurotrophic factors in primary neurons. We have previously shown that cytoplasmic introduction of the oncogene product p21^{ras} into cultured chick embryonic neurons can mimic the survival and neurite outgrowth-promoting effects of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF) and ciliary neuronotrophic factor (CNTF). To assess the possible signal-transducing role of endogenous p21^{ras} we now introduced function-blocking anti-ras antibodies and their biologically active, affinity purified Fab fragments into cultured chick embryo neurons.

The antibodies blocked BDNF-induced neurite outgrowth in E12 nodose ganglion neurons, and the Fab fragments inhibited NGF-induced survival of E9 dorsal root ganglion neurons in a dose-dependent manner. Both effects could be reversed by saturating the epitope-binding sites with biologically inactive p21^{ras} prior to microinjection or trituration. In E12 sympathetic neurons, which interestingly do not respond to trituration with oncogenic ras, the Fab fragments did not affect NGF-induced survival. Neither did they inhibit CNTF-induced survival of E8 ciliary neurons, which do respond to oncogenic ras.

Together with our data on NGF-induced increases of GTP-bound ras in neurons, these results support the concept of an involvement of p21^{ras} in the intracellular signal transduction of neurotrophic factors. We suggest the existence of multiple signal transduction pathways for NGF in different neuronal populations.

PZ 222 HISTOLOGY ON MICROWAVE-FIXED CELLS SHOWS RAPID REORGANIZATION OF CELLULAR PROTEINS AND CYCLIC NUCLEOTIDES. **Stephen J. Marx & Julia Barsony, Mineral Metab Sect, NIDDK, Bethesda, MD 20892.**

Microwave radiation (MiR) (approx 10 seconds) in a standard Amana oven stabilized cell structures and retained cAMP and cGMP for immunostaining (PNAS 87:1188, 1990). A custom made microwave oven provided better fixation after only 2.5 s radiation. Subcellular patterns of cAMP or cGMP accumulation were time- and agonist-dependent after calcitonin, forskolin, atrial natriuretic peptide, or sodium nitroprusside (SNP). MiR aided study of the effects (I) of insulin or of SNP on *Tetrahymena pyriformis* (a free-swimming ciliate) or (II) of steroid-related agonists on cell lines from several organs.

(I) On *Tetrahymena*: SNP raised cGMP diffusely in cytoplasm and macronucleus. Insulin rapidly raised cGMP: near plasma membranes, cilia, and kinetosomes at 0-1 min; within cytoplasm & perinuclear zones but not in macronucleus at 1-5 min (Experientia 48:476, 1992).

(II) On monolayers: estrogen slowly (18+ hr) redistributed VDRs from mainly cytoplasmic to mainly nuclear sites. 1,25-Dihydroxyvitamin D_3 (D) reorganized VDRs rapidly. A portion of cytoplasmic VDRs became clumped within 15 s, aligned along cytoplasmic fibrils at 30-45 s, near nuclear membrane at 1 min, and inside nucleus at 1-5 min (J Cell Biol 111:2389, 1990). Estrogen caused similar rapid reorganization of estrogen receptors. D increased cGMP as aggregates: intensely in cytoplasm at 15 s, aligned along cytoplasmic filaments at 30-45 s, perinuclear at 1 min, nucleolar and otherwise intranuclear maximally at 3-5 min. Androgen, estrogen, glucocorticoid, iodothyronine, or retinoid induced similar cGMP patterns (with important variations). The changes after a steroid-related agonist showed analog-specificity expected for that agonist's receptor. Double immunostaining showed that cGMP aggregates colocalized with clumped VDRs during their D-induced rapid reorganization. D-treated fibroblasts from patients with mutations in the VDR gene showed an appropriate spectrum of defects in VDR reorganization (PNAS 88:1436, 1991). Similar uses of microwave fixation could contribute to studies on neuronal signal transduction, developmental biology of the cardiovascular system, and other topics.

PZ 223 TRANSGENIC STUDIES ON THE GENE FOR THE PERIPHERAL MYELIN PROTEIN, PMP 22. Peter R. Maycox, Danny Ortuno, Maude White, Rainer Kuhn & Greg Lemke, Molecular Neurobiology Lab., Salk Institute, 10010 North Torrey Pines Rd., La Jolla, CA 92037.

The peripheral myelin protein PMP 22 (also called gas-3, CD 25) is a 22kD glycoprotein that is incorporated into the compact regions of the peripheral myelin sheath. Relatively little is known about the function of this protein. However, two interesting neuropathies are related to this gene. The mouse *Trembler* phenotype is thought to be due to a single base missense mutation,which introduces a charged amino acid into the fourth putative membrane spanning domain of PMP 22. The human neuropathy, Charcot-Marie-Tooth disease type 1A (CMT 1A), is thought to be due to a relatively large interstitial duplication on chromosome 17. The PMP 22 gene is wholly contained within the duplicated region and the CMT 1A phenotype is thought to result from inappropriate gene dosage (i.e. 2-3x).

In order to elucidate the role of PMP 22 in myelination we have synthesized a series of constructs to generate transgenic animals. We placed the rat PMP 22 gene, in sense and antisense orientations, under the control of the rat P0 promoter. In addition, we have made similar constructs modifying the rat PMP 22 gene to have a *Trembler* -type mutation. Using these constructs we have generated transgenic founder animals (screened by PCR tail analysis and Southern blotting). We have subsequently bred F2 generation animals, which we are presently analysing for morphological and genetic changes. Many of the animals containing the antisense constructs show clear *Trembler* phenotypes as characterised by a lack of tonicity in the extended hind limbs and tail. We will describe these initial results in this poster.

PZ 224 A COMPARISON OF $GABA_A$ RECEPTORS FROM RAT BRAIN WHICH CONTAIN γ_1,γ_2 AND γ_3 SUBUNITS. **Ruth M. McKernan, Kate Quirk, Nigel P. Gillard, Paul Whiting and C. Ian Ragan. Neuroscience Research Centre, Merck,Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Rd., Harlow, Essex, CM20 2QR U.K.**

Antibodies specific for the γ_1, γ_2 and γ_3 subunits of the $GABA_A$ receptor have been made by bacterially expressing the unique putative cytoplasmic regions of the receptor, purifying them by preparative SDS/PAGE and using them as antigens to raise polyclonal antisera in rabbits. The antibodies have been used to probe the composition and pharmacology of naturally occurring $GABA_A$ receptors in the rat brain.

More than 90% of all $GABA_A$ receptors were found to contain one of these three subunits. The percentage of each population, determined by immunoprecipitation of [^{3}H]muscimol binding, was 11 ± 1%, 21 ± 3% and 59 ± 3% for γ_1, γ_2 and γ_3 subunits respectively.

Receptors containing only γ_2 or γ_3 subunits bound the benzodiazepine site ligands [^{3}H]Ro 15-1788 and [^{3}H]flunitrazepam with high affinity whereas all γ_1 containing receptors were labelled by [^{3}H]muscimol with equal affinity. The pharmacology of receptors immunoprecipitated by γ_2 and γ_3 antibodies was compared. They had similar affinities for Ro 15-1788 (γ_2=1.0nM; γ_3=1.15nM) and flunitrazepam (γ_2=1.1nM; γ_3=3nM). Competition for [^{3}H]Ro 15-1788 binding by zolpidem revealed multiple populations of receptor which were deduced to arise from pairing of γ_2 or γ_3 with multiple α-subunits. Immunoprecipitation of $GABA_A$ receptors from cerebellar and whole rat brain preparations was consistent with there being only one type of γ subunit in a receptor monomer. This would indicate a potential subunit composition for the receptor of (2 x α, 2 x β and 1 x γ).

$GABA_A$ receptors were purified on α-subunit specific immunoaffinity columns and the enriched populations were eluted and subjected to Western blot analysis with the γ-antibodies. $\alpha_2\gamma_1$ was the major population of γ_1-containing receptors whereas several α-subunits were present in combination with γ_2 and γ_3-subunits.

PZ 225 castor ENCODES A NOVEL ZINC FINGER PROTEIN REQUIRED FOR THE DEVELOPMENT OF A SUBSET OF CNS AXONS IN DROSOPHILA. Dervla M. Mellerick-Dressler*, Judith A. Kassis#, Shang-Ding Zhang*, and Ward F. Odenwald*. Lab. of Neurochemistry, NINDS, NIH* and Ctr. for Biologics Evaluation and Research, FDA #, Bethesda, MD 20892.

Enhancer detection screening has identified two closely spaced Drosophila genes, castor and pollux, that are expressed in the developing CNS. The two genes, located at chromosomal subdivision 83C, are transcribed in converging directions and separated by only 99bp.

DNA sequence analysis of pollux indicates that it encodes a leucine-rich protein, containing a leucine zipper motif. pollux transcripts are detected in oocyte nurse cells and are ubiquitous in early embryos. After germ band retraction pollux transcripts are restricted to CNS neurons and the developing trachael system.

The putative castor gene product contains a novel zinc-binding domain and multiple transactivation domains. Its expression is restricted to ventral midline glioblasts and to a subset of delaminated neuroblasts. Embryos lacking the castor gene product have a diminished CNS axonal network and express engrailed abberantly in late CNS neurons. Thus castor expression appears necessary for the development of a subset of neuronal precursors.

PZ 226 PHOSDUCIN-LIKE PROTEIN (PhLP): AN ETHANOL-RESPONSIVE MODULATOR OF G-PROTEIN FUNCTION. Michael F. Miles, Steve Barhite, Norb Wilke and Michael Sganga, Dept of Neurology and the Ernest Gallo Clinic and Research Center, University of California at San Francisco, San Francisco, CA 94110

Chronic exposure to ethanol produces the development of tolerance and dependence in the central nervous system (CNS). Other investigators have documented changes in several signal transduction pathways accompanying CNS adaptation to ethanol. We have investigated the hypothesis that regulation of neuronal gene expression by ethanol may be the ultimate mechanism underlying the development of CNS tolerance and dependency. Using the NG108-15 neural cell line, we have recently isolated specific ethanol-responsive genes (EtRGs) using subtractive hybridization cloning. One prominently induced EtRG showed significant homology to phosducin, a known modulator of G-protein function. This phosducin-like protein (PhLP) had a 54% identity with phosducin at the amino acid level. Comparison of PhLP and previously cloned phosducin genes showed several regions of extremely high sequence conservation, suggesting functional correlation. Multiple forms of PhLP exist due to alternate splicing and polyadenylation. In contrast to phosducin, which is expressed only in retina and pineal, PhLP mRNA was seen in all tissues surveyed with testis and brain having particularly high levels on Northern blot analysis. Southern blot hybridizations showed a high degree of evolutionary conservation of PhLP. Treatment of NG108-15 cells with ethanol (25-50 mM) for 24 hours produced a 3-fold increase in PhLP mRNA. Induction of PhLP was also seen in brains from mice exposed to ethanol vapor for 24 hours. Thus, induction of PhLP by ethanol may play a functional role in the known modulation of signal transduction cascades, such as cyclic AMP, seen with chronic ethanol exposure.

PZ 227 INHIBITION OF β_2-ADRENERGIC RECEPTOR DESENSITIZATION, Scott W. Miller, Taraneh N. Haske and Harry LeVine III, Department of Neuroscience Pharmacology, Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co., Ann Arbor, MI 48105

The G-protein-linked β_2-adrenergic receptor undergoes homologous desensitization in response to agonists. The mechanism is thought to be by the uncoupling of receptor and G-protein mediated by phosphorylation of the receptor by ß-adrenergic receptor kinase (ß-ARK). ß-arrestin can potentiate the inhibitory effects of receptor phosphorylation by ß-ARK. To further elucidate the components of desensitization we have examined the effects of inhibitors of desensitization that act on the related rhodopsin system. Inositol hexaphosphate (IP_6), (thought to be a selective arrestin antagonist) and inositol hexasulfate (IS_6), heparin and suramin (ß-ARK inhibitors) were added to permeabilized A_{431} cells prior to desensitization with agonist. Suramin, heparin and IP_6 completely inhibit desensitization at 0.1, 0.1 and 1 μM, respectively. IS_6 inhibited desensitization by a maximum of 80% using concentrations $\geq$ 0.1 μM. The IC_{50} for IP_6 was 0.1 μM, which is 100-fold more sensitive than IP_6 binding to visual arrestin. Suramin, IS_6 and heparin inhibit purified ß-ARK mediated phosphorylation of rhodopsin/brain-$\beta\gamma$ at concentrations known to inhibit desensitization while IP_6 inhibits β-ARK activity at levels $\geq$3 mM. The sulfated compounds may inhibit desensitization through β-ARK inactivation while IP_6 clearly acts in another manner.

PZ 228 STUDIES ON TRYPTOPHAN HYDROXYLASE GENE EXPRESSION

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The tryptophan hydroxylase gene codes for the rate-limiting enzyme in the biosynthesis of serotonin in the raphe neurons of the brain. This neurotransmitter has been shown to be involved in intolerance to delay, temperature and sleep control. To define the mechanisms controlling tryptophan hydroxylase gene expression, we have been studying its gene regulation *in vitro*.

Tryptophan hydroxylase gene expression is being studied in TT cells, a medullary thyroid carcinoma cell line, and in P815 cells, a mastocytoma cell line, by Northern and RNA dot spot hybridization analysis. The effects of various drugs on tryptophan hydroxylase mRNA content in these cells are being investigated to elucidate the factors controlling its expression. We have found several drugs that induce the expression of tryptophan hydroxylase mRNA in these cell lines. Fusion genes have been constructed with various deletions of the tryptophan hydroxylase gene promoter fused to the luciferase reporter gene. These have been transfected into the TT, P815 as well as NIH3T3 cells (a fibroblast cell line). Regions necessary for tissue specific expression and regulation by the various drugs are being identified. This work is being complemented by footprinting studies of the tryptophan hydroxylase promoter with nuclear proteins, isolated from the various cells grown with the drugs, to identify transcription factor binding sites.

PZ 229 DISTRIBUTION OF AGRIN mRNA IN THE CNS OF THE ADULT RAT AND THE EFFECT OF SEIZURE ACTIVITY ON ITS EXPRESSION. L.T. O'Connor, J.C. Lauterborn, C.M. Gall, and M.A. Smith. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Agrin is an extracellular matrix protein thought to play a critical role in development and maintenance of the neuromuscular junction (NMJ). When applied to cultured myotubes, agrin induces the clustering of acetylcholine receptors (AChRs) and other molecules normally present in the postsynaptic appparatus of the NMJ. In addition to motor neurons, agrin mRNA has been detected in the brains of adult (electric ray) and embryonic (rat and chick) vertebrates raising the possibility that this molecule may play a role in the formation of synapses between neurons in the central nervous system (CNS). As a first step towards understanding agrin's role in the CNS, we have used probes derived from a rat agrin cDNA (Rupp *et al.*, 1991) to analyze the regional and cellular distribution of agrin mRNA in the adult rat brain. Northern blot analysis shows a single band of approximately 8kb present in RNA isolated from all regions of adult brain examined. Consistent with this observation, *in situ* hybridization studies using ^{35}S-labeled cRNA probes reveal that agrin mRNA is widely distributed throughout the adult brain. Recent studies indicate that RNA isolated from rat embryonic spinal cord contains at least 4 isoforms of agrin mRNA (designated $agrin_0$, $agrin_8$, $agrin_{11}$, and $agrin_{19}$) which result from alternative splicing events that occur at a single site within a common mRNA precursor. Agrin proteins encoded by these isoforms exhibit quanitative differences in their ability to cluster AChRs on cultured myotubes suggesting they may have different physiological or developmental roles. We utilized primers flanking the site of alternative splicing and PCR techniques to analyze the regional distribution of these transcripts in the adult rat brain. Three of these transcripts ($agrin_0$, $agrin_8$, and $agrin_{19}$) are expressed in all regions of the brain analyzed. $Agrin_{11}$, however, was detected only in RNA isolated from regions of the forebrain where it is expressed at low abundance in comparison with the other isoforms. These results indicate that alternatively spliced agrin transcripts are differentially regulated in the adult CNS. Using *in situ* hybridization techniques we have observed that agrin mRNA in the adult rat brain can also be regulated by recurrent limbic seizures induced by placement of a electrolytic lesion in the hilus of the dentate gyrus. We are currently analyzing whether changes in synaptically-mediated electrical activity can differentially regulate expression levels of the individual agrin isoforms. *Supported by* NIH NS-27563 to M.A.S., NS-07351-01 to L.T.O., and NS-26748 to C.M.G.

PZ 230 **Differential Association of SH-2 Proteins with the pp140$^{c\text{-}trk}$ NGF receptor.** Masahide Ohmichi*@, Stuart J. Decker#@, and Alan R. Saltiel*@. Departments of *Physiology and #Microbiology, University of Michigan School of Medicine, Ann Arbor, MI 48109, @Department of Signal Transduction, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

Growth factor receptor tyrosine kinases can form stable associations with intracellular proteins that contain *src* homology (SH) 2 domains. We investigated the association of the pp140$^{c\text{-}trk}$ NGF receptor with GST fusion proteins containing SH2 domains in both PC-12 and NIH3T3 cells transfected with the human pp140$^{c\text{-}trk}$ cDNA (3T3-*c-trk*). Although there was no detectable association of pp140$^{c\text{-}trk}$ with the SH2 domain of the GTPase Activating Protein of *ras* (GAP), NGF rapidly induced the association of its receptor with the SH2 domains of PLC-γ1. Interestingly, 38 kd tyrosine phosphorylated protein was also detected in this complex. This phosphorylation and subsequent association with both receptor and PLC-γ1 SH2 domains occur only in response to NGF. Moreover, the NGF-dependent pp38-SH2 binding is specific for the SH2 domains of PLC-γ1 and is not detected using other SH2 domain fusion proteins. Using a fusion protein containing the N-terminal SH2 domain of the p85 regulatory subunit of PI-3 kinase or anti-p85 antiserum, we observed NGF-dependent association of two tyrosine phosphorylated proteins, although association of the receptor with this protein was not detected. Nevertheless, NGF produces the rapid activation of PI-3 kinase detected with anti-phosphotyrosine antiserum, although there is no evidence for tyrosine phosphorylation of p85. These results indicate that the high affinity NGF receptor, pp140$^{c\text{-}trk}$, can differentially associate with a variety of SH2-containing proteins, *via* a direct mechanism, as in the case of PLC-γ1, or an indirect mechanism, as in the case of PI-3 kinase.

PZ 231 EXCITATORY AMINO ACIDS DOWN-REGULATE GENE EXPRESSION POST-TRANSCRIPTIONALLY IN HIPPOCAMPAL NEURONS, D.M. Panchision, C.M. Gerwin, R.J. DeLorenzo, and E.R. Jakoi, Department of Neurology, Medical College of Virginia, Richmond, VA 23298.

In primary cultures of hippocampal neurons, an acute cytotoxic activation of excitatory amino acid (EAA) receptors results in the rapid and long-lasting down-regulation of ligatin, a receptor for phosphoglycoproteins. Northern and slot hybridization analyses of total RNA revealed that this decrease in receptor number was accompanied by a selective reduction (40-80%) in steady state levels of mRNA coding for ligatin; other mRNAs encoding neuron-specific enolase (NSE) and cyclophilin showed no change. Nuclear transcription assays revealed that ligatin hnRNA levels did not decrease, demonstrating a post-transcriptional regulation of mRNA by EAA receptor activation. *In situ* hybridization studies with ligatin antisense probes demonstrated down-regulation of cytosolic ligatin mRNAs occurred in EAA-treated neurons with no increase in nuclear content, implicating an accelerated destabilization of cytosolic mRNA may be an early consequence of EAA receptor activation. Fractionation of mRNA on the basis of poly(A) content showed essentially all of the ligatin mRNA failed to bind to poly(U) paper. These observations demonstrate that ligatin mRNA belongs to the poly(A)$^-$ class of mRNA which accounts for 50% of adult brain mRNA, and additionally show that these poly (A)$^-$ mRNAs can be modulated by EAA receptor activation. The data support the hypothesis that control of mRNA stability may be part of the normal regulatory program underlying some of the effects of EAA receptor activation on neuronal plasticity as well as susceptibility to pathophysiological conditions.

PZ 232 XENOPUS *DISTAL-LESS* RELATED HOMEOBOX GENES ARE EXPRESSED IN THE DEVELOPING FOREBRAIN AND ARE INDUCED BY PLANAR SIGNALS. Nancy Papalopulu and Chris Kintner. Molecular Neurobiology Laboratory, The Salk Institute, 10010 North Torrey Pines Rd. La Lolla, CA 92037.

The polymerase chain reaction (PCR) was used to isolated five *Xenopus* homeobox clones (*X-dll1* to 5) that are related to the *Drosophila Distal-less* (*Dll*) gene and we propose a subdivision of the vertebrate *distal-less* gene family according to sequence similarities. cDNA clones were isolated for *X-dll2, 3* and *4* and their expression was studied by RNase protection and in situ hybridization. *X-dll2*, which belongs to a separate subfamily than *X-dll3* and *4*, is not expressed in the neural ectoderm. *X-dll3* and *X-dll4*, which belong to the same subfamily, have similar but not identical pattern of expression that is restricted to anterior ectodermal derivatives, namely the ventral forebrain, the cranial neural crest and the cement gland. *X-dll3* is also expressed in the olfactory and otic placodes while *X-dll4* is expressed in the developing eye. *X-dll3* differs from the other *Xenopus* genes and the previously isolated *Dll* related mouse genes, in that localized expression can de detected by in situ hybridization very early in development, in the anterior-transverse ridge of the open neural plate. Based on that early expression pattern, we suggest that *X-dll3*, marks the rostral-most part of the neural plate, which gives rise to the ventral forebrain and the olfactory placodes. Finally, we have used one of the *Xenopus distal-less* genes (*X-dll3*) to show that signals that spread within the plane of neural ectoderm are sufficient to induce the anterior-most neural plate.

PZ 233 STRUCTURE AND FUNCTION OF THE TRANSCRIPTIONAL CONTROL REGION OF THE SEROTONIN 5-HT1A RECEPTOR GENE, Christopher L. Parks and Thomas Shenk, Howard Hughes Medical Institute, Department of Molecular Biology, Princeton University, Princeton, NJ 08544-1014

To begin determining what mechanisms are responsible for limiting expression of the serotonin 5-HT1a receptor to the brain, we have started to characterize the transcriptional control region of this gene. Genomic DNA sequences 5' of the protein coding region for the mouse and human 5-HT1a receptor have been cloned and sequenced. Within the cloned genomic DNA, a promoter region has been identified by locating the transcription initiation sites through cDNA cloning and nuclease protection experiments, and demonstrating the ability of this sequence to drive expression of a reporter gene in transient expression assays. These results have established the boundaries of a complex promoter that contains ten or more transcription initiation sites, but lacks identifiable TATA box sequences. Currently, we are characterizing potential DNA binding proteins that may participate in regulating expression from this promoter.

The promoter region described above can direct expression in a variety of cell types suggesting that this region alone is not sufficient for conferring cell-type specific expression, and that it is likely that DNA sequences flanking the promoter will be important for selective expression of the gene. To analyze the regulatory sequences necessary for cell-type specific expression with transient expression assays, it is ideal to have a cell line that expresses the gene of interest in culture. Therefore, we have used targeted tumorigenesis in an attempt to establish cell lines from transgenic mouse brain that do express the genomic 5-HT1a receptor gene. Presently, we are characterizing immortalized cells that express an SV40 T-antigen transgene under the control of the 5-HT1a receptor gene transcriptional control region.

PZ 234MOLECULAR AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A KAINATE-PREFERRING RECEPTOR EXPRESSED IN RAT DORSAL ROOT GANGLION (DRG) NEURONS, K.M. Partin, L.A. Wong, M.L.Mayer, & *A. Buonanno. Lab. Cell. & Molec. Neurophysiol. and *Lab. Dev.Neurobiol., NICHD, NIH.

Glutamate receptors in rat DRGs are different from those in the hippocampus and cortex in that they produce a rapidly desensitizing response when activated by kainate *(Huettner (1990), Neuron, 5:255).* 5-substituted willardiine analogs activate rat DRG receptors with a potency sequence that is the reverse of that observed in the hippocampus *(Patneau et al.(1992) , J. Neurosci.,12:595); Wong et al.(1992), Soc. Neurosci. Abs. , 18(1):86;*). We now show that concanavalin A but not cyclothiazide or aniracetam blocks desensitization at rat DRG glutamate receptors and potentiates kainate-activated currents. In order to understand the molecular components mediating the pharmacological differences between DRG and hippocampal neuron glutamate receptors, we performed northern blot analyses on DRG total RNA, hybridizing with cDNA's to the glutamate receptor clones. In parallel experiments, we reverse transcribed DRG total RNA and used subunit-specific primers in PCR reactions, to ascertain with greater sensitivity which subunits are expressed in DRG's. These experiments show that although most glutamate receptor subunits are expressed (some at low levels) in DRG's, GluR5 is predominant. Furthermore, since GluR5 exists as a family of isoforms, with heterogeneity mapped to the extracellular domain (5-1 vs. 5-2); to the TMII domain (Q vs. R); and, to the carboxyl domain (a, b, c carboxyl tails), experiments are being carried out utilizing PCR analysis of reverse transcribed total RNA to compare isoform distribution in DRGs with brain tissue. Experiments utilizing single-cell PCR on electrophysiologically-characterized neurons, and mimicry of the *in vivo* response by the co-expression of receptor subunits, are currently being performed to identify possible receptor subunit composition.

PZ 235 ISOLATION AND CHARACTERIZATION OF TWO NOVEL HIPPOCAMPAL NUCLEAR HORMONE RECEPTORS. Sandra Peña de Ortiz and Gordon A. Jamieson Jr. Department of Environmental Health, University of Cincinnati College of Medicine, 3223 Eden Avenue, Cincinnati, OH 45267-0056.

Alterations in gene expression are considered to be a major mechanism for eliciting long-term responses to external stimuli in neuronal tissues. These changes often occur subsequent to hormone or morphogen-dependent activation of specific transcription factors. Previously characterized ligand-dependent transcription factors (*i.e* nuclear hormone receptors) are known to function in the hippocampus. Herein we demonstrate the existence of novel, hippocampal-specific, nuclear receptors. Rat hippocampal cDNA was prepared and degenerate oligonucleotide primers used to successfully amplify and subsequently isolate partial cDNAs encoding the DNA binding domain of ligand-dependent transcription factors. Among our isolates, we identified cDNAs which encode the zinc finger region of the rat glucocorticoid and thyroid hormone receptors. More interestingly two novel cDNA isolates, which we designate as hippocampal zinc fingers (HZFs) 2 and 3 were identified. These novel cDNA clones are 58% to 79.8% homologous to the zinc finger region of known members of the superfamily of nuclear receptors. Northern blot analysis detected transcripts for both HZF-2 and HZF-3 in the rat brain. Reverse transcription-PCR analysis determined the distribution of HZF-2 and HZF-3 transcripts in the rat brain and other rat tissues. In the brain, mRNAs encoding these nuclear receptors are preferentially localized in the hippocampus. To isolate full length HZF-2 and HZF-3 cDNA, a hippocampal cDNA library was screened using a solution hybridization enrichment strategy coupled to PCR. *In situ* hybridization will allow us to define the precise cellular localization of our novel isolates in the hippocampus. Currently we are using chimeric receptors in conjunction with appropriate reporter gene constructs in order to identify neuronal stimulants which directly or indirectly activate the transregulatory function of HZF-2 and HZF-3. Together, these studies will allow us to determine whether these novel nuclear receptors function to regulate important aspects of hippocampal physiology. *Supported by ONR N00014-90-J-1898 and a Ford Foundation Dissertation Fellowship for Minorities.*

PZ 236 MUTATIONS IN *LOZENGE* PERTURB THE DEVELOPMENT OF THE FRUIT FLY VISUAL SYSTEM: A MODEL FOR CELLULAR INTERACTION. John A. Pollock*, Philip Batterham† and Jennifer R. Crew*. †Department of Genetics, The University of Melbourne, Parkville, Victoria 3052, Australia; *The Center for Light Microscopic Imaging and Biotechnology and the Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213.

Mutations in the *lozenge* gene cause a pleiotropic range of phenotypes including severe morphological defects in the adult compound eye. Our developmental and genetic studies of *lozenge* mutant flies have shown that defects become apparent early in eye development during the late third larval instar and the "white pre-pupal" stages. We have carried out a detailed cell biological analysis of mutant eye imaginal discs from these stages of development employing light and electron microscopic techniques. Our findings indicate that the mutations in the *lozenge* gene perturb the highly choreographed development of the eye and brain. The development of identifiable neurons and supporting cells are affected by mutations in this gene. Furthermore, defects apparent in the eye are associated with altered patterns of cell death that occur during critical periods of cell fate determination.

Cell Death. Using acridine orange to assay cell death, we observe that developing *lozenge* mutant retinas show a dramatic and coordinated increase in the normal amount of cell death posterior to the morphogenetic furrow. For most alleles there is a correlation between the amount of cell death and the severity of the adult compound eye phenotype.

Neuron & Supporting Cell Recruitment. Ommatidial facets in the adult retina of *lozenge* mutant flies lack specific cell types that may be the target of normal *lozenge* gene function. Affected cell types include photoreceptor neuron R7, cone cells and 2° and 3° pigment cells. Our developmental analysis supports the hypothesis that the product of the *lozenge* gene exerts its function during the process of cell-cell interaction that recruits these specific cell types. A model for the normal cellular interactions that occur during eye development will be discussed in light of the *lozenge* eye phenotype.

The molecular cloning of *lozenge* is underway in our laboratory; results of this work-in-progress will be presented as well.

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Molecular Neurobiology III

PZ 300 MAMMALIAN CELL LINES FUNCTIONALLY EXPRESSING GLUTAMATE RECEPTOR SUBUNITS.
J.S.Rasmussen, L.S.Nielsen, E.Boel, K.Hansen, A.Hansen, K.M.Houamed, B.Holm, C.E.Stidsen, P.H.Andersen. Molecular Biology II and Molecular Pharmacology, Pharmaceutical Biotechnology, Novo Nordisk A/S, Novo Allé, DK-2880, Bagsvaerd, Denmark.

Rat Glu A-D flip and flop subunits were cloned by PCR using 1. strand cDNA derived from rat cerebellum or hippocampus as template and sequence specific oligonucleotides as primers. The receptor cDNAs under the control of a constitutively active metallothionine promoter were transfected into BHK cells. Following selection, resistant subclones expressing high fmol - low pmol of receptor/mg membrane protein were isolated.
Using binding of ^{3}H-AMPA the pharmacological profile of the subunits was investigated. These results indicated no difference between flip and flop subunits. The profiles of Glu A - D were comparable, showing high affinity for AMPA (10-30 nM) and quisqualic acid (2-20 nM), medium affinity for kainate (300-4000 nM) and very low affinity for ibotenic acid (>30 µM).
Whole cell patch clamp recordings, at a holding potential of -70 mV of cell lines expressing glutamate receptor subunits, revealed sustained inward currents activated by kainate indicating formation of functional ion channels.
In conclusion, the pharmacological and functional profile of the recombinant receptors resembles the high affinity AMPA receptors found in rat brain.

PZ 301 LEUKEMIA INHIBITORY FACTOR (LIF) PROMOTES THE DEVELOPMENT OF MAMMALIAN SPINAL CORD PRECURSOR CELLS *IN VITRO*, Linda J. Richards, Trevor J. Kilpatrick, Perry F. Bartlett and Mark Murphy, Neuroimmunology Laboratory, The Walter and Eliza Hall Institute of Medical Research, PO, Royal Melbourne Hospital,, Victoria, 3050, AUSTRALIA.
In the course of studying the development of mammalian spinal cord neurons, we have found that LIF stimualtes a profuse neuritic outgrowth when neuronal precursors, derived from embryonic day 10 (E10) murine spinal cord primordium, are placed as explants *in vitro*. To determine if this effect was due to an increase in the number of neurons generated, neuronal precursors were plated at low cell density (1500 cells/cm^2) onto NIH 3T3 fibroblast monolayers in the presence or absence of LIF. LIF stimulated a significant increase in the number of neurons which were found, by lack of ^{3}H-Thymidine incorporation, to be generated from non-diving precursors. Further, those neurons generated in the control cultures probably arose due to endogenous LIF, as when inhibitory anti-LIF receptor antibody was added, no neurofilament (150kD) positive neurons were detected. These data show that LIF is required for the generation of spinal cord neurons in these cultures. To assess the phenotype of these neurons we stained the cultures for choline acetyltransferase (ChAT). Whereas LIF has been reported to act as a cholinergic switching factor for sympathetic neurons (Patterson and Chun, 1977), LIF did not selectively increase the percentatge of ChAT positive neurons in the spinal cord cultures and appeared to act on the neuronal population in general.
In addition to defining the effects of LIF on the whole spinal cord primordium, we have begun to investigate which of the component cells respond directly to LIF. We have utilised a method for sorting cells on the basis of their ability to express major histocompatability antigens (Bartlett et al, 1990), to obtain a purified population of neuronal precursor cells, and have found that LIF potentiates the generation of differentiated neurons in these cultures. Preliminary results show that in the presence of serum, LIF also increases the number of astrocytes in long-term cultures derived from E10 embryos. We are now investigating the presence of LIF receptors on the surface of these cell-types to determine which cells are specifically responding to LIF within the spinal cord primordium.
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PZ 302 DIFFERENTIATING AND ANTIPROLIFERATIVE EFFECTS OF GAMMA-IFN AND NGF ON SUBPOPULATIONS IN A NEUROBLASTOMA CELL LINE: FLOW CYTOMETRIC AND MORPHOLOGICAL ANALYSIS, J.Ridge, D.Terle, E.Dragunsky and I.Levenbook, CBER, FDA, Bethesda, MD 20892
To determine the differentiating effects of gamma-interferon (IFN) and nerve growth factor (NGF) on neuroblastoma (NB) we selected SH-SY5Y, a human NB line. These cells are known to have receptors for NGF; contain a single copy of the N-myc proto-oncogene; extend neurites in culture; and respond to differentiating agents. Using NGF, IFN or IFN+NGF we found earlier that combined treatment resulted in morphologically and immunohisto-chemically defined differentiation. Since NB is a neural crest-derived cell line it contains, in addition to neuroblasts, precursors of both melanocytes and Schwann cells. In this study we examined the effects of treatment on all three cell populations using markers to cell -specific, or- associated, proteins. With fluorescence activated flow cytometry cells were analyzed for expression of the 200kD neurofilament, S-100 and myelin basic protein. In addition, morphological changes were observed and correlated with the protein changes after 8 and 14 days of continuous treatment. Results showed IFN+NGF induced the largest increase in marker expression and the greatest changes morphologically compared to controls. IFN alone induced effects similar to those with combined treatment, but to a lesser degree. There was little difference in these parameters between NGF alone and non-treated cells. Moreover, when growth rates were examined, while NGF and control cultures increased 4 to 20 fold over seeding densites from 8 to 14 days, cell numbers remained constant in IFN and IFN+NGF-treated cultures. Nude mouse experiments confirmed our *in vitro* data demonstrating a delay in tumor formation only from inoculum of cells with the latter pretreatments. These results indicate that IFN, alone or in combination with NGF, induces differentiation in SY5Y subpopulations concomitant with extensive and sustained inhibition of proliferation resulting in delayed tumor formation in nude mice.

PZ 303 ANALYSIS OF C-FOS REGULATION IN TRANSGENIC MICE, Linda M Robertson, Richard J. Smeyne, Montserrat Vendrell, Daniel Luk, James I. Morgan, and Tom Curran, Departments of Molecular Oncology and Neurosciences, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.
The protooncogenes, c-*fos* and c-*jun*, are cellular immediate-early genes that are induced in response to a variety of extracellular stimuli. Their protein products (Fos and Jun) form heterodimers that regulate transcription of target genes containing AP-1 and CRE binding sites. We have developed a transgenic mouse line containing a c-*foslacZ* fusion gene. The bacterial ß-galactosidase (*lacZ*) gene was fused, in frame, into the carboxyl-terminal region of c-*fos*, without disrupting any of the known regulatory sequences. This construct was then introduced into mice by microinjection and founder strains were derived and characterized for ß-galactosidase (ß-gal) activity. To characterize the molecular basis of c-*fos* regulation in vivo, we also produced transgenic mice carrying a c-*foslacZ* gene containing mutations in the known regulatory elements. Constituitive expression of the intact transgene was observed in specific regions of the bone and skin. Other areas, including the central nervous system, expressed little or no ß-gal activity. Injection with the seizure-inducing agents, kainic acid or pentylentetrazole resulted in high levels of ß-gal activity in neurons in many regions of the central nervous system. In contrast to the observations with the intact transgene, the majority of the mutants exhibited little or no constituitive expression in vivo. Induced expression of the mutant transgenes varied dramatically. For example, injection with kainic acid or pentylentetrazole induced high levels of neuronal expression in transgenic lines derived from the construct containing a mutation in the AP-1 element. In contrast, transgenic lines derived from constructs containing a mutation in the SRE, SCM, or CRE sites exhibited low or aberrant patterns of expression. Primary fibroblasts were prepared from each transgenic line and ß-gal activity was monitored in response to a variety of stimuli. Exposure to high serum, TPA, and PDGF increased expression of ß-gal activity in fibroblasts from transgenic lines containing the intact c-*foslacZ* gene. Induction of ß-gal activity was also seen in fibrobasts derived from the mutants, but at a lower level. In summary, constituitive expression of the c-*foslacZ* transgene in vivo was dramatically reduced by mutations in most of the known regulatory elements. Future studies will involve the characterization of the regulatory mutants in other physiological contexts known to affect expression of c-*fos* in vivo.

PZ 304 β-ADRENERGIC RECEPTOR KINASE-2 AND β-ARRESTIN-2 AS MEDIATORS OF ODORANT-INDUCED DESENSITIZATION, Jane Roskams*, Robert Bakin*, Ted Dawson*, Robert Lefkowitz‡ and Gabriele Ronnett†, Departments of Neuroscience* and Neurology†, Johns Hopkins University School of Medicine, Baltimore, MD 21205 and Howard Hughes Medical Institute, Department of Medicine and Biochemistry, Duke University Medical Center‡, Durham, NC 27710

β-adrenergic receptor kinase (β-ARK) and β-arrestin (β-ARR) function in homologous or agonist-activated desensitization of G protein-coupled receptors. We have localized the isoforms β-ARK-2 and β-ARR-2 as highly enriched in and localized to the dendritic knobs and cilia of the olfactory receptor neurons (ORN), where the initial events of olfaction occur. Preincubation of rat olfactory cilia with neutralizing antibodies raised against β-ARK-2 and β-ARR-2 increases the odorant-induced elevation of cAMP and attenuates desensitization. Using rat β-ARK-2 and β-ARR-2 clones, we have identified olfactory β-ARK and β-ARR isoforms. We are currently performing in situ hybridization to localize the expression of olfactory β-ARK and β-ARR. In addition, we have generated antibodies directed against the putative family of odorant receptors in order to localize their expression in the ORN and elucidate their function in olfactory signal transduction. Olfactory signal transduction is monitored by measuring odorant-induced adenylyl cyclase activity in ORN cilia preparations and primary cultures of olfactory neurons. Although the ORN is specialized for the function of odorant detection and transduction, it appears to utilize signal transduction cascades common to other neuronal systems, and therefore olfaction serves as an interesting model system for neuronal signal transduction.

PZ 305 VARIATIONS IN THE EXPRESSION OF $GABA_A$/BZ RECEPTOR SUBUNIT mRNAs IN STAGGERER MUTANT MOUSE CEREBELLUM. Andrej Rotter, Vera Luntz-Leybman and Adrienne Frostholm. Department of Pharmacology, The Ohio State University, Columbus, OH 43210.

Recent studies have identified several subunits (α, β, γ, δ and ρ) of the gamma aminobutyric acid$_A$/benzodiazepine receptor; each consists of several variants. The expression of $GABA_A$/BZ receptor subunit mRNAs was studied by in situ hybridization with [^{35}S] cRNA probes in normal and staggerer mutant mice. In normal animals, the α_1, β_2 and γ_2 subunits were present in Purkinje cells which, in adult animals, form a monolayer at the interface of the granule cell and molecular layers. In the staggerer mutant, the cerebellum is reduced in size and is relatively unfoliated. There are fewer Purkinje cells; those that remain become embedded in the granule cell layer during migration and are unable to form synapses with granule cells. Although normal high levels of α_1 subunit mRNA expression were observed in staggerer Purkinje cells, no hybridization signal was present with β_2 or γ_2 probes. Furthermore, both [^{3}H] muscimol and [^{3}H] flunitrazepam binding sites (GABA and benzodiazepine ligands, respectively) were considerably reduced in the staggerer cerebellar cortex. These studies support a previous finding (Pritchett et al, 1989) that the γ_2 subunit mediates the interaction between α and β subunits, making the receptor capable of modulation by benzodiazepines.

PZ 306 LIGAND SPECIFICITY AND TRANSACTIVATION PROPERTIES OF STEROID RECEPTORS EXPRESSED IN THE CNS, Rainer Rupprecht, Thorsten Trapp, Hans Reul, Maija Castren, Florian Holsboer and Klaus Damm, Dept. of Neuroendocrinology, Max-Planck-Institute of Psychiatry, Clinical Institute, Munich, Germany.

Adrenal steroids and sex steroids have a major influence on maintaining central nervous system homeostasis, cell proliferation and differentiation. The actions exerted by these hormones are mediated mainly by the respective intracellular hormone receptors. Because of the near identity in the DNA binding domain, the glucocorticoid (GR), mineralocorticoid (MR) and progesterone (PR) receptors can interact with similar target DNA sequences. In addition, the three receptors are very similar in their ligand binding domains (57% and 55% identity) and only a few hormones can discriminate selectively between these receptors. In functional cotransfection assays using the human neuroblastoma cell line SK-N-MC we characterized the pharmacological profiles of the three receptors for corticosteroids, progestins and synthetic ligands with regard to binding profiles and agonistic or antagonistic properties at the gene expression level. For example, we demonstrate that the antiprogestin and antiglucocorticoid RU486 displays a partial agonistic activity with the GR, suggesting a direct interaction of antihormone-receptor complexes with the response elements on the DNA. Our results also show that the hMR stimulation of the MMTV promoter was only 2-4% of the maximum level obtained with the hGR. To examine the molecular basis of this differential response, a series of hMR/hGR hybrid receptors were created and their properties determined. These experiments revealed that the hMR amino terminus is lacking the strong trans-activation function present in the equivalent hGR domain. In addition, the amino termini of hGR and hMR differentially promote or inhibit synergistic trans-activation functions of the DNA- and ligand binding domain of both receptors.

PZ 307 **NEUROENDOCRINE-SPECIFIC TRANSCRIPTION OF THE CALCITONIN/CGRP GENE INVOLVES SYNERGY BETWEEN AN HLH FACTOR AND AN ASSOCIATED FACTOR** Andrew F. Russo and Lois Tverberg, Department of Physiology and Biophysics, University of Iowa, Iowa City IA 52242

The calcitonin/CGRP gene is transcribed in thyroid C-cells and a subset of peripheral and central neurons. We have localized the sequences required for cell-specific enhancement of calcitonin/CGRP transcription to an 18 bp region between -1025 and -1043 bp upstream of the transcriptional start site. This fragment confers a 50-80-fold enhancement in the 442C rat C-cell line, in contrast to less than 2-fold enhancement in the heterologous Hela cell line. It also contains the sequences required for cell-specific repression by glucocorticiods. Based on sequence homologies, the enhancer contains a helix-loop-helix (HLH) transcription factor binding site flanked by a motif similar to a composite GRE/AP1 element identified in the proliferin gene. Point mutations in either the HLH site or the flanking motif essentially abolished enhancer activity. Separation of the elements by either a half or full helical turn also eliminated enhancer activity. The functional contribution of HLH proteins was demonstrated by cotransfection of an expression vector containing the MASH HLH factor gene with the calcitonin/CGRP enhancer reporter into Hela cells. Cotransfection with MASH yielded a 3-4-fold enhancement relative to a reporter lacking the enhancer or cotransfection with a control plasmid. These results suggest that both the HLH and associated factor(s) act in a synergistic manner to stimulate transcription in C-cells. Electrophoretic mobility shift assays revealed two specific DNA-protein complexes that could form on the 18 bp calcitonin/CGRP element. The complexes were assigned as the HLH or HLH flanking complexes based on using the mutant oligonucleotides both as probes and as competitors in the mobility shift assay. The HLH complex was further confirmed by competition with oligonucleotides containing binding sites for the AP4 and Pan HLH proteins. The nature of the flanking complex is not yet clear since it was not competed by oligonucleotides containing a consensus AP1 binding site. These studies indicate that calcitonin/CGRP gene enhancer activity is controlled by synergistic interactions between an HLH factor and an associated factor.

PZ 308 CALCIUM SIGNALING IN IMMORTALIZED HYPOTHALAMIC (GT1-7) NEURONS. Michael J. Sanderson, Tim Hales, and Andrew Charles. Departments of Anatomy and Cell Biology, Anesthesiology, and Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Immortalized hypothalamic (GT1-7) neurons in culture show spontaneous, transient increases in $[Ca^{2+}]_i$ which are observed with fura-2 fluorescence imaging. These Ca^{2+} spikes are abolished by TTX and by the removal of extracellular Ca^{2+}. They are temporally correlated with spontaneous bursts of action potential observed in parallel studies using the patch clamp technique in the cell attached patch configuration. These Ca^{2+} spikes are generally asynchronous in individual cells in the culture, although some cells show a coordinated pattern of spikes. The patterns of the Ca^{2+} spikes vary from cell to cell, although the Ca^{2+} spikes in each individual cell have a characteristic and consistent pattern. The periodicity of the spikes ranges from 1-60 seconds in individual cells and the peak $[Ca^{2+}]_i$ of each spike ranges from 100 to 400 nM. Bath application of 1 μM gamma-amino butyric acid (GABA) results in a simultaneous increase in $[Ca^{2+}]_i$ in all cells in the culture, followed by an increase in the frequency of Ca^{2+} spikes in each cell. Bath application of 10 μM GABA results in either a further increase in the frequency of Ca^{2+} spikes, or a plateau of increased $[Ca^{2+}]_i$. The GABA-evoked increase in $[Ca^{2+}]_i$ is blocked by bicuculline and is inhibited but not abolished by TTX. Application of GABA evokes bursts of action potentials observed in the cell attached patch configuration. These observations suggest that spontaneous Ca^{2+} spikes in GT1-7 cells are initiated by spontaneous action potentials, which result in the influx of Ca^{2+} through voltage gated Ca^{2+} channels. Activation of $GABA_A$ receptors increases the frequency of Ca^{2+} spikes by increasing the frequency of action potentials. These spontaneous and GABA-evoked Ca^{2+} signals may be involved in the regulation of release of GNRH in the hypothalamus. Supported by Smokeless Tobacco Research Council, Inc., the Tobacco Related Disease Research Program of the University of California, the American Academy of Neurology (ACC) and a Veterans Administration Career Development Award (ACC).

PZ 309 CLONING OF NEW HUMAN BRAIN Ca^{2+}-CHANNEL cDNA's RELATED TO P- AND N-TYPE SUBFAMILIES OF VOLTAGE ACTIVATED Ca^{2+}-CHANNELS, Toni Schneider, Xiangyang Wei, Phil Palade[@], Edward Perez-Reyes, Stanley H. Appel, and Lutz Birnbaumer; Molecular Physiol. & Biophysics, Cell Biology, Neurosciences and Neurology, Baylor College of Medicine, Houston, TX 77030; and [@]Physiology and Biophysics, University of Texas Medical Branch at Galveston, Galveston, TX 77555.

Voltage activated Ca^{2+}-channels (=CaChs) are classified by electrophysiological and pharmacological criteria into T-, L-, P-, and N-type Ca^{2+}-channels. They contain high affinity receptors for the classical Ca^{2+}-antagonists (L-type Ca^{2+}-channels) or for different peptide toxins like the aga- and conotoxins (P- and N-type, but also L-type Ca^{2+}-channels). In accordance with the order of molecular cloning of their ion conducting α_1-subunits they are named as CaCh-1, -2, -3, etc. To some extent, a larger number of different genes of the α_1-subunit has been identified than suspected on the basis of electrophysiological or pharmacological criteria.

This report focuses on nucleotide sequences related to putative P- and N-type Ca^{2+} channels of human brain. Using oligonucleotides related to conserved regions in L- and P- type Ca^{2+} channels as well as in voltage-activated Na^+ channels, we amplified from a mouse brain-derived cell line several ≈1kb long DNA's. These were subcloned and shown to be homologous to published sequences of the CaCh-3 (2 known splice variants), CaCh-4 (2 splice variants, one new) and CaCh-5 (one variant) α_1 subunits. The partial sequence from CaCh-5 was used as a probe to screen two human brain cDNA libraries. It hybridized to DNA containing partial sequences of CaCh-4 and CaCh-5, as well as of a new Ca^{2+}-channel related sequence, which we call CaCh-6. The human CaCh-6 nucleotide sequence shows regions with high homology to the published sequences, but also contains regions with strong differences, and is derived from a separate gene.

In an evolutionary tree, two subfamilies of voltage dependent Ca^{2+}-channels are distinguished: 1) the α_1-subunits of CaCh-1, CaCh-2 and CaCh-3 are members of one subfamily, and 2) the α_1-subunits of the new human CaCh-6 appears to belong to a second subfamily together with the CaCh-4 and CaCh-5 subunit.

PZ 310 MOLECULAR DISSECTION OF ACTIVE SITE PROPERTIES IN IMMOBILIZED CHOLINESTERASES PREDICTS SEQUENCE SPECIFIC SUSCEPTIBILITY FOR CHOLINERGIC POISONS, Mikael Schwarz[1], Yael Loewenstein[1], Jian Liao[2,3], Avraham Yaron[1], Bent Norgaard-Pedersen[2], Urs Brodbeck[3], and Hermona Soreq[1], [1]Dept. Biological Chemistry, Hebrew University of Jerusalem, Jerusalem , Israel, and [2]Dept. Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark and [3]Institute of Biochemistry and Molecular Biology, University of Bern, Bern Switzerland,

To investigate the molecular mechanisms underlying the neuropathological consequenses of cholinesterases (CHE's) inhibition, (i.e. by organophosphorous (OP) insecticides) we immobilized the enzymes to monoclonal antibodies which allowed for their enrichment and enabled removal of ligands prior to enzymatic assays. This is important since ligands used therapeutically in cases of CHE's intoxication, such as the oxime reactivator Pyridine-2-aldoxime Methiodide (2-PAM), are known to interact with enzyme-substrate tertiary conformations, thereby accelerating the rate of substrate hydrolysis by CHE's and exaggerating the apparent rate of reactivation. Pre-incubation with, and subsequent removal of 2-PAM, reduced activity of immobilized acetylcholinesterase (ACHE) but not of butyrylcholinesterase (BCHE) with increased 2-PAM concentration. When 2-PAM was used to reactivate CHE's which were previously treated with the OP inhibitor diisopropylfluoro phosphate (DFP), ACHE displayed a bell shaped reactivation rate dependence on 2-PAM concentration with an optimum at 1mM. In contrast, reactivation rate of BCHE was increased up to at least 100mM 2-PAM. Since neither DFP nor 2-PAM were present when enzyme activity was assayed, these results suggest that 2-PAM interacts transiently with BCHE but binds irreversibly to the active site of ACHE, perhaps through the ACHE-characteristic peripheral site which accomodates excess substrate. These observations may explain the neurotoxic effects reported for oxime therapy of OP intoxication and foreshadow the use of this approach with natural human CHE mutants (Soreq et al., TIBS 17: 353-358, 1992) to disclose inherited predisposition for neuropathological damage due to OP poisoning.

PZ 311 NERVE GROWTH FACTOR (NGF) AND INTERLEUKIN-6 (IL-6) MEDIATE AN INTRATHYMIC NEUROMODULATORY LOOP. Isabella Screpanti, Daniela Meco, Susanna Scarpa, Stefania Morrone, Luigi Frati, Alberto Gulino and Andrea Modesti, Department of Experimental Medicine, University La Sapienza, Rome, Italy.

Neural crest derivatives are involved in thymus development. We established thymic stromal cell cultures (TC) which support T cell differentiation and display phenotypic and biochemical markers specific for neuronal cells, such as neurofilament mRNA and 68 and 160 kDa neurofilament proteins as well as 74 kDa synapsin I isoform. The two known neurotrophic factors, NGF and IL-6 are produced in an autocrine way by TC cells and both enhanced the expression of the above described neuronal markers. Finally we found that IL-6 gene expression in TC cells is enhanced by NGF. Evidence is thus offered of a novel neuromodulatory loop within the thymic stromal cell population supported by local production of NGF and IL-6 and involving neural crest-derived cell elements. Interestingly, IL-6, which is known to be implicated in thymocyte differentiation, also displays a neuromodulatory activity on thymic stromal cells, suggesting a multivalent role of this cytokine within the thymus.

PZ 312 NEUROTROPHINS IN DEVELOPING CEREBELLUM.

Rosalind A. Segal*, Hiroshi Takahashi+, Ron McKay+, and Charles Stiles*. *Division of Microbiology and Molecular Genetics, Harvard Medical School, and Dana Farber Cancer Institute, Boston, Ma. 02115; +Department of Biology, Massachusetts Institute of Technology, Cambridge, Ma. 02139.

The developmental functions of the neurotrophins can be studied by identifying the time in differentiation when a cell can, and does, respond to a particular neurotrophin. We have been investigating the functions of the neurotrophins during cerebellar development. Cerebellar granule cells respond to BDNF and NT3 at distinct stages in differentiation, as assessed by induction of the immediate early gene product, FOS. BDNF causes FOS induction in more immature cells, while NT3 affects more fully differentiated granule neurons. The functions of the neurotrophins were studied in dissociated and organ cultures of developing cerebellum. BDNF, but not NT3, acts as a survival factor for early granule cells in culture. The changes in receptor expression and activity that may be responsible for the developmental progression in neurotrophin responsiveness are being defined.

PZ 313 CHARACTERIZATION OF NOVEL METABOTROPIC GLUTAMATE RECEPTORS ISOLATED FROM RAT OLFACTORY BULB. *Thomas P. Segerson, Julie A. Saugstad, J. Mark Kinzie, Eileen R. Mulvihill, Gary L. Westbrook. Vollum Institute, Portland, OR 97201 and Zymogenetics, Seattle, WA 98105.* Excitatory transmission by glutamate appears to involve a complicated interplay of glutamate-gated ion channels (ionotropic receptors: NMDA, AMPA/kainate) and a growing family of G protein-coupled or metabotropic glutamate receptors (mGluRs). mGluRs may couple to multiple intracellular signaling pathways in neurons and function at both pre- and postsynaptic sites. Nowhere in the brain is this interaction likely to be as important as in the olfactory system, which expresses at high levels nearly all the members of the mGluR family characterized thus far. The demonstration of a G protein-coupled glutamate receptor specific for the conformationally restricted glutamate analog L-AP4 in mitral/tufted cells of the olfactory bulb (Trombley and Westbrook, 1992) suggested the existence of still other mGluRs in olfactory neurons. To isolate novel mGluRs, oligonucleotide mixes containing all possible codons for conserved regions of the second and sixth transmembrane domains of mGluR1 and mGluR4 (degenerate oligonucleotides) were synthesized and used to amplify rat olfactory bulb first strand cDNA. Amplificants were digested with restriction enzymes that bisect this region of mGluR1 and 4 to enrich for novel sequences. Intact fragments were subcloned and sequenced. This analysis identified at least three sequences that encode fragments of molecules with similarity to other mGluRs. Using these fragments, we probed an olfactory bulb cDNA library to isolate full-length cDNA clones. Probe *olf1*, which was closely related to mGluR1 isolated a full-length cDNA for mGluR5 (Abe, 1992). Probe *olf2* isolated a full-length cDNA encoding a member of the mGluR family whose sequence is 67% identical to mGluR4. Preliminary *in situ* hybridization of rat brain using this cDNA as a probe shows expression of mRNA for this novel mGluR in dentate gyrus and regions CA1-4 of the hippocampus and in the glomerular and mitral layers of the olfactory bulb. This pattern is distinct from the known mGluRs and resembles more that of mGluR1 than mGluR4. Expression in *Xenopus* oocytes resulted in no glutamate-dependent current, suggesting that this receptor does not couple to phosphatidylinositol turnover. Given its sequence similarity to mGluR4, we suspect that this may be an L-AP4 specific member of the family. Probe *olf8* isolated yet another novel mGluR sequence that is presently being characterized. As intact neurons express multiple subtypes of mGluRs, we are investigating the effects of individual mGluRs on neuronal excitability by expression of mGluR cDNAs in neuronal cells.

PZ 314 DEVELOPMENTAL EXPRESSION OF THE D_{1A} DOPAMINE RECEPTOR, Diana M. Severynse and Marc G. Caron, Department of Cell Biology, Duke University Medical Center, Durham, NC 27510

Dopamine is a neurotransmitter that interacts with receptors to activate a number of physiological responses such as motor control, emotion, affect and neuroendocrine function. Binding of dopamine to the D_1 class of receptors results in a stimulation of adenylyl cyclase and phospholipase C. Although D_1 receptors are the most abundant dopamine receptors in the brain, their role in the functioning of the central nervous system is unclear. The goal of this study is to gain insight into the possible role of the D_{1A} receptor subtype, by examining the temporal and spatial patterns of D_{1A} expression during neural development.

Transgenic mice were generated that contain the β-galactosidase gene fused to a 6.5 kb fragment containing the human D_{1A} receptor promoter. This promoter has been previously characterized in our laboratory using a series of CAT deletion constructs. Preliminary results indicate that the transgene is expressed in the brain as early as GD 13, which is consistent with in-situ data that has detected D_{1A} receptor transcripts at GD 14. β-galactosidase can be detected in the mesencephalon and ventriculus lateralis of transgenic embryos. In addition, the transgene can be detected in several peripheral areas which are known to have tissue-specific expression of D_{1A} receptors.

PZ 315 **IEGs are heterogeneously expressed in individual neurons and are specifically regulated by patterned neuronal activity.**

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To address the issue of how a relatively small number of IEGs can be induced in a wide range of cell types in a stereotypic fashion, yet at the same time show specificity in inducing cellular growth, differentiation or neuronal adaptive responses (Sheng & Greenberg, 1990) we examined the expression of seven IEGs, c-fos, c-jun, jun-B, jun-D, nur/77, SRF, and zif/268 in individual mouse DRG neurons using a two-stage amplification procedure. It was found that individual neurons in an apparently homogeneous population expressed extremely diverse patterns of IEGs. Of the 80 neurons examined, we detected 30 different combinations of IEGs. Furthermore, using a semi-quantitative-PCR assay, we show that IEG expression is sensitive to the pattern of electrical stimulation. Dissociated DRG were stimulated with electrical pulses organized in various patterns. A constant number of stimuli (180) were delivered in 3 different patterns for 30 min.: 1) steady 0.1 Hz; 2) bursts of 6 stimuli at 10 Hz delivered every minute; 3) 12 stimuli at 10 Hz delivered every 2 minutes. The 0.1 Hz stimulation produced a small increase in c-fos expression over control, (69%, $p<0.02$; 22+5.8 vs. 13+2.8, mean O.D.+SD), while bursts of 6 pulses/min. at 10 Hz produced a significantly larger increase (168%, $P<0.001$; 34.8+3.8). By contrast 12 pulse bursts every 2 min. at 10 Hz produced no significant increase in c-fos expression (14.8+1.9). Differences in c-fos activation did not correlate with the peak intracellular calcium $[Ca^{++}]i$ produced by the different stimulation patterns, or with residual $[Ca^{++}]i$ following stimulation. However, the net increase in [Ca++]i was greater for the pulsed stimulus that was effective in activate c-fos (6 imp./min.), compared to the ineffective stimulus (12 imp./2 min.). It appears that both with regard to the rich diversity in the patterns of IEG expression in individual neurons and the subtlety and specificity of their responses to neural signals, this system of genes is admirably suited to mediating the coupling between a large number of different stimuli (both chemical and electrical) and large number of functional genes.

*M. Sheng, M. E. Greenberg, *Neuron* 4, 477-485 (1990).

PZ 316 CONTROL OF VCAM-1 EXPRESSION IN NEURAL DIFFERENTIATION. Allan M. Sheppard, Jay J. McQuillan, and Douglas C. Dean. Departments of Medicine and Cell Biology, Washington University School of Medicine, St. Louis, MO 63110

Classically, the integrin $\alpha4\beta1$ and its counter receptor vascular cell adhesion molecule-1 (VCAM-1) mediate cell-cell interactions that are important for maturation and function of immune cells. However, we found recently that these receptors have a role in myogenesis. Here we show that VCAM-1 is expressed in a developmental-specific pattern in the brain—it is present on glial cells and/or uncommitted bipotential cells, but not on neurons, during embryogenesis and it dissipates after birth. We turned to a cell culture system to examine mechanisms that control VCAM-1 expression during glial differentiation. The P19 embryonic carcinoma cell line can be induced by treatment with retinoic acid to differentiate into neurons and glial. VCAM-1 was not detected on undifferentiated cells, but, as in the brain, it appeared on glial and/or uncommitted bipotential cells after differentiation. The level of VCAM-1 mRNA paralleled that of VCAM-1, suggesting that increased expression may occur at the level of gene transcription. In a series of transfection assays we found that the VCAM-1 gene promoter was active in differentiated cells but not in undifferentiated cells. Deletion of promoter sequence from position -2.1 kb to position -288 bp caused activation of the gene in undifferentiated cells without affecting expression in the differentiated cells. This deletion then appears to remove a silencer(s) that prevents VCAM-1 expression in the undifferentiated cells (this silencer is not active in the differentiated cells). Interestingly, we have demonstrated that this region of the VCAM-1 gene promoter contains three octamers that act as transcriptional silencers which prevent VCAM-1 expression on "resting" endothelial cells. Thus, it is possible that the activity of these octamers controls VCAM-1 expression in differentiating P19 cells: they are silencers in undifferentiated cells but they are inactive after differentiation.

PZ 317 BIOLOGICAL ACTIVITIES OF INTRACELLULAR SIGNAL TRANSDUCTION PATHWAY INTERMEDIATES. Mark R. Smith, BCDP, PRI/DynCorp, BRMP, Frederick Cancer Research and Development Center, Frederick, MD 21702.

Polypeptide hormone-mediated signal transduction is an important mechanism used by organisms to coordinate the metabolism of individual cells. The metabolic network is controlled by factors that evaluate and regulate important decisions in cell behavior, such as proliferation and differentiation. Many of the pathway intermediates have been shown to be involved in the development and maintenance of neoplasia. Key oncogenes in the pathway include hormones, receptors with and without TK activity, G proteins, ser/thr kineses, and transcription factors. I will summarize my findings involving microinjection of a number of purified pathway intermediates into fibroblasts, macrophages, and neuronal cells. Oncogenes induced varied responses depending upon the cell type and end point evaluated. Ras protein induced DNA synthesis and morphologic transformation of fibroblasts, Ia expression on the surface of macrophages, and neuronal differentiation of PC12 cells. Recently, the eukaryotic translational initiation factor-4E (eIF-4E) was shown to be a proto-oncogene. This protein binds the mRNA cap and is the limiting factor, controlling the overall rate of protein synthesis. Over expression of the gene in cells by transfection or microinjection resulted in transformation, DNA synthesis-induction, and tumor development in mice. The DNA synthesis-inducing activity of eIF-4E was increased by PKC and phorbol ester and inhibited if Ras activity was blocked. The activity of eIF-4E depends upon phosphorylation, and other factors that affect eIF-4E activity will be discussed. A transcription factor has been discovered that is associated with human adenocarcinomas and is oncogenic. The factor binds to and activates transcription from the promotor of the c-neu gene (erbB2). Elevated levels of ErbB2, a surface receptor-like protein have been observed on transformed cells. Although the ligand for this receptor is not known, overexpression may transform cells by increased aggregation which activates its TK activity.

PZ 318 PD90780: A SELECTIVE NON-PEPTIDE INHIBITOR OF NGF BINDING TO THE P75 NGF RECEPTOR. K. Spiegel, J.M. Hopkins, M.R. Dickerson, T.W. Hepburn, R.E. Davis, J. Fergus, J. Marks, H. LeVine, J. Jaen and W. Moos, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48106.

A series of novel non-peptide ligands has been discovered that selectively inhibits the binding of NGF to its p75 binding protein (BP). PD90780, characteristic of this series, inhibits binding of ^{125}I-NGF to the extracellular domain of the p75BP in a cell-free system, as well as to cells expressing full-length p75BP. PD90780 inhibits DSS cross-linking of ^{125}I-NGF to p75BP, but does not inhibit NGF cross-linking to gp140trk. In PC12 cells expressing both p75BP and gp140trk, most but not all ^{125}I-NGF binding was inhibited by PD90780 (with an IC50 of approx. 200 nM). The apparent numbers of PD90780-sensitive and -insensitive NGF binding sites are proportional to the amounts of p75BP and gp140trk immunoprecipitated from PC12 cells. These results suggest that PD90780 inhibits binding of NGF to p75BP, but does not inhibit NGF binding to gp140trk. In addition, ^{125}I-PD90780 bound to NGF-sepharose, but not to cytochrome-C sepharose or to NGF receptors on PC12 cells, suggesting that PD90780 inhibits NGF interaction with p75BP by binding to a p75-specific domain on NGF. Despite the ability of PD90780 to inhibit NGF binding to p75, PD90780 did not inhibit the ability of NGF to elevate ChAT and MAP kinase activities in PC12 cells or to promote the survival of rat SCG neurons in culture. Furthermore, PD90780 (and related compounds) did not inhibit retrograde transport of ^{125}I-NGF from the eye to the SCG in adult rats.

PZ 319 *The Cloning and Characterization of ETS Domain cDNAs From Muscle*

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The Ets gene family encodes sequence specific transcription factors. Some of these factors have been demonstrated to associate with SRF and have been implicated in signal transduction. We are interested in elucidating the mechanisms by which extracellular signals modulate gene expression in muscle. As an initial step in understanding the role of Ets family transcription factors in muscle gene expression several Ets cDNAs were cloned and characterized. Nucleotide sequencing revealed the existence of two unique Ets domain cDNAs in Rat. We are currently characterizing these cDNAs with respect to their tissue-specific distribution, transactivation potential, and interaction with other transcription factors in muscle.

PZ 320 IDENTIFICATION OF TWO PROTEIN TYROSINE KINASES IN TORPEDO ELECTRIC ORGAN THAT ASSOCIATE WITH THE ACETYLCHOLINE RECEPTOR, Sheridan L. Swope and Richard L. Huganir, HHMI, Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Essentially all neurotransmitter receptors examined to date are regulated by protein phosphorylation or contain consensus sequences for phosphorylation by protein kinases. Classically, the nicotinic acetylcholine receptor (AChR) has been a model for investigations of the structure, function, and modulation of ligand-gated ion channels. Both in vitro and in vivo, the AChR is phosphorylated on tyrosine residues. This phosphorylation appears to regulate the rate of receptor desensitization and is associated with AChR clustering induced by the neuronal extracellular matrix protein agrin. To identify the protein tyrosine kinase(s) which phosphorylates the nicotinic receptor, we have used molecular cloning techniques to identify and characterize two protein tyrosine kinases that are highly expressed in Torpedo californica electric organ, a tissue enriched in AChR. One of the kinases was identified as the Torpedo homolog of neuronal *fyn* while the other was a novel kinase we have named *fyk* due to its homology to both *fyn* and *yes* protein tyrosine kinases. Using antibodies to these two kinases, *fyn* was shown to be a 55kD protein phosphorylated on tyrosine residues, while *fyk* was a 56kD/53kD doublet phosphorylated on serine and tyrosine residues. At the mRNA and/or protein level, *fyn* and *fyk* were present in Torpedo electric organ, skeletal muscle, and brain suggesting an involvement in the regulation of synaptic transmission. Both kinases were present in membranes enriched in the AChR and together comprised almost 50% of the protein tyrosine kinase activity in these postsynaptic membranes. In addition, immunoprecipitation experiments demonstrated that *fyn* and *fyk* were specifically associated with the AChR. These results demonstrate that *fyn* and *fyk* are highly expressed in the electric organ of Torpedo and may catalyze tyrosine phosphorylation of the AChR.

PZ 321 IL6, CNTF, LIF AND ONCOSTATIN M INDUCE VIPmRNA THROUGH SIMILAR MOLECULAR MECHANISMS, Aviva Symes and J. Stephen Fink, *Molecular Neurobiology Laboratory, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Boston, 02114.*

Ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OM) and interleukin 6 (IL6) may represent members of a distinct cytokine family. This proposal is based on their similar protein structure, shared receptor subunits and overlapping functions. We have previously demonstrated that CNTF, LIF and OM can all induce VIP mRNA expression in a human neuroblastoma cell line, NBFL, and that these inductions are mediated through the same 180bp region in the VIP promoter, the cytokine response element (CyRE). This CyRE is both necessary and sufficient to mediate the response to all three cytokines. The signal transducing subunit of the IL6 receptor, gp130, has been implicated in mediating the response to CNTF, LIF and OM yet it is unclear whether the signal transduction pathways utilized by these cytokines are the same as those used by IL6. Indeed IL6 treatment of NBFL cells does not induce VIP mRNA. We present data showing that treatment of NBFL cells with both IL-6 and soluble IL-6 receptor induces VIP mRNA and that this induction is mediated through the CyRE. These data suggest that the IL6-binding receptor subunit is absent in these cells but that when the IL6 response is reconstituted with a soluble receptor, IL6 utilizes similar signal transducing pathways to those employed by CNTF, LIF and OM.

As the transcription factor, c/ebpβ, has been shown to be involved in mediating the IL6 response of acute phase genes in hepatoma cells we investigated its role in the induction of the VIP gene in response to CNTF, LIF and OM. Through gel shift and footprint analyses we show that members of the c/ebp family can bind to the 180bp CyRE, and cotransfection experiments indicate that c/ebp family members are able to transactivate the CyRE. These results suggest that c/ebp proteins may be involved in the transcriptional activation of the VIP gene by CNTF, LIF and OM.

PZ 322 CHARACTERIZATION OF POSITIVE AND NEGATIVE REGULATORY ELEMENTS WHICH CONTROL NGF-INDUCED PERIPHERIN GENE EXPRESSION, Mary Ann Thompson, Allen Adams, Donna Choate, and Todd Patton, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

We are studying the transcriptional regulation of the peripherin gene, which is a late response gene induced by NGF in PC12 cells. The peripherin gene, encoding a neuronal-specific intermediate filament protein, is induced when PC12 cells begin to terminally differentiate into neurons. Previously, we have defined three regulatory elements in the peripherin promoter: a distal positive element, a proximal positive element, and a negative regulatory element which in part prevents peripherin expression in undifferentiated and non-neuronal cells.

By 5' and 3' fine deletion mapping of the distal positive region, we have defined a 26 bp element which is necessary for full responsiveness to NGF. In transient transfections of peripherin-CAT constructs into untreated or NGF-treated PC12 cells, deletion of this element reduces the level of induction of the reporter gene by NGF from 7-fold down to 2-fold. This NGF- responsive element, which is greater than 2300 bp upstream of the transcriptional start site, contains an inverted repeat sequence. DNA mobility shift assays are underway to determine whether proteins unique to differentiated PC12 cells bind to this element.

By DNA mobility shift assay, we have previously demonstrated specific protein complex formation at the negative regulatory element (NRE). Although these assays indicate a change in the complex formed at the NRE between undifferentiated and differentiated PC12 cell extract, laser UV-crosslinking experiments indicate identity of proteins which actually contact the DNA. As a first step in purification of repressor proteins, undifferentiated PC12 cell nuclear extracts have been fractionated by elution from a heparin-agarose column with a linear KCl gradient. When fractions are analyzed by the DNA mobility shift assay, combination of two separate fractions is necessary to reconstitute the specific DNA-protein complex. One of these two fractions has no DNA-binding activity alone, whereas the second has a weak and non-specific DNA binding activity. This result confirms that at least two proteins must interact to form the putative repressor complex at the NRE.

PZ 323 AGONIST-INDEPENDENT ACTIVATION OF ADENYLYL CYCLASE BY THE D_{1B} DOPAMINE RECEPTOR SUBTYPE, Mario Tiberi, Keith R. Jarvie, Susanna Cotecchia and Marc G. Caron. HHMI Labs, Department of Cell Biology, Duke University, Durham, NC 27710

D_1 dopamine receptor subtypes namely D_{1A} and D_{1B} belong to the superfamily of G-protein coupled receptors. It has been shown that these two receptor subtypes are coupled to the activation of adenylyl cyclase. Sequence comparison within the third intracellular loop and the carboxy tail, regions involved in the coupling of receptors with the G-proteins, reveal the highest degree of divergence between the two receptors. This likely suggests differences in the coupling to G-proteins and activation of adenylyl cyclase. The present study was undertaken to examine possible differences in the coupling properties between D_{1A} and D_{1B} receptor subtypes. Binding studies performed in human kidney embryonic cells (293) membranes expressing either D_{1A} or D_{1B} receptor revealed differences in the binding profile of agonists and antagonists. Indeed, agonists displayed a higher affinity at the D_{1B} receptor than at the D_{1A} subtype. On the other hand, antagonists had a lower affinity at the D_{1B} subtype when compared to the D_{1A} receptor subtype. Basal levels of cAMP in 293 cells expressing the D_{1B} receptor were about 2-3 fold higher than the basal levels of cAMP obtained by the expression of the D_{1A} receptor subtype. Activation of D_{1B} receptor by agonists gave a lesser maximal stimulation (Vmax) of adenylyl cyclase than the activation of the D_{1A} receptor. Dose-response curves performed in whole cells expressing the D_{1B} receptor revealed an increased potency of agonists for stimulation of adenylyl cyclase when compared to cells expressing the D_{1A} receptor. These properties are reminescent of those of constitutively active G-protein coupled receptors obtained by site-directed mutations (Cotecchia et al., PNAS 87, 2896, 1990). Preliminary experiments with chimeric D_{1A}/D_{1B} receptors suggest that part of the constitutive activity and increased affinity for agonists might reside in sequences of the third intracellular loop.

PZ 324 DISINHIBITION OF NEURONAL CALCIUM CHANNELS DURING WHOLE-CELL PATCH-CLAMP RECORDING

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The barium current flowing through voltage-dependent calcium channels was recorded from cortical neurons using the whole-cell configuration of the patch-clamp technique. Neurons were obtained from 18 days old fetal rats and cultured for 2 to 3 weeks on astrocyte monolayers.

The current evoked by depolarizing voltage pulses from -80 mV to 0 mV (duration 0.5 s) was divided into an inactivating and a non-inactivating component. In most cells (48 of 62) the inactivating component increased from a low amplitude (< 0.1 nA) to an average value of 1.0 nA over the first few minutes of whole-cell recording. The non-inactivating component (mean amplitude 0.5 nA) remained unchanged or was subject to a slow "run-down".

The increase of the inactivating component was prevented when the "perforated patch technique" (Horn & Marty, 1989, J. Gen. Physiol. 92: 145) was used suggesting that some intracellular factor inhibiting the calcium channels was lost during the conventional whole-cell experiments. When GTP (0. 5 mM) or GTP-γ-S (0.1 mM) were added to the pipette solution no or only a weak rise of the inactivating current was seen (final amplitude 0.4 nA), whereas GDP-β-S (0.5 mM) accelerated and enhanced its increase (to 1.6 nA).

The results suggest that part of the calcium channels of cultured cortical neurons is inhibited by a G-protein even in absence of any added neurotransmitter and that the current increase observed during whole-cell recording is due to the loss of intracellular GTP.

PZ 325 MULTIPLE MECHANISMS OF COUP-TF-DEPENDENT REPRESSION OF STEROID RECEPTOR TRANSACTIVATION.

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COUP-TF is a member of the steroid/thyroid hormone receptor superfamily. Two members, COUP-TF1 and COUP-TF2 have been identified which bind a wide variety of A/GGGTCA repeats with different spacings between the half sites. Recently we and others have demonstrated that COUP-TF is able to repress hormonal induction of target genes by Vitamin D receptor (VDR), Thyroid hormone receptor (TR) and retinoic acid receptor (RAR). This repression is dose-dependent on COUP-TF. Inhibition of VDR, TR, and RAR activities occurs through natural physiological response elements found in the osteocalcin, myosin heavy chain, and βRAR promoters, respectively. Here we show that the mechanisms of repression by COUP-TF does not involve the formation of detectable functionally inactive heterodimers between COUP-TF and VDR, TR, and RAR. Instead, we show that the mechanism of repression could occur at three different levels : active silencing of transcription, and dual competition for, (a), occupancy of DNA binding sites, and (b), heterodimer formation with RXR, the coregulator of VDR, TR, and RAR. In addition, COUP-TF is expressed in various regions of the brain during mouse development.

PZ 326 INDUCTIONS OF TRE- AND CRE-BINDING ACTIVITIES IN CULTURED MOUSE CEREBELLAR GRANULE CELLS STIMULATED VIA GLUTAMATE RECEPTORS.

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Using nuclear mini-extracts prepared from cultured cerebellar granule cells in a gel-shift assay, we found that exogenous NMDA (N-methyl-D-aspartate) or kainate increased TRE (TPA responsive element)- and CRE (cyclic-AMP responsive element)-binding activities specifically through NMDA or non-NMDA receptors. The increase of TRE-binding activity appeared to be caused by c-fos induction followed by de novo synthesis of c-Fos protein. The induction of TRE-binding activity by NMDA or kainate was triggered by influx of extracellular Ca^{2+} into the cells. The dose-dependencies of increase of TRE-binding activity and Ca^{2+} uptake into the cells on stimulation with NMDA or kainate showed good coincidence. Activation of protein kinase C could contribute to these increases of TRE- and CRE-binding activities. Competition and proteolytic bandshift experiments revealed that the increases in TRE- and CRE-binding activities were both mediated by the same DNA-binding complexes whose binding affinities were higher for CRE than for TRE. Super-gel shift assays with anti-c-Fos antibody revealed that the DNA-binding complexes formed on CRE as well as on TRE involved c-Fos or Fos-related proteins. Thus, stimulation of cerebellar granule cells with NMDA or kainate induced TRE-binding activity cross-binding to CRE. Recently, we found that intraperitoneal administration of NMDA or kainate to mice caused increases of both TRE- and CRE-binding activities in the CA1, CA3 and dentate gyrus of the hippocampus associated with seizures. We are now characterizing these DNA-binding activities expressed in mouse brain and investigating their relationships with the expressions of some genes that might be regulated by activation of glutamate receptors.

PZ 327 ACTIVATION OF C-MYC AND C-FOS BY EGF IN MITOTIC VERSUS POSTMITOTIC NEURONAL H19-7 CELLS.

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Epidermal growth factor (EGF) functions in a bimodal capacity in the nervous system, acting as a mitogen in neuronal stem cells and a neurotrophic factor in differentiated adult neurons. In order to understand how the EGF signalling pathways lead to such different endpoints, we utilized a hippocampal cell line, H19-7. This neuronal cell line is mitogenically responsive to EGF only before differentiation, despite an increase in EGF binding after differentiation. Initially, we investigated an early event in the EGF signalling pathway, the activation of MAP kinase. MAP kinase activity was induced by EGF in H19-7 independent of differentiation state. To investigate where the signalling pathways diverge, we looked at c-*myc* and c-*fos* proto-oncogene induction by EGF. Both c-*myc* and c-*fos* transcription were induced before and after differentiation of H19-7 cells. These results indicate that neither MAP kinase activation nor c-*fos* and c-*myc* induction are sufficient for EGF-stimulated mitogenesis in H19-7 cells.

PZ 328 TRANSGENIC STUDIES OF MYELINATION: THE ROLE OF *SCIP* AS A REGULATORY SWITCH, David E. Weinstein and Greg Lemke, Department of Molecular Neurobiology, The Salk Institute for Biological Studies, La Jolla, CA 92037.

The myelin-specific structural genes encoding myelin basic protein, proteolipid protein, protein zero (P_0) and myelin associated glycoprotein, are expressed exclusively in the myelinating cells of the central and peripheral nervous systems, and are under tight developmental control. SCIP (suppressed cAMP inducible POU), a *trans*-acting transcriptional regulator expressed predominantly in oligodendrocyte and Schwann cell progenitors has recently been identified and cloned in Dr. Greg Lemke's laboratory. In cultured Schwann cells, SCIP is exquisitely and rapidly sensitive to increased intracellular cAMP levels. Co-transfections of SCIP and the P_0 promoter driving a reporter CAT gene strongly suggest that SCIP represses P_0 activated CAT expression in cultured Schwann cells. As is the case with cultured Schwann cells, during development, SCIP expression also precedes the expression of the myelin genes. However, unlike cultured Schwann cells, SCIP expression falls by ~90% within 21 days after birth, whereas transcription of the myelin genes remains elevated. In sciatic nerve transection experiments, SCIP is rapidly and transiently reinduced and the myelin genes are transiently turned off, suggesting a reciprocal pattern of expression consistent with the expression in dividing progenitors. To study the role of SCIP in the myelination and proliferation in the peripheral nervous system we have made transgenic mice that express a dominant- negative form of the gene under the transcriptional regulation of the P_0 promoter. This form of the SCIP protein binds cognate DNA but fails to act as a transcriptional regulator. Founder animals demonstrate a phenotype consistent with a defect in peripheral myelination. These animals are currently being bred to homozygosity and lines generated to further study the role of SCIP *in vivo*.

PZ 329 CONVERGENT MODULATION OF NEURONAL NA^+ CHANNELS BY PROTEIN KINASE C AND cAMP-DEPENDENT PROTEIN KINASE, James W. West*, Randal Numann*, Ming Li*, Raymond D. Smith#, Alan L. Goldin#, Todd Scheuer*, and William A. Catterall*. *Department of Pharmacology, University of Washington, Seattle, WA 98115, #Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717

Phosphorylation of the rat brain Na^+ channel by protein kinase C (PKC) or cAMP-dependent protein kinase (cA-PK) leads to modulation of its activity. Phosphorylation of the large α subunit by PKC causes slowing of inactivation and decreases peak Na^+ current. Phosphorylation of serine 1506 is required for both effects, however the two effects can be resolved in a dose dependent manner in that activation of PKC with low concentrations of 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) leads to slowing of inactivation with no change in peak current while higher concentrations of OAG lead to both slowing and reduction in peak current. These results suggest the involvement of at least two phosphorylation sites for modulation by PKC. We show here that a phosphorylation site within the intracellular loop between homologous domains I and II of the Na^+ channel α subunit is required for the reduction of peak current but not for slowing inactivation. Phosphorylation of serine 1506 causes slowing of inactivation and also is required for reduction of peak current. In contrast, phosphorylation of the Na^+ channel α subunit by cAMP-dependent protein kinase (cA-PK) results in only a reduction in peak current. Although phosphorylation by cA-PK is restricted to five sites within the intracellular loop between domains I and II and serine 1506 is not phosphorylated by cA-PK in biochemical experiments, mutation of serine 1506 to alanine blocks the reduction in activity caused by cA-PK phosphorylation indicating that phosphorylation of this residue by PKC is required for cA-PK regulation. The PKC phosphorylation site centered at serine 1506 was converted to a consensus cA-PK phosphorylation site by mutation of lysine 1507 and lysine 1508 to glutamine. In contrast to wild type Na^+ channel, cA-PK caused both slowing and strong peak current reduction. These results are consistent with the conclusion that phosphorylation of serine 1506 by PKC causes slowing of inactivation and regulates the reduction of Na^+ channel activity by both PKC and cA-PK.

PZ 330 NEUROTROPIN GENE EXPRESSION BY CELL LINES DERIVED FROM, HUMAN GLIOMAS. Manfred Westphal, Wolfgang Hamel, Eva Szoyi, Enrique Escandon and Karoly Nicolics. Department of Neuroscience, Genentech Inc., South San Francisco CA 94080, and Department of Neurological Surgery, University Hospital Eppendorf, Hamburg, Germany.

Glial cells and neurons show a profound interaction in the developing nervous system. The interaction is mediated via molecules of the extracellular matrix and soluble compounds like growth factors and neurotropins. In the present study, the production of neurotrophic factors by glioma cells was studied in 24 permanent glioma cell lines by Northern analysis. Nearly all cell lines expressed BDNF, and about 2/3 of the cell lines expressed NGF and NT3. 50% of the cell lines expressed all three neurotropins. In addition, active secretion od NGF was detected into the medium of several of the cell lines. In contrast to these findings, no expression of trk or trkB was found in any of the cell lines by gene expression analysis and receptor crosslinking. Consequently, no effect was seen by the neurotropins on stimulation of cell proliferation.

It is concluded, that gliomas constitutively secrete neurotrophic factors because neuronal support is one of the main physiological functions of glial cells and this is maintained in the transformed state. The absence of functioning receptors is a clear indication that the secretion of neurotropins does not serve an autocrine purpose as the secretion of other growth factors such as PDGF.

PZ 331 TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF NPY EXPRESSION IN PC12 CELLS. J.D. White, G.L. Yount, G. Sanacora, R.A. Maikis and P. Camp, Div. Endocrinology, SUNY Stony Brook, Stony Brook, NY 11794

Neuropeptide Y (NPY) is a 36 amino acid peptide that is expressed widely in central and peripheral neurons, in chromaffin cells of the adrenal medulla and in the rat pheochromocytoma cell line PC12. PreproNPY gene expression in PC12 cells, as measured by preproNPY mRNA content, can be increased by treatment with nerve growth factor (NGF), cAMP analogs or phorbol ester and at least some of this increase in preproNPY mRNA content is transcriptionally mediated. In the present studies we investigated NPY gene regulation by two tyrosine kinase-linked receptor systems: NGF and insulin. In the first series of studies, plasmids were constructed containing regions -702 to + 110 of the rat NPY gene fused to rabbit β-globin, as a reporter gene, and transfected into PC12 cells by electroporation. In the first construct, NGF treatment was found to increase β-globin mRNA levels by 2.5 fold, while endogenous preproNPY mRNA levels rose 6-fold. An 81 basepair deletion mutant, from positions -176 to -95, was not inducible by NGF treatment and, in fact, NGF treatment was found to decrease reporter gene expression. In the third construct, sequences -103 to -97 (TGACTGC) were specifically mutated. This construct was completely unresponsive to NGF treatment, suggesting that these bases were required for NGF-induction of preproNPY gene expression. In contradistinction to NGF-signalling, insulin treatment reduced preproNPY mRNA levels. This reduction was relatively rapid (within 4 hr) and did not appear to involve *transcriptional* mechanisms. Measurement of preproNPY mRNA turnover, in experiments using actinomycin D, revealed that insulin treatment led to a significant increase in preproNPY mRNA turnover. These data demonstrate that NPY expression in PC12 cells is differentially regulated by tyrosine kinase linked receptors and that this regulation is expressed at both the transcriptional and post-transcriptional levels.

PZ 332 ACTIVATION OF THE GLUTAMINE SYNTHETASE GENE IN EMBRYONIC RETINA BY PROTEIN KINASE A, Anthony Young, Haiying Zhang, and Yi-Chen Li, College of Pharmacy, and Ohio State Biotechnology Center, Ohio State University, Columbus, OH 43210

Despite the presence of glucocorticoid receptors throughout development, the glutamine synthetase (GS) gene does not become hormonally responsive in the chicken retina until embryonic day 8 (E8). Prior to hatching at day 21, GS begins a sharp constitutive rise that plateaus at a level several hundred fold higher than that observed in early embryonic tissue.

Glucocorticoids produce a 5 fold increase in CAT activity after electroporation of a GS-CAT fusion gene into intact E10 retina. However, when E51/2 retina are utilized as the recipient tissue, only a 1.3 fold induction is observed. If an expression vector clone encoding protein kinase A (PKA) is cotransfected with the GS-CAT fusion gene into E51/2 retina, glucocorticoid responsiveness increases dramatically. A similar increase in hormonal responsiveness is observed after transfection of E51/2 retina with the GS-CAT fusion gene alone, followed by treatment with forskolin, a direct activator of adenyl cyclase. Forskolin treatment will also render pG46TCO glucocorticoid inducible in E51/2 retina. This plasmid contains the CAT reporter gene under transcriptional control by the thymidine kinase promoter and glucocorticoid response elements (GREs) derived from the mouse mammary tumor virus LTR. Based on these results, we postulate that E51/2 retina contain dormant glucocorticoid receptors whose signaling properties are liberated by PKA.

PKA has a different effect on expression of a GS-CAT fusion gene in transfected primary cultures of Müller glial derived "flat cells" Here, cotransfection with PKA or forskolin treatment produces a 400-fold increase in transient expression of the GS-CAT fusion gene in the absence of glucocorticoids. Promoter "bashing" studies indicate that a negative element flanked by two distinct positive regulators mediate this effect. The distal enhancer has been partially characterized and shown to also mediate the glucocorticoid response and contain a GRE juxtaposed to a CRE. The proximal enhancer has not yet been identified but sequence analysis does not reveal a conventional CRE. Additional "bashing" studies are in progress. Since PKA produces dramatically different effects on expression of a GS-CAT fusion gene in intact E5.5 retina and in primary cultures of Müller glial derived "flat cells", it would appear that "flat cell" specific factors are required to mediate the direct induction by PKA. If so, the effect of PKA on expression of the GS-CAT fusion gene in flat cells might prove to be of profound relevance to the constitutive activation of GS transcription during retinal development.

PZ 333 EXPRESSION OF $G_{olf}\alpha$ AND $G_{t2}\alpha$ IN PANCREATIC ISLETS, Jeffrey M. Zigman, Gunilla T. Westermark, Joseph LaMendola, Esper Boel and Donald F. Steiner, The Howard Hughes Medical Institute and the Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637 and Novo Research, Denmark

The insulin-producing pancreatic ß-cell is a chemosensory cell which possesses a number of attributes characteristic of neurons. Because novel G-proteins have been found in visual, olfactory and gustatory cells, it is of interest to explore the possibility of a novel G-protein in the ß-cell. Although we have not been able to detect a novel G-protein in the ß-cell, we have found some G-proteins previously thought to have very limited patterns of expression. Here we report evidence suggesting that the ß-cell expresses $G_{olf}\alpha$, a G-protein hitherto believed to mediate signal transduction mechanisms exclusively within the olfactory sensory neuron, and $G_{t2}\alpha$, a G-protein previously thought to be expressed solely in retinal cone cells, where it mediates visual signal transduction. These findings suggest roles for $G_{olf}\alpha$ and $G_{t2}\alpha$ outside of the olfactory neuroepithelium and retina, respectively. We propose models for their roles in the pancreatic ß-cell.

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PZ 334 **AUTOCRINE REGULATION OF NEUROTROPHIN SECRETION IN SCHWANN CELLS.** Astrid Zimmermann♋, Arne Sutter♍and Ulrich Stephani♌, ♋Zoologisches Institut der THD, Schnittspahnstr.3, 61 Darmstadt, FRG; ♍Neuroimmunology Group (Immunepharmacology) E.MERCK Frankfurter Str. 250, 61 Darmstadt FRG, ♌Klinik für Neuropädiatrie, Schwanenweg 20, 23 Kiel, FRG,

Schwann cells purified from sensory ganglia of embryonic chicken were cultured in vitro. In such cultures the fibroblast contamination was 1% after one day as judged by anti-fibronectin, E1 (SGIII-1) and D2 (SGII-2) immunofluorescence and NGF-receptor autoradiography using ^{125}I-NGF. Secretion of neurotrophic factors by these Schwann cell cultures was assayed, quantified and characterized after 3 days by adding the Schwann cell culture supernatants to purified sensory neurons (>95% pure) in the presence or absence of anti-NGFantibodies. It was found that Schwann cells display only a low secretory activity for neurite promoting/neurotrophic factors which were not or only to a very small degree inhibitable by anti-NGF antibodies. If, however, NGF was added to the Schwann cell cultures, NGF (neurotrophin) secretion was greatly increased. Neurotrophic activity inhibitable by anti-NGF antibodies in the Schwann cell cultures was up to 50 fold higher than that of the NGF added initially. Surprisingly, in view of the present knowledge on NGF receptor representation on Schwann cells, NGF excerted its autocrine effects already at $2x10^{-12}$M, suggesting the presence of high affinity binding sites for NGF on Schwann cells.

PZ 335 AXONAL RECEPTOR-LINKED PROTEIN TYROSINE PHOSPHATASES IN THE EMBRYONIC DROSOPHILA CENTRAL NERVOUS SYSTEM. Kai Zinn, Shin-Shay Tian, Chand Desai, Bruce Hamilton, and Sarah Fashena. Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Receptor-linked protein tyrosine phosphatases (PTPases) often contain adhesion molecule-like extracellular domains, and may thus couple cell recognition to signal transduction *via* control of tyrosine phosphorylation. We previously showed that three adhesion molecule-like PTPases are selectively expressed on CNS axons during the period of axon outgrowth (Tian et al. (1991), Cell 67, 675-685). We have now found that another *Drosophila* PTPase, DPTP69D, is also expressed primarily on CNS axons. DPTP69D contains two immunoglobulin-like domains and two fibronectin type III (FN) domains. To define the roles these PTPases play in neural development, we are making mutations in the genes encoding them. We have developed a 'local transposition' method for isolating insertions of P-element transposons at specific locations, and have been able to use this method to make a mutation in the DPTP99A gene. We are currently characterizing the phenotype of this mutation. We have shown that DPTP99A and DPTP10D do not act as homophilic adhesion molecules in transfected tissue culture cells, suggesting that if they are involved in cell recognition they must recognize other cell surface ligands. In preliminary experiments, we have also identified proteins that may be substrates for one of the PTPases.

Late Abstracts

PREFERENTIAL INTERACTIONS BETWEEN D2 DOPAMINE RECEPTORS AND G-PROTEINS.

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The D2 dopamine receptor, an inhibitor of adenylyl cyclase, belongs to the family of seven transmembrane domain G-protein coupled receptors. This receptor is encoded by two mRNAs produced from the same gene by alternative splicing, here referred to as D2L and D2S. The resultant proteins are identical except for an insertion of 29 amino acids (aa) in the putative third intracytoplasmic domain. This domain has been shown to be important for the coupling of this family of receptors to G-proteins. We have previously shown, that there is differential inhibition of the adenylyl cyclase activity when these two receptors are produced in JEG3 cells; D2S being more efficient than D2L. In order to understand the molecular basis of such differential activity, we analyzed the G-proteins expressed in these cells. We show that Gai2 is absent in this cell line. Moreover, it is possible to restore the same inhibitory activity obtained by D2S, when an expression vector encoding this a-subunit is cotransfected with D2L. In addition, transfections of the two receptors in a recipient cell line containing the three Gai-subtypes confirm that the two receptors behave similarly. We conclude that the 29 aa insertion present in D2L allows it to interact specifically with Gai2. These data suggest that, in vivo, the function of activated D2 receptors is exerted by specific interactions with Gi-proteins subtypes.

mRNA FOR MURINE SEROTONIN 5HT3 RECEPTOR: EVIDENCE FOR ALTERNATIVE SPLICING AND IMPLICATIONS FOR RECEPTOR FUNCTION, E.H. Kawashima#, F.Boess*, P.Werner# and K.A.Jones#, Glaxo Institute for Molecular Biology#, Geneva, CH1228 Switzerland and Department of Pharmacology*, Univ. of Alberta, Edmonton, Canada T6G 2H7

cDNAs for the murine 5HT3 receptor were isolated from a library made from the NG108-15 murine neuroblastoma X rat glioma cell line. Upon analysis of more than 80 hybridizing clones, it was found that in addition to the original 5HT3a cDNA reported by Maricq et al (Science, v.254, p.432, 1991), a second cDNA was isolated which was identical except for a specific deletion of 6 amino acids within the second intracellular loop. Sequence and PCR analysis of the isolated 5HT3 cDNAs show that approximately 80% are missing these 6 amino acids, indicating that perhaps a specific splicing event is taking place which synthesizes and incorporates one full length 5HT3 subunit and 4 subunits deleted in these 6 amino acids in the putative pentameric receptor complex. The deleted peptide sequence is predicted to contain a site for phosphorylation by casein kinase II. Analysis of mRNA from brain stem and cortex by reverse transcriptase and PCR reveals that the majority of mRNA transcripts encode 5HT3 receptors which delete these 6 amino acids. Electrophysiological studies using the wild-type and deleted forms of receptor cDNA transiently expressed in X.laevis oocytes and HEK-293 cells reveal receptor characteristics similiar to those observed in the NG108-15 cell line. Functional implications for 5HT3 receptor responses will be discussed.

DIFFERENTIAL INDUCTION OF EARLY GENES IN THE CNS BY CLOZAPINE AND DOPAMINE D2 RECEPTOR ANTAGONISTS, Patrick Rogue, Anant N. Malviya and Guy Vincendon, L.N.M.I.C. (UPR 416 CNRS), Centre de Neurochimie, 5 rue Blaise Pascal, Strasbourg 67084 France.

Dopamine D_2 receptors regulate the expression of a specific set of immediate early genes (IEG) in the rat striatum. A single injection I.P. of haloperidol (2 mg/kg) or sulpiride (100 mg/kg) produces a rapid and transient increase in c-*fos*, c-*jun*, *jun* B and zif268 mRNA, but has no influence on the expression of ETR1 or *jun* D (Brain Res Bull 29, 469). These inductions are specifically blocked by pretreatment with a D_2 agonist (1 mg/kg quinelorane). We have further studied the effect of clozapine and dopamine D_2 receptor antagonists on IEG expression in different regions of the CNS by northern analysis and ISH. Both clozapine (20 mg/kg) and haloperidol (2 mg/kg) induce zif268, c-*fos*, and *jun* B in the nucleus accumbens. However, only haloperidol induces all of these proto-oncogenes in the striatum, whereas in the frontal cortex clozapine induces c-fos but not zif268. The effects of the prolonged administration of these compounds will also be presented. The significance of these specific IEG activation patterns will be discussed with respect to the mechanism of action of antipsychotics and to the induction of dopamine D_2 receptor gene expression upon prolonged treatment with antagonists (European J Pharmacol 207, 165).

RAT HOMOLOGS OF THE DROSOPHILA NEUROGENIC LOCUS ENHANCER OF SPLIT.
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Mammalian homologs of the transducin like Drosophila neurogenic locus Enhancer of split have been isolated from a rat hippocampal cDNA library. One clone, R-espl, encodes a protein of 196 amino acids that is highly similar to the amino terminal region of the Drosophila Enhancer of split protein. However, the encoded protein lacks the carboxy terminal domain which is similar to the transducin guanine nucleotide binding protein β subunit. The 1.3 kB R-espl cDNA clone hybridizes with a 1.3 kB mRNA, indicating that this cDNA is full length. Northern blot analysis revealed expression of R-espl in adult heart, brain, kidney and lungs. Antibodies have been raised against R-espl which immunoprecipitate a 24 kDA. protein when R-espl is translated in vitro in the rabbit reticulocyte lysate. These antibodies also precipitate a 24 kDa protein from [^{35}S]-methionine labeled PC-12 cells. Finally, we have also isolated a second clone, R-esp2, which encodes a 741 amino acid protein and contains the transducin homology region.