MOLECULAR BIOLOGY OF NEUROTRANSMITTERS AND THEIR RECEPTORS

Organizers: Robert Steiner, Huda Akil and Stanley Watson
April 17-23, 1990

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Molecular Biology of Neurotransmitters and their Receptors

Stress

CP 001 MOLECULAR REGULATION OF THE STRESS AXIS, Huda Akil and Stanley J. Watson, Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109. The mammalian stress axis includes brain, pituitary and adrenal components which are well orchestrated in producing an optimal response when the organism is challenged. This talk will focus on integrating molecular mechanisms in the context of the physiological system of stress responsiveness. The expression, brain localization and regulation of multiple gene products including CRH, vasopressin and glucocorticoid receptors will be described. The role of multiple types of feedback mechanisms (genomic and non-genomic) on gene expression of CRH and POMC will be covered. Thus, the role of several regulatory mechanisms including control of secretion, biosynthesis, peptide processing and mRNA will be described in the context of brain circuits. Finally, the regulation of this system by circadian rhythms and by aging will be used to exemplify more subtle regulatory controls.

CP 002 SIGNAL TRANSDUCTION BY THE GLUCOCORTICOID RECEPTOR. M.J. Garabedian, D. Picard, M. Schena and K.R. Yamamoto, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448.

The glucocorticoid receptor protein interacts selectively with a series of ligands via a large (~250 aa) C-terminal hormone binding domain. Ligands with hormonal activity (agonists) trigger, at moderate temperatures, receptor "transformation", which results in nuclear localization, specific DNA binding and transcriptional regulation by the hormone-receptor complex; antagonists are tightly bound but do not effect transformation. To investigate this signal transduction pathway, we are exploiting the capacity of the rat glucocorticoid receptor to function in yeast. We found that the relative activities of various ligands are strikingly different between mammalian cells and yeast; certain antagonists, for example, display agonist activity. These differences do not merely reflect interspecies distinctions in ligand permeability or metabolism. Thus, components in addition to the receptor and ligand must also act as determinants of ligand efficacy. Genetic experiments in yeast are consistent with the idea that the heat shock protein hsp90 participates in the mechanism of signal transduction by steroid receptors, although expression of mammalian hsp90 in yeast is not alone sufficient to generate the mammalian ligand pattern. In a second approach, we have screened in yeast a large number of point mutations within the steroid binding domain, and have discovered a class of mutants with novel spectra of ligand efficacy. The mutations cluster within discrete subregions of the hormone binding domain; some may define receptor-ligand contacts, and others may correspond to interactions between the receptor and other factors. This novel combination of genetics and pharmacology is revealing new insights into the structure and activity of the hormone binding domain.

Molecular Biology of Neurotransmitters and their Receptors

CP 003 TRANSCRIPTIONAL REGULATION OF CORTICOTROPIN-RELEASING HORMONE GENE EXPRESSION, Audrey F. Seasholtz, Mental Health Research Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109. Corticotropin-releasing hormone (CRH) is the major hypothalamic releasing factor in the mammalian stress response. CRH stimulates the synthesis and secretion of ACTH from the anterior pituitary which in turn stimulates the production and release of glucocorticoids from the adrenal cortex. The regulation of CRH expression in the paraventricular nucleus of the hypothalamus is most complex since these CRH-producing cells receive inputs from many different regions of the brain and these inputs must be integrated at the hypothalamic neurosecretory cell to control synthesis and release of CRH. Its secretion is regulated by catecholamines, opioid peptides, acetylcholine, serotonin, glucocorticoids, angiotensin II, and interleukin I, but the effects of most of these compounds on CRH transcription is unknown. In order to more systematically evaluate the role and mode of action of many of these potential transcriptional regulatory agents on CRH expression, we have utilized CRH gene transfer experiments in well characterized cell lines. The regulation of the rat and human CRH genes (or CRH-chloramphenicol acetyltransferase (CRHCAT) fusion genes) by cAMP, glucocorticoids, and several other regulatory compounds in various cell lines will be described. The glucocorticoid regulation of the CRHCAT fusion constructs is particularly interesting since both positive and negative glucocorticoid regulation have been observed in different cell lines. The localization of these cis-acting transcriptional control elements and analyses of the proteins which interact with these sequences and with one another in different cells and tissues will help us to better understand the unique promoter specificity and regulation of this important gene, not only in the hypothalamus but also in other regions of the brain and periphery where CRH may mediate other aspects of the mammalian stress response.

Growth, Development, and Reproduction

CP 004 THE OXYTOCIN SYSTEM OF THE HYPOTHALAMUS: TOPOGRAPHY OF REGULATION OF PEPTIDE AND RECEPTORS BY ESTRADIOL AND PROGESTERONE IN FEMALE RATS, Bruce S. McEwen, Hector Coirini, Maya Frankfurt and Michael Schumacher, Laboratory of Neuroendocrinology, Rockefeller University, 1230 York Avenue, New York, N.Y. 10021. The induction of mating behavior in the female rat is a coordinated activity by two ovarian hormones, estradiol (E) and progesterone (P) acting upon the ventromedial nuclei of the hypothalamus (VMN). The actions of E take many hours and involve genomic activation, as demonstrated directly and by the use of RNA and protein synthesis inhibitors. The actions of P are more rapid, within 1 hour, and may involve a combination of genomic and non-genomic mechanisms. E priming induces massive increases in the capacity of VMN neurons to make proteins, and the identification of the induced proteins is an important task. Receptors for P are one of the induced proteins and represent an important link in the synergism of the two steroids. Induction of synaptic plasticity is another event, involving induction of spines on dendrites and increased numbers of synaptic profiles. And the induction of receptors for oxytocin is another important consequence of E actions in the VMN. Oxytocin receptor induction parallels in dose and timecourse the induction of progestin receptors, but the receptors are only evident initially in the ventrolateral VMN where there is no evidence of oxytocin immunoreactive nerve fibers. E treatment causes oxytocin fibers to become immunoreactive in the region lateral to the VMN. With the passage of time after E treatment, the induced field of oxytocin receptors spreads beyond the boundaries of the VMN in a lateral direction and then appears to stop. This may involve transport of receptors along the dendrites of VMN neurons. However, the application of P causes a rapid additional spread of the oxytocin receptor field in a lateral and dorsal direction. These changes occur within 30' of P application. Because they can be mimicked by P application in vitro prior to oxytocin receptor binding, it is likely that P effects are at the membrane level and do not involve genomic activation. These rapid actions of P may represent either an activation of pre-existing oxytocin receptors to a high affinity state or further stimulation of rapid dendritic transport. The physiological significance of these changes in oxytocin receptor spread is indicated by two principal observations: 1) P treatment after E priming is required to allow intracranial oxytocin to actiAviate female sexual behavior; 2) Intracranial oxytocin application is only effective in facilitating sexual behavior when infused at the more caudal VMN where we have also seen the spread of oxytocin receptors induced by E+P treatment. More rostral VMN shows no evidence of the spread of oxytocin receptors beyond the boundaries of the VMN itself. Taken together these observations suggest that the VMN is an enormously plastic brain region in which the coordinated actions of E and P orchestrate reversible morphological and neurochemical changes which represent the substrate by which reproductive behavior is facilitated in temporal coordination with the signal for ovulation. Supported by NIH Grant NS07080 and by fellowship support from the NIH Fogarty program.

Molecular Biology of Neurotransmitters and their Receptors

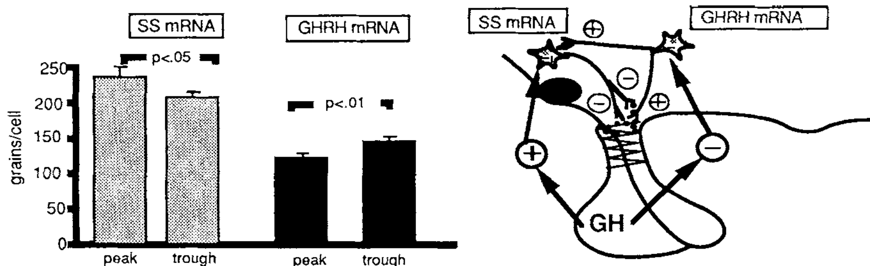
Introduction into transgenic mice of hybrid genes which express the oncogene, SV40 T-antigen, from the regulatory regions of the human α and ß subunits of luteinizing hormone or the rat GnRH gene, produces tumors of the pituitary gonadotrope and the GnRH-secreting neurons of the hypothalamus, respectively. From these tumors, we have derived immortalized, differentiated cell lines representing steps in the developmental lineage of the pituitary gonadotrope and the hypothalamic GnRH-secreting neuron.

In the pituitary, targeting of oncogene expression by the regulatory region of the α-subunit gene produces tumors and cell lines which express only the endogenous mouse α-subunit mRNA, while targeting expression of the oncogene with the regulatory region of the ß-subunit gene produces tumors which express not only the ß LH mRNA but also the α and ß FSH mRNAs. Cells derived from these tumors respond to the releasing hormone GnRH and process and secrete the hormones. By transfection into these cells, we have determined the regions of the α-subunit gene which confer gonadotrope specificity and GnRH responsiveness. A gonadotrope specific nuclear protein has been identified which binds not only to the α-subunit gene but to the LH ß-subunit gene as well.

The GnRH-secreting neuronal cell lines maintain many of the differentiated characteristics of CNS neurons. They extend neurites, process GnRH and GnRH-associated peptide, and secrete in response to depolarization. Transfections with a hybrid gene containing the GnRH regulatory region have demonstrated that GnRH gene expression is tightly restricted to the GnRH neuronal cell lines; no expression is seen after transfection into glial cells, fibroblasts, or PC12 cells. A tissue-specific enhancer region has been identified which is required for this restricted expression and binds to specific nuclear proteins.

CP 006 ULTRADIAN TRANSCRIPTIONAL OSCILLATIONS IN NEUROTRANSMITTER GENE EXPRESSION AS A MECHANISM SUBSERVING NEUROENDOCRINE PULSE GENERATOR ACTIVITY. R.A. Steiner, P. Zeitler, G. Tannenbaum, and D.K. Clifton; Ob & Gyn and PBio, Univ. of Washington, Seattle, WA 98195, USA, and Dept. Peds, McGill Univ., Montreal, Canada.

Pulsatile neurosecretory rhythms coordinate the processes of growth and reproduction in mammals; however, the cellular mechanisms responsible for generating these low frequency, ultradian oscillations in neuronal activity are unknown. We examined whether a transcriptional component within a network of interacting neurotransmitters may subserve the generation of ultradian neuroendocrine rhythms. In the male rat, pituitary growth hormone (GH) secretion is characterized by a 3.3 h secretory rhythm. This rhythm is governed by the secretory interplay of growth hormone-releasing hormone (GHRH) and somatostatin (SS) from the hypothalamus, with the secretion of each neuropeptide being 180° out-of-phase from the other. We tested the hypothesis that GHRH and SS gene expression vary on an ultradian basis and that, like GH secretion, can be entrained by photoperiod. Male rats were entrained to a regular photoperiod and killed at times associated with presumptive peaks (1100h; n=4) and troughs (1300h; n=4) of the endogenous GH rhythm. GHRH mRNA and SS mRNA were measured by *in situ* hybridization with ^{35}S-labeled cRNA probes in individual neurons in the arcuate and periventricular nuclei, respectively. Cellular mRNA levels (~150 cells/animal) were estimated with a computer-assisted image analysis system. Results show that SS mRNA and GHRH mRNA vary significantly as a function of plasma GH levels and are out-of-phase of one another (figure on left).



Conclusions: The cooperative and reciprocal interplay among GH, SS, and GHRH may involve **rate-limiting transcriptional processes**, which could provide the necessary kinetics and, therefore, a mechanism for generating ultradian neurosecretory events and for governing the frequency of their occurrence (figure on right).

Molecular Biology of Neurotransmitters and their Receptors

Transcription Factors Involved in Tissue Determination and Tissue-Specific Expression (joint)

CP 007 TRANSCRIPTIONAL REGULATION BY FOS AND JUN: ZIPPERS, BASIC REGIONS AND OTHER BITS.
Tom Curran, Cory Abate, Donna R. Cohen, Pascale Macgregor and Frank J. Rauscher III. Department of Molecular Oncology & Virology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.
The leucine zipper motif, which consists of a heptad repeat of leucine residues, defines a class of transcriptional regulators that includes proteins that bind to the AP-1 and CRE regulatory sites. The leucine zipper association involves a parallel interaction of alpha helical regions in a structure similar to the coiled-coil. In the case of Fos and Jun, dimerization results in a juxtaposition of basic regions of each protein adjacent to the zipper that constitute a DNA binding domain. A combination of mutagenesis and domain-swapping analysis has revealed that amino acids outside of the zipper and basic regions also contribute to dimerization and DNA binding. The biochemical properties of Fos and Jun have been investigated using purified proteins overexpressed in *E. coli* and a novel assay has been developed for direct cloning of zipper-containing proteins. These studies have revealed an unexpected level of complexity in the DNA binding and dimerization affinities and specificities of Fos and Jun.

CP 008 TRANSCRIPTIONAL REGULATION OF NEUROPEPTIDE GENES, Jack E. Dixon, Ourania Andrisani and Zheng Zhu, Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907

The cAMP signalling pathway regulates the expression of a number of neuropeptide hormone genes in the mammalian brain and nervous system. The genetic element, named CRE for cAMP responsive element in combination with CRE-specific DNA binding protein(s) mediate the transcriptional events triggered by changes in the intracellular cAMP levels.

A 43 kDa DNA binding protein (CREB) which recognizes the TGACGTCA (CRE) element of the rat somatostatin gene as well as other cAMP regulated genes has been purified from rat brain. The purified protein specifically stimulates transcription from the somatostatin promoter *in vitro*. This protein has been cloned from mRNA isolated from CA-77 cells, a cell line which expresses elevated levels of somatostatin mRNA.

Using procaryotic expression systems, we have overexpressed the 43 kDa CREB protein and have obtained it in relatively large amounts in a homogeneous form. The bacterially expressed 43 kDa CREB is currently being examined for both transcriptional activity *in vitro* as well as a substrate for post-translational modifications. In addition to the 43 kDa protein, the CRE motif is recognized by other CRE-specific DNA binding proteins.

A novel 120 kDa polypeptide has been purified by combination of conventional and CRE-affinity chromatography and shown to display, by a number of assays, CRE-specific binding. The role of the 120 kDa CRE-binding protein in the cAMP-signal transduction pathway is not currently understood.

The 120 kDa protein is readily phosphorylated by PKC but not PKA. In addition, in *in vitro* transcription/complementation assays, the 120 kDa protein does not transactivate the cAMP-responsive somatostatin promoter, suggesting that this molecule may mediate, through the CRE motif, responses distinct from those of the 43 kDa CREB.

CP 009 **POU-Domain Gene Products in Development of Cellular Phenotypes in Neural and Endocrine Tissues,**
Holly A. Ingraham, Xi He, Jeffrey Voss, Maurice Treacy, Vivian Albert, Bryan Crenshaw, & Michael G. Rosenfeld, Eukaryotic Regulatory Biology Program, Howard Hughes Medical Institute, & School of Medicine, M-013, University of California, San Diego, La Jolla, CA 92093.
The molecular strategies utilized to activate gene expression in specific cell types within an organ have been investigated using anterior pituitary cells as a model. The evolutionarily highly-related growth hormone and prolactin genes are expressed in distinct cell types, referred to as somatotrophs and lactotrophs, respectively. Tissue-specific *cis*-active elements in the growth hormone and prolactin promoters bind to a tissue-specific transcription factor, Pit-1, that contains a POU-domain. Analysis of the ontogeny of Pit-1 gene expression reveals it to be expressed in all anterior pituitary cells on e16, but with a lag of detectable protein expression until the time of initial activation of prolactin and growth hormone gene expression. In the mature pituitary, Pit-1 transcripts are present in high concentrations in all cell types, but Pit-1 protein is detectably expressed in only three cell types, apparently reflecting regulation at the level of mRNA translation. Prolactin and growth hormone gene expression in distinct cell types therefore appear to reflect the actions of further restrictive and activating factors in addition to Pit-1. The Pit-1 gene itself exhibits a concentration-dependent pattern of positive and negative autoregulation. We have identified a large number of POU-domain genes that are expressed during neurogenesis in mammals and in *Drosophila*. These genes exhibit distinct temporal and spatial patterns of expression, consistent with potential functions in activation of gene transcription. *Cis*-active elements to which several of the brain-specific POU-domain proteins bind have been identified and binding elements within promoters of genes encoding different classes of neural proteins. Co-transfection analyses have documented the ability of the POU-domain genes to regulate transcription of these promoters in heterologous cell types. We speculate that the POU-domain gene family exerts important developmental functions in determining neuronal phenotypes in the forebrain of higher eukaryotes.

The POU-domain contains a unique region, the 76 aa POU-specific domain that is necessary for high affinity, site-specific binding, and cooperative protein interactions; the POU-homeodomain is necessary for DNA binding, although it alone is capable of only low affinity binding and with highly relaxed intersite specificity. The major transactivation domains reside outside the POU-specific domain.

Occupancy of binding sites is not alone sufficient to account for gene activation events, and a series of experiments suggest that the precise nature of the DNA binding site dictates the consequence of transactivation factor binding, further specified by contributions from a series of potential homodimeric and heterodimeric complexes. The potential diversity afforded by the specificity of heterodimers may be particularly exploited in the regulation of morphogenesis.

Neurodevelopment (joint)

CP 010 MECHANISMS REGULATING PROLIFERATION AND DIFFERENTIATION OF CNS STEM CELLS. Ron McKay, Lyle Zimmerman, Martha Marvin, Myles Cunnigham, Nicole Valtz, Elena Cattaneo and Thor Noregaard; Dept. of Brain and Cognitive Science, Dept. of Biology, MIT, Cambridge, MA02139.

In many regions of the CNS multipotential stem cells give rise to neurons and non-neuronal cells (McKay, Cell 58: 815-821, 1989). The great variety of cell types in the central nervous system are determined by the mechanisms which control the proliferation and differentiation of multipotential stem cells. To identify CNS stem cells we have defined a gene which is specifically expressed in the stem cells and not in the differentiated cell types of the CNS. The deduced amino acid sequence shows the gene codes for a new class (type VI) of high molecular weight intermediate filament protein, nestin (Lendahl et al., Cell, in press, 1990). Northern blots suggest that the nestin gene is strongly transcribed in the precursor cell but not in differentiated neurons or glia. The regulation of proliferation and differentiation of primary cells expressing the nestin gene has been studied in vivo and vitro. These experiments show that defined growth factors can influence the number and differentiated state of nestin positive cells. Our data show that we can count, identify and significantly manipulate the external environment of cells of the early nervous system. Cloned immortal cell lines which represent neuronal, astrocytic and oligodendrocytic precursors have been generated using a temperature conditional oncogene. These cell lines differentiate at the non-permissive temperature, which is the body temperature of rodents. Cloned immortal cell lines which can be induced to differentiate in vivo and in vitro offer powerful genetic and biochemical approaches to the lineage choices of CNS stem cells.

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CP 011 NEURAL CELL LINEAGE ANALYZED WITH A RECOMBINANT RETROVIRUS,
Joshua R. Sanes, Department of Anatomy and Neurobiology, Washington University Medical School, St. Louis, Missouri, 63110.

We have used recombinant retroviral vectors to analyze cell lineage in the nervous systems of mice and chickens. A virus infects an individual progenitor in the neuroepithelium following injection of viral concentrate into the neural tube. The viral genome integrates into a chromosome of the infected cell and is inherited by all of that cell's progeny. Because the vectors are replication-defective, they cannot produce new virions and infect other cells; they are, therefore, true clonal markers. Because the vectors encode an enzyme, β-galactosidase (lacZ), which is detectable histochemically, clonal relatives can be identified at any desired developmental stage. Using this strategy, we have marked progenitors and analyzed their progeny in mouse cerebral cortex and peripheral nerve, and in chicken optic tectum, forebrain, dorsal root ganglia, and spinal cord. Others have used this method to study rat retina and cortex. In general, the retroviral method has allowed three issues to be addressed: 1) What range of cell types arises from a single progenitor? 2) What is the distribution of clonally related cells --i.e., how do cells migrate from their sites of generation to their final positions? 3) How does naturally occurring cell death affect clonal relatives? In addition, new vectors that encode lacZ plus a second, putatively neuroactive protein allow analysis of the behavior of small cohorts of "transgenic" cells in an unperturbed (wild-type) environment.

General reference: Sanes, J.R., TINS 12:21-28 (1989).

Nigrostriatonigral Loop

CP 012 **MOLECULAR ANALYSIS AND REGULATION OF DOPAMINE SYNTHESIZING ENZYMES,**
Tong H. Joh, Joanne M. Carroll, Un Jung Kang, Andrew Towle* and Himadri Samanta*. Laboratory of Molecular Neurobiology, Cornell University Medical College /Burke Rehabilitation Center, White Plains N.Y., 10605 and *Eugene Tech International, Inc., Allendale, N.J. 07401

Dopamine biosynthesis is catalyzed by tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC). The regulation of the rate limiting enzyme TH involves transcriptional and posttranslational processes. To analyze the transcriptional component we have isolated cDNA and genomic clones for rat TH. Fusion genes containing portions of the 5'upstream region linked to the reporter gene chloramphenicol acetyltransferase (CAT) were introduced into mammalian cells by transfection. In several cell lines including C6 glioma, BE(2)C neuroblastoma and HeLa, TH sequences within 500 bp of the TH cap site efficiently directed CAT transcription. Constructs containing 151 bp of upstream sequence allowed for CAT expression at lower efficiency. With either construct, treatment with activators of protein kinase A or C signal transduction pathways induced CAT expression. The 151 bp construct contains a putative cAMP regulated element at -45. AP-1 and AP-2 consensus sequences are found within 500 bp at -206 and -221, respectively. Moreover, in transfected CV1 or Ltk$^-$ fibroblasts, where the basal level of expression did not exceed that of the promoterless plasmid, treatment with forskolin or TPA led to dramatically enhanced CAT expression. These data suggest that elements conferring cell type specific expression lay at least in part beyond this 500 bp region.

AADC, while not highly regulated by physiological parameters, is the only enzyme of the pathway remaining active in neurodegenerative disorders such as Parkinson's disease. AADC, therefore, is responsible for the conversion of exogenously administered L-DOPA to dopamine. To investigate the kinetics of dopamine production in normal and diseased striatonigral system, TH and AADC cDNA were transfected into a mouse fibroblast cell line. Active enzymes were expressed in separate lines of transfected cells. In vivo production of dopamine from these cells transplanted into the striatum of rats with 6-OHDA lesion is currently under investigation.

Molecular Biology of Neurotransmitters and their Receptors

CP 013 TACHYKININ PEPTIDERGIC NEURONS IN THE STRIATONIGRAL SYSTEM: MOLECULAR BIOLOGICAL ANALYSIS OF PRESYNAPTIC SUBSTANCE P PRODUCTION AND POSTSYNAPTIC RECEPTOR MECHANISMS, James E. Krause, Andrew D. Hershey and Yasuo Takeda, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.
Substance P (SP) and multiple related tachykinins can be produced and secreted from neurons which express the preprotachykinin (PPT) I gene. In the rat, SP cell bodies in the striatum project via the globus pallidus to an extensive nerve terminal field within the substantia nigra pars reticulata, where they synapse upon the dendrites of nigrostriatal dopamineric neurons. Previous work has established that dopaminergic neurotransmission in the striatum increases SP gene expression in striatal neurons. Both blockade of dopamine receptors or lesions of dopamine neurons lead to motor deficits and decreases in basal ganglia content of tachykinins and their mRNAs. Moreover, there exists a rapid compensatory activation of SP biosynthesis by L-DOPA in striatonigral neurons of neonatal dopaminergic denervated rats, suggesting a high turnover rate of tachykinin peptides in this system. In the striatum, the γ- and β-PPT mRNAs predominate and SP, neurokinin A (NKA), neuropeptide γ (NPγ), neuropeptide K (NPK) and NKA (3-10) are processed posttranslationally from the γ- and β-PPT polyprotein precursors. Despite the extensive SP terminal field in the substantia nigra, few receptors exist in this region for the tachykinins. In the striatum, a high density of SP binding sites exist that have the characteristics of a NK-1 tackykinin receptor. We have recently cloned this rat SP receptor, and its mRNA is expressed at relatively high levels in the striatum. The receptor protein possesses seven transmembrane domains as a member of the G protein-coupled receptor superfamily, and it consists of 407 amino acid residues with a molecular mass of 46,385 daltons and an average isoelectric point of 6.1. This receptor protein couples with a G regulatory protein to activate phospholipase C as a consequence of ligand binding, though the functional relevance of this in terms of basal ganglia function is not currently appreciated. Studies on the relationship between dopaminergic neurotransmission and SP receptor mRNA expression are currently being carried out to define the mechanisms underlying SP receptor regulation. Consequently, these and others studies provide evidence for the regulatory control of dopamine to the development and maintenance of striatonigral SP neurons and SP-receptive neurons in the basal ganglia.

CP 014 ANATOMY AND REGULATION OF DOPAMINE D_2 RECEPTOR mRNA in CNS, by S. J. Watson, J. Meador-Woodruff, A. Mansour, O. Civelli* & H. Akil, Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109, Vollum Institute for Advanced Biomedical Research, Oregan Health Sciences University, Portland Oregan 97201*. The anatomy of dopamine (DA) systems in the brain are well understood in terms of their cell bodies and projections. The tissues innervated by DA neuronal systems are known to contain two types of receptors, the D_1 and D_2 subtypes. The D1 is thought to be mainly post-synaptic and positively coupled to adenylate cyclase. The D_2 receptor is probably both post-synaptic, either uncoupled or negatively coupled to adenylate cyclase, and presynaptic, produced in DA neurons themselves in cell bodies and terminals. Last year, the D_2 receptor was cloned (1) from rat brain, and more recently its gene structure has been determined (2). Using cRNA clones prepared against the third cytosolic loop we have studied the distribution of the D_2 mRNA in rat CNS, using *in situ* hybridization (3) and tyrosine hydroxylase immunocytochemistry. D_2 mRNA is found heavily concentrated in the caudate, n. accumbens, olfactory tubercle and the DA producing cells of the A9 and A10 (substantia nigra, VTA) and the zona incerta. In part this distribution corresponds to post synaptic cells which produce D_2 receptors (caudate, n. accumbens) and receive DA proteins. The DA cell groups (A9, A10 and ZI) express their own D2 receptors, presumably playing an autoreceptor role in these cells.

Currently we are comparing the localization of D_2 mRNA and D_2 and D_1 binding sites. Is there a tight match between D_2 binding and it's mRNA? We are also trying to determine the cells of origin of D_2 using lesioning methods such as, 6-hydroxydopamine and ibotenic acid, in caudate and substantia nigra. These studies demonstrate that D_2 producing cells are found in the nigra, intrinsic to the caudate and from extra-caudate cells (cortex, thalamus, etc).

Future studies involve evaluation of mRNA splicing variants of the D_2 mRNA including studies of their regulation in a variety of neural systems. Beyond these basic studies are efforts to study mental illnesses associated with dopamine receptor dysfunction.

1. Bunzow, J.R., Van Tol, H.H.M., Grandy, D.K., Albert, P., Salon, J., Christie, M., Machida, C.A., Neve, K.A., and Civello, O.: Nature 336, pp. 783-787, 1988.
2. Grandy, D.K., Marchionni, M.A., Makam, H., Stofko, R.E., Alfano, M., Frothingham, L., Fischer, J.B., Burke-Howie, K.J., Bunzow, J.R., Server, A.C. and Civelli, O.: Proc. Natl. Acad. Sci. USA, in press.
3. Meador-Woodruff, J.H., Mansour, A., Bunzow, J.R., Van Tol, H.H.M., Watson, S.J. and Civelli, O.: Proc. Natl. Acad. Sci. Vol. 86, pp. 7625-7628, 1989.

Molecular Biology of Neurotransmitters and their Receptors

Transcriptional Regulation

CP 100 REGULATION OF NEURAL OXYTOCIN mRNA BY GONADAL STEROIDS.

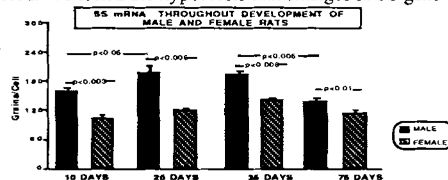
R. Chibbar, J. Toma, B.F. Mitchell, F.D. Miller

The nonapeptide oxytocin (OT) has been implicated in a number of reproductive functions as well as in various maternal and sexual behaviors. We have previously demonstrated (Miller, F.D. et al P.N.A.S. 86;2468) that in female rats, oxytocin mRNA increases during puberty and is decreased in adult females following ovariectomy. To determine whether gonadal steroids play a role in the pubertal increase in oxytocin mRNA, prepubertal male and female rats were gonadectomized and replaced with estradiol, testosterone or dihydrotestosterone. Forty days later animals were sacrificed and total cytoplasmic RNA preparations from the brain were analyzed on Northern blots using 32P labelled oxytocin riboprobes. These experiments indicate that the pubertal increase in OT mRNA is partially inhibited by gonadectomy. This inhibition is completely reversed by administration of exogenous steroids. To determine whether oxytocinergic neurons were equally responsive to steroid hormones at all developmental stages, 10 day old male and female rats were treated with estradiol, testosterone or dihydrotestosterone for 10 days. Although the levels of OT mRNA increased as compared to control prepubertal rats, they remained low as compared to adults. These studies suggest that

a) levels of circulating gonadal steroids regulate neural OT mRNA, and

b) some additional factor(s) also play a role in the observed developmental increase.

CP 101 DEVELOPMENTAL AND SEXUALLY DIMORPHIC PATTERNS OF SOMATOSTATIN GENE EXPRESSION IN THE PERIVENTRICULAR NUCLEUS OF THE RAT BRAIN, J.A. Chowen-Breed, J. Argente, R.A. Steiner and D.K. Clifton, Dept. of OB & PBio., Univ. of Wash., Seattle, WA 98195.
With the onset of the puberty in the male rat growth hormone (GH) secretion becomes demonstrably pulsatile; however, the mechanisms underlying this developmental change remain unknown. Although somatostatin (SS) is involved in the orchestration of GH secretory patterns in the adult, its possible role in mediating the developmental change in GH secretion has not been explored. We tested the hypothesis that changes in SS gene expression in neurons of the periventricular nucleus (PeN) become manifest over the course of development and that this would be reflected in changes in levels of SS mRNA. Using *in situ* hybridization and a computerized image analysis system, we measured and compared levels of SS mRNA in individual cells of the PeN between male and female rats 10, 25, 35, and 75 days of age. In the male rat, the signal level of SS mRNA in cells of the PeN inceased significantly between 10 and 25 days of age and then declined with pubertal maturation. In the female animal, a significant increase in SS mRNA levels was observed at 35 days of age which declined at 75 days of age. In addition, at all ages studied male rats had significantly more SS mRNA in neurons of the PeN than female animals. Conclusion: These observations suggest that changes in SS gene expression are involved in the development of adult GH secretory profiles and may also be involved in the development of sexually dimorphic GH secretory patterns.



CP 102 ANALYSIS OF mRNA EXPRESSION IN RAT ADRENAL GLAND GANGLION CELLS

Å. Dagerlind, M. Schalling, K. Friberg and T. Hökfelt. Department of Histology and Neurobiology, Karolinska Institutet, P.O. Box 60400, S-104 01 Stockholm, Sweden.

The medulla of the rat adrenal gland contains a population of ganglion cells but little is known about their projection and function. Therefore we combined indirect immunofluorescence, retrograde tracing and in situ hybridization to analyse transmitters/neuropeptides expressed in these cells. Neuropeptide tyrosine-immunoreactive (NPY-IR) and vasoactive intestinal polypeptide-immunoreactive (VIP-IR) ganglion cells were identified in addition to NPY-IR chromaffin cells. The NPY-IR ganglion cells formed islands consisting of 5-30 large cell profiles, preferentially located to the center of the medulla. NPY-IR nerve fibers were found in the zona glomerulosa of the cortex. VIP-like immunoreactivity (VIP-LI) was found in ganglion cells, mostly distributed closer to the periphery of the medulla. The VIP-IR cells were somewhat smaller than the NPY-IR cells and were found in islands with 1-5 clustered cells. VIP-IR nerve fibers were seen in the zona glomerulosa with a similar pattern to those containing NPY-LI. No evidence for coexistence of these two peptides was found. Application of the retrograde tracer Fast Blue to the surface of the adrenal gland labelled NPY-positive ganglion cells suggesting that these cells give rise to cortical NPY fibers. Using in situ hybridization, several synthetic oligonucleotide probes directed against mRNA for NPY, VIP, choline acetyltransferase, tyrosine hydroxylase, phenylethanolamine N-methyltransferase, AADC (L-aromatic amino acid decarboxylase) or D2-R (dopamine type 2 receptor) were tested. VIP as well as NPY mRNA positive ganglion cells were seen with a similar distribution as shown with immunohistochemistry. In addition, NPY containing ganglion cell groups were found to express mRNA for the catecholamine synthesizing enzyme AADC. The traditional view that these ganglion cells are cholinergic must therefore be further scrutinized.

CP 103 LEVELS OF GROWTH HORMONE-RELEASING HORMONE mRNA AND SOMATOSTATIN mRNA IN THE HYPOTHALAMUS OF THE GENETICALLY OBESE ZUCKER RAT, J. A. Finkelstein, I. Ahmad, A. W. Steggles, P. Chomczynski, T. R. Downs, and L. A. Frohman, Depts. of Anatomy and Biochemistry, Northeastern Ohio Univ. College of Medicine, Rootstown, OH 44272 and Dept. of Internal Medicine, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267.
Levels of both circulating growth hormone (GH) and its pituitary messenger ribonucleic acid (mRNA) are depressed in genetically obese Zucker rats in comparison to lean littermates. In order to determine the role of the hypothalamic factors which regulate GH release, we measured the levels of growth hormone-releasing hormone (GHRH) mRNA and somatostatin (SOM) mRNA in lean and obese male Zucker rats. Separate analyses were performed on animals of ages 11, 14, and 24 weeks. Total RNA was extracted from pooled samples for each group and analyzed by Northern blotting. The same membrane was sequentially hybridized with probes for GHRH, SOM, and ß-actin. At all ages studied, GHRH mRNA levels were lower in obese rats as compared to lean littermate controls, but no difference was seen in levels of SOM or B-actin mRNA. Densitometric quantitation of Northern blot analyses of GHRH mRNA in individual hypothalami and of GH mRNA in individual pituitaries of 20 week animals revealed a 47 $\pm$ 5% reduction in GHRH mRNA ($p<0.02$) and a 61 $\pm$ 5% reduction in GH mRNA ($p<0.01$). These results, in conjunction with previous reports of hypothalamic peptide content in genetically obese Zucker rats, which have shown decreased levels of GHRH, though no difference in SOM levels, suggest that the decreases in GH synthesis and release are attributable to decreased production of GHRH. Supported by funds from the Ohio Board of Regents Research Challenge (J.A.F.) and NIH grant DK 30667 (L.A.F.).

CP 104 TYROSINE HYDROXYLASE mRNA EXPRESSION IN FETAL SUBSTANTIA NIGRA DOPAMINERGIC GRAFTS, I. Mendez, C. Naus, K. Elisevich and B. Flumerfelt. Departments of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Canada
Evidence for restoration of the nigrostriatal dopaminergic input in rats with 6-hydroxydopamine (6-OHDA) nigral lesions by fetal substantia nigra grafts is well documented. Tyrosine hydroxylase (TH) immunoreactive neurons grafted into the dopamine deafferented neostriatum have been shown to synaptically interact with host neurons. There is also evidence that the behavioural recovery observed in lesioned rats after grafting correlates with the degree of restoration of the dopaminergic input to the host striatum by the graft. The purpose of this study was to analyse the TH gene expression in nigral grafts. The presence of TH-mRNA in grafted nigral cell suspensions was shown by in situ hybridization using a ^{35}S-radiolabelled anti-sense oligonucleotide probe. Grafted animals showing behavioural recovery at three, six and nine months post-grafting were used in the study. TH immunohistochemistry at the light and electron microscope levels was performed on alternate sections. A direct correlation between the sites of TH-mRNA expression and TH immunoreactvity was observed. Thus, the grafted dopaminergic neurons retain the gene expression for the production of TH and actively synthesize the gene product following transplantation into the deafferented striatum.
Supported by the Medical Research Council of Canada

CP 105 DOPAMINE RECEPTOR MEDIATED CHANGES IN RAT STRIATAL C-FOS mRNA EXPRESSION, Jeannette C. Miller, Department of Psychiatry, Millhauser Laboratories, New York University School of Medicine, New York, New York, 10016
In previous Northern hybridization studies, I have shown that neuroleptic drugs which are dopamine (DA), D2 receptor antagonists produce a rapid and transient increase in the expression of the proto-oncogene c-fos in rat striatum. This effect on c-fos mRNA expression is dose-dependent and is blocked by concomitant treatment with the D2 agonist, LY171555. Treatment with LY171555 alone had no effect on striatal c-fos expression. These findings support the idea that c-fos expression in striatum is under DA, D2 receptor mediated inhibitory control and that the induction of c-fos may be an important early event in the initiation of the neuroleptic-induced increase in D2 receptor number. Recently, Robertson & Robertson, Neurosci. Abst., 15:782, 1989, reported that the D1 agonist, SKF38393, activates striatal c-fos expression; however, this stimulation was shown in striatum of 6-hydroxydopamine lesioned rats. The data to be presented will include my more recent studies of the effects of selective D1 and D2 agonists/antagonists on the expression of striatal c-fos mRNA and a comparison of the effects found in naive animals with those of animals demonstrating neuroleptic induced D2 receptor supersensitivity. A possible basis for the differential response of c-fos to D1 and D2 agents in 6-hydroxydopamine vs. neuroleptic-induced supersensitivity will be presented.

CP 106 ANALYSIS OF MESSENGERS AND THEIR RECEPTORS IN THE BASAL GANGLIA OF RAT, CAT, MONKEY AND HUMAN. Martin Schalling*, Katarina Friberg*, Åke Dagerlind*, Kim Seroogy, Mikael Djurfelt, Edward Bird, Menek Goldstein, Michelle Erlich, Paul Greengard and Tomas Hökfelt*. *Department of Histology and Neurobiology, Karolinska Institute, 104 01 Stockholm, Sweden.

The ventral mesencephalon contains a dense population of dopaminergic neurons. These neurons have in man been implicated to play a role in several diseases such as Parkinson's disease and schizophrenia. In many neurons in the rat mesencephalon the neuropeptide cholecystokinin (CCK) and its mRNA are extensively colocalized with tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, and evidence along several lines suggests a functional interaction between CCK and dopamine in experimental animals. In this study we have analysed the coexistence of CCK mRNA and TH enzyme in the ventral mesencephalon of cat, monkey and human using a combination of in situ hybridisation and indirect immunofluorescence histochemistry on the same section. CCK mRNA was shown to be extensively colocalized with TH both in cat, monkey and in a human with the diagnosis of schizophrenia. Several nonschizophrenic brains have been analysed along these lines with no or only very weak expression of CCK mRNA in dopamine cells.

At the postsynaptic level we have investigated the distribution and regulation of the dopamine D2 receptor mRNA as well as the mRNA coding for a dopamine and cyclic AMP regulated phosphoprotein with a molecular weight of 32 kilodalton (DARPP-32). DARPP-32 has previously been shown to be associated with the dopamine D1 subtype receptor and may be considered a third messenger in the postsynaptic neuron. Both DARPP-32 mRNA and D2 mRNA were shown to be extensively colocalized in many brain areas. In some instances such as the septum, cerebellum, brainstem and adrenal gland marked differences were found. In the caudate nucleus and nucleus accumbens, the regulation of DARPP-32 mRNA and D2 mRNA was studied following various pharmacological manipulations.

CP 107 MULTIPLE mRNAs FOR THE TYPE I CORTICOSTEROID RECEPTOR IN RAT HIPPOCAMPUS, Robert C. Thompson, Paresh D. Patel, Thomas G. Sherman, Sueng P. Kwak, Huda Akil and Stanley J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109. We have recently cloned a cDNA for the type I, mineralocorticoid receptor (MR)-like, binding protein from rat hippocampus (Patel et al., Neurosci. Abst., V.14, 1988). Comparison to human MR cDNA (Arriza et al., Science, 237:268, 1987) demonstrates uniformly high homology throughout, except in the 5'-untranslated (5'-UT) region. RNase protection of cRNA with hippocampal mRNA revealed 5'-UT sequence heterogeneity, suggestive of multiple mRNAs. To determine the source of this observation, a genomic fragment spanning the 5'-translated/flanking regions of the rat MR gene was isolated and partially sequenced. Sequences were found corresponding to the 5'-UTs for both the rat (β) as well as the human (α) MR cDNAs, and appear to exist as distinct exons separated by approximately 2 kb, situated more than 4 kb upstream of an exon coding for the N-terminal domain. Both the α and β 5'-UT exons display consensus donor sites for proper splicing to produce the observed mRNAs. Taq polymerase chain reaction is being used to determine if the homolog of the human MR 5'-UT is expressed in mature rat MR mRNA.

It is not yet known what role alternate 5'-UT sequences play in expression of the MR. Quantitation of α and β forms does not reveal a clear tissue specific distribution, although there is a tendency for the β form to be expressed at higher levels in CNS tissues. Potential correlations may be found with development of or in the stability/translatability of the variant mRNAs.

CP 108 LONG-TERM IMIPRAMINE ADMINISTRATION INCREASES MINERALOCORTICOID RECEPTOR (MR) MRNA IN THE HIPPOCAMPUS AND DECREASES CORTICOTROPIN RELEASING HORMONE (CRH) MRNA IN THE HYPOTHALAMUS OF RATS, Harvey J. Whitfield, Linda S. Brady, Robert J. Fox, Philip W. Gold and Miles Herkenham, Unit on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892

Alterations in CNS gene expression produced by chronic administration of tricyclic antidepressant drugs may provide insight into their mode of action. *In situ* hybridization was used to assess the effects of daily imipramine administration (5mg/kg, ip) in rats. Imipramine for 8 but not 2 weeks increased MR mRNA levels (70%, $p<0.003$) in CA fields1-4 and in the dentate gyrus of the dorsal hippocampus and decreased CRH mRNA levels (37%, $p<0.0001$) in the parvocellular region of the paraventricular nucleus (PVN). Glucocorticoid receptor (GR) mRNA levels were not altered in the hippocampus, PVN, or locus coeruleus (LC). GR (51%, $p<0.03$) and proopiomelanocortin (POMC, 38%, $p<0.03$) mRNA levels were decreased in the anterior lobe of the pituitary. Tyrosine hydroxylase mRNA was decreased in the LC after 2 (20%, $p<0.03$) and 8 weeks (40%, $p<0.003$) of imipramine. Changes in brain MR, CRH, GR and POMC gene expression that occur after long-term (8 weeks) imipramine administration may be relevant to the delayed onset of clinical efficacy (3-6 weeks) of tricyclic antidepressant drugs in major depression.

Molecular Biology of Neurotransmitters and their Receptors

Neurotransmitters and Their Receptors: Development and Expression

CP 200 ASSEMBLY INTERMEDIATES OF THE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTOR, Paul Blount, McHardy M. Smith, and John Paul Merlie, Department of Pharmacology, Washington University School of Medicine, St Louis, MO 63110. By stably co-expressing muscle nicotinic acetylcholine receptor (AChR) subunits in fibroblasts we found previously that the γ and δ, but not the β subunit associated efficiently with α subunit, and they extensively modified its binding characteristics (Neuron, 3, 349-357). Now we have studied the cellular localization and stoichiometry of the $\alpha\gamma$ and $\alpha\delta$ complexes expressed in these fibroblasts. In clonal cell lines expressing complexes of $\alpha\gamma$ or $\alpha\delta$ subunits, α-bungarotoxin binding sites were restricted to an intracellular location. Lectin binding and pharmacological studies suggested that complex sugars, normally present on the AChR, are absent from these $\alpha\gamma$ and $\alpha\delta$ complexes. Since complex oligosacaride modifications occur in the Golgi, and γ and δ are the only subunits thought to contain complex sugars, these data suggest that assembly intermediates are formed in and confined to a pre-Golgi compartment. Sucrose gradient fractionation demonstrated that discrete assembly intermediates were formed in these fibroblast clones. Both the $\alpha\gamma$ and $\alpha\delta$ complexes labeled with ^{125}I-BTX had sedimentation coefficients of approximately 6.5S, a value between ^{125}I-BTX labeled 5S α subunit and the ^{125}I-BTX labeled 9.5S AChR. An additional peak at 8.5S was observed for $\alpha\gamma$ complexes suggesting that γ may have more than one binding site for α, leading to the formation of trimers or tetramers.

CP 201 ISOLATION AND CHARACTERIZATION OF THE MOUSE D2 DOPAMINE RECEPTORS. E. Borrelli, N. Amlaiky*, L. Maroteaux, R. Hen, P. Bausero, and J.P. Montmayeur. LGME/CNRS - U184/INSERM - Institut de Chimie Biologique (*Institut de Pharmacologie, URA DO 589 CNRS), Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex, France.
Dopamine receptors belong to the family of neurotransmitter and hormone receptors that are linked to G-proteins for their signal transduction. Two types of Dopamine receptors have been described: D1 and D2. D1 stimulates adenylyl cyclase while D2 inhibits it. D2 in addition inhibit the phosphatidylinositol turnover as well as it affects K^+ and Ca^{2+} transport. This diversity of response induced by D2 receptors suggest the existence of multiple receptors or different forms of it. Therefore we decided to look for the pituitary D2 receptor, being this tissue very enriched in it. A cDNA library was constructed from mouse pituitary RNA and screened at low stringency with two oligonucleotide sequences obtained from a consensus sequence of already cloned G-protein-coupled receptors. 12 clones were isolated, 3 of them were related to the rat D2 Dopamine receptor isolated from the brain. Sequence analysis revealed one major difference of these clones, from the rat receptor; that consists in a single insertion of 87 base pair in the third cytoplasmic loop of the receptor, probably originated by alternative splicing. S1 nuclease analysis of RNAs from different parts of mouse brain revealed the presence of both cDNAs showing a differential tissue specific expression pattern. The D2 receptor we isolated is the most abundant form. Mouse cells expressing this receptor shown [^{3}H] spiperone binding with appropriate D2-dopaminergic pharmacology. The differential expression and studies on the G-protein-coupling of this receptor form will be discussed.

CP 202 TRANSIENT LAMINAR AND COLUMNAR EXPRESSION OF SEROTONIN RECEPTORS IN THE DEVELOPING CAT VISUAL CORTEX, Richard H. Dyck and Max S. Cynader, Department of Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada V5Z 3N9
Developmental changes in the distribution of many neurotransmitter/neuromodulator receptors in the cat visual cortex are age- and lamina-specific. For some receptors, significant changes in laminar distribution occur in layer IV during the period within which the visual cortex exhibits ocular dominance plasticity. It is not known if the relationship between neurotransmitter receptor expression and plasticity is simply correlational or whether the redistribution plays a significant mechanistic role. This study was undertaken to assess the ontogenetic distribution of serotonin (5-HT) receptor subtypes in cat visual cortex.
In vitro autoradiography was used to study the distribution of serotonin (5-HT) receptor subtypes in the cat visual cortex. Cats aged postnatal day 0 (P0), P13, P30, P42, P50, P62, P90, and adult were anaesthetized, perfused through the ascending aorta with phosphate-buffered saline; then the brain was quickly removed, frozen in isopentane at -70°C, and stored at -20°C until sectioning. Three sets of adjacent, 16µm coronal sections through the visual cortex were thaw-mounted onto gelatin-coated slides. Following preincubation, each set of sections was incubated in buffer with either [^{3}H]-8-OHDPAT, [^{3}H]-mesulergine or [^{3}H]-ketanserin to assess 5-HT$_{1A}$, 5-HT$_{1C}$, or 5-HT$_2$ receptor subtype distribution, respectively. Several sections from each set were incubated in the presence of µM concentrations of competitor to determine binding specificity.
All 5-HT receptor subtypes demonstrated transient laminar distributions, each with a different time course. While 5-HT$_{1A}$ and 5-HT$_2$ receptors presented changes in distribution similar to other receptors we have previously studied, the 5-HT$_{1C}$ receptor was demonstrable in layer VI from P13 through P62, but more remarkably, was **transiently confined to columns in layer IV between 32 and 60 days of age.** Until now, no receptor distribution has been demonstrated as participating in the columnar organization of cat visual cortex.

Molecular Biology of Neurotransmitters and their Receptors

CP 203 MOLECULAR BIOLOGY OF NEUROPEPTIDES DERIVED FROM THE RAT MELANIN-CONCENTRATING HORMONE PRECURSOR, Dominique Fellmann, Christophe Breton, Malika Bahjaoui, Brigitte Cormery, Claude Bugnon, Laboratoire d'Histologie Embryologie, Université de Franche-Comté, Faculté de Médecine, 25030 Besançon France.
We previously demonstrated that a neuron population of the lateral and dorsal areas of the posterior hypothalamus coexpress distinct peptides stained by antisera to human somatocrinin 1-37 (GRF37), to α-melanotropin (α-MSH) and to salmon melanin-concentrating hormone (sMCH). In order to elucidate the primary structure of the neuropeptide related to rMCH, we have screened rat hypothalamus cDNA expression libraries with sMCH antiserum. Three immunopositive clones were sequenced and enabled us to deduce the sequence of the 96 C terminal residues of the rat MCH (rMCH) precursor. The presence of three possible sites for enzymatic cleavage suggest that rMCH and two additional peptides may be generated from this precursor. These potential peptides display sequence homologies with the C terminal segment of GRF37, with the amidated C terminus of α-MSH, and with sMCH respectively. Biochemical results suggest that these putative neuropeptides are actually released from the pre-pro-rMCH.

CP 204 CLONING AND SEQUENCING OF cDNA ENCODING BLACK WIDOW SPIDER NEUROTOXIN, Nikita I. Kiyatkin, Irina E. Dulubova, Elena A. Chekhovskaya and Eugene V. Grishin, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR
Latrotoxin is a potent neurotoxic protein of m.m. 118 kD, which specifically binds to a presynaptic receptor stimulating neurotransmitter release. We isolated a cDNA library prepared from poly(A^+) RNA extracted from the venom glands of the black widow spider (*Latrodectus mactans tredecimguttatus*). The RNA analysis showed the absence of 28S ribosomal RNA, that appears to be typical of arthropods. Northern blot analysis of polyA^+RNA showed that latrotoxin RNA is larger than 28S RNA.
Specific oligonucleotide probes synthesized on the basis of amino acid sequences of toxin tryptic peptides were used to select recombinant clones, containing latrotoxin structural gene fragments. The subsequent structural analysis of isolated clones allowed us to determine a virtually complete nucleotide sequence for the latrotoxin structural gene.

CP 205 MOLECULAR BIOLOGY OF THE SEROTONERGIC SYSTEM IN THE RODENT BRAIN, H. Lübbert, M. Foguet, J. Hartikka, L. Merguin, and M. Staufenbiel, Preclinical Research, Sandoz Pharma AG, 4002 Basel, Switzerland
We have cloned a mouse brain 5-HT1c receptor using an electrophysiological assay and a novel hybrid depletion strategy (PNAS 84:4332, 1987). The cloned receptor is functional and displays 5-HT1c pharmacology when expressed in Xenopus oocytes. By cross-hybridization we then isolated cDNA clones for the 5-HT2 receptor which is pharmacologically and functionally related to the 5-HT1c receptor. We found that the genes for both receptors have the same exon-intron pattern which distinguishes them from other members of the family of G-protein coupled receptors. We have also isolated genomic clones from a new receptor with exon-intron boundaries identical to those of the 5-HT1c and 5-HT2 receptors. This is interesting because there is no other receptor known to be pharmacologically and functionally related to these two receptors. By northern blots and in situ hybridization of brain slices we examined the expression pattern of every exon of the 5-HT1c receptor.This did not reveal any evidence for differential splicing which should cause a different distribution of hybridisation signal of each exon-specific probe. Recently, we attempt to follow the expression of serotonin receptors and of other specific markers in cultures of raphe neurons alone or together with their target neurons. The goal is to identify parameters and factors influencing the expression of these markers.

Molecular Biology of Neurotransmitters and their Receptors

CP 206 PRE-PRO NEUROPEPTIDE Y mRNA EXPRESSION IN CULTURED GLIAL CELLS OF THE RAT.
Brian A. Masters[1], Corinne R. Pruysers[2], William J. Millard[3] and Jennifer J. Poulakos[2], Departments of Physiology[1], Pharmacology[2] and Pharmacodynamics[3], University of Florida, Gainesville, FL 32610
Neuropeptide Y (NPY) is found throughout the brain and is important in several physiological and behavioral processes. However, the role of non-neuronal NPY in the brain with respect to these processes is largely unknown. Therefore, it was of interest to determine if glial cells had the ability to synthesize NPY. The expression of pre-pro NPY mRNA was examined in glial and primary neuron-enriched cell cultures by Northern blot analysis. Total RNA was isolated from astrocytes, oligodendrocytes and neurons prepared from whole brains of 1 day old Sprague Dawley rats by the guanidinium thiocyanate phenol extraction method. Additionally, cultures of astrocytes prepared from whole brains of 21 day old rats were included. A Northern blot containing 10, 25 and 50 µg of total RNA from each cell type was hybridized with a ^{32}P labelled cDNA probe encoding the rat NPY gene (generously provided by Dr. J. Allen). Computer image analysis (Betascope, model 603) indicated that a 0.8 Kb pre-pro NPY mRNA was detected in neurons (28 counts/µg RNA) and astrocytes (10 counts/µg RNA) prepared from one day old rats but not in astrocytes prepared from 21 day old rats. Oligodendrocytes showed a faint 0.8 Kb band (3 counts/µg RNA) in only the 50 µg lane which may be due to the presence of astrocytes (10%) in these cultures. Furthermore, NPY was not detectable in pooled samples of oligodendrocytes when measured by RIA. These results indicate that pre-pro NPY mRNA is present in astrocytes, the significance of which remains unknown.

CP 207 PURIFICATION AND CHARACTERISATION OF THE 5-HT_3 RECEPTOR FROM NCB 20 CELLS.
R M McKernan, N Gillard, K Quirk, C Kneen, G Stevenson, C Swain, C I Ragan. Departments of Biochemistry and Chemistry, Neuroscience Research Centre, Merck Sharp & Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex. CM20 2QR, U.K.
The 5-HT_3 receptor is a novel member of the family of ligand gated ion channel receptors. While the electrophysiological and ligand binding properties of this receptor are well characterised, little is known of its structure or sequence. We have demonstrated binding of the selective 5-HT_3 ligand (^3H)Q ICS 205 930 to membranes prepared from NCB 20 cells. These sites have been solubilised using 0.5% DOC (Bmax for solubilised receptors = 1.19 ± 0.31 pmol/mg protein, K_d = 0.43 ± 0.07 nM; n = 4). The molecular size of the receptor has been determined using the combined techniques of gel filtration and sucrose density gradient centrifugation. (M_r = 249,000; n = 4). The receptor has also been purified to > 50% purity using a novel affinity resin, L-685,603 -agarose. This resin retains 90% of solubilised receptor in 0.1% triton, and after extensive washing in high salt buffer 54.9 ± 9% of the receptor (n = 8) can be eluted by 1mM quipazine. The specific activity of the purified receptor is 2-4 nmoles/mg protein, and the pharmacology of this receptor is essentially unchanged after purification. This purification procedure should provide a method for obtaining sufficient protein for cloning and sequencing studies.

CP 208 **CHANGES IN BIOCHEMICAL MARKERS DURING NEUROBLASTOMA CELL DIFFERENTIATION.**
M. Ponzoni, M. Lanciotti, P. G. Montaldo, P. Longone, and P. Cornaglia-Ferraris, Pediatric Oncology Research Laboratory, G. Gaslini Children's Hospital, L.go G. Gaslini 5, 16148 Genova, Italy.
In order to define some neuronal differentiation-related biochemical markers we studied the effects of retinoic acid (RA), γ-interferon (γ-IFN), cytosine arabinoside (ARA-C), nerve growth factor (NGF), and tumor necrosis factor (TNF) on the human neuroblastoma (NB) cell line, LAN-5. Intracellular levels of acetylcholinesterase (Achase), neuron-specific enolase (NSE), and catecholamines have been quantitated. RA caused a dramatic decrease (80%) in norepinephrine (NE); DOPA and dopamine were also diminished. Concomitantly, a five fold increase in Achase was observed. Morphologic maturation was marked with complete loosening of cell clusters and emission of long neurites. The effects induced by γ-IFN were similar to RA both on catecholamines and Achase, but less pronounced. Also the morphologic differentiation of γ-IFN treated cells was similar but less evident, to that induced by RA. ARA-C induced a different morphology mainly characterized by enlargment of cell body, did not affect catecholamines, but increased by 60% the level of serotonine. TNF did not alter the cell shape, but decreased NE by 40%. Finally, NGF treatment induced a few cells to sprout short and synaptic dendritic processes, and slightly increased NE and DOPA levels. Noteworthy, intracellular NSE was unaffected by all the inducers tested.
Our findings indicate that the most striking biochemical change accompanying LAN-5 cell differentiation is a shift from a catecholaminergic to a cholinergic phenotype. Small changes may be independent of the morphologic maturation. Other human NB cell lines and inducing agents are currently under evaluation in our laboratory to extend the generality of the observed phenomena.
Supported by AIRC and by Ricerca Corrente Gaslini 891701C.

Molecular Biology of Neurotransmitters and their Receptors

CP 209 FORMATION OF ANXIOLYTIC STEROIDS FOLLOWING ACUTE STRESS, Robert H. Purdy, A. Leslie Morrow, Perry H. Moore, Jr., and Steven M. Paul, Southwest Foundation for Biomedical Research, San Antonio, TX 78284 and Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892. The endogenous anxiolytic steroids, 3α,21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone, THDOC), a metabolite of deoxycorticosterone, and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, AP), a metabolite of progesterone, are amongst the most potent known ligands of the $GABA_A$-receptor complex in the CNS. These steroids potentiate $GABA_A$ receptor-mediated chloride ion conductance in neurons, and have anxiolytic, sedative, and analgesic effects *in vivo*. We have developed specific radioimmunoassays to measure >25 pg amounts of THDOC and AP in extracts of plasma and brain tissue after purification of the steroids by HPLC. Control levels in plasma (ng/ml) and cerebral cortex (ng/g) of adult male Sprague-Dawley rats were 0.19 and 0.15 for THDOC and 0.06 and 0.39 for AP. Within 5 min after exposure to ambient temperature swim stress there was a 7-20 fold increase in circulating THDOC and AP respectively, and a concomitant 4-7 fold increase of THDOC and AP in cerebral cortex. These increases in plasma and brain were maintained for 1 hr following stress, and then declined to baseline levels by 2 hr. We conclude that THDOC and AP are modulators of central $GABA_A$-receptors which may play an important role in the response of the CNS to stress.

CP 210 cDNA CLONES DEFINE BRAIN α-BUNGAROTOXIN-BINDING PROTEINS AS LIGAND-GATED ION CHANNELS, Ralf Schoepfer, William Conroy, Paul Whiting, Jon Lindstrom, The Salk Institute, P.O. Box 85800, San Diego, California 92138

α-bungarotoxin (αBgt) is a potent, high affinity antagonist for muscle type nicotinic acetylcholine receptors (AChR), but not for AChRs from neurons. High affinity αBgt-binding proteins (αBgtBP) are found in vertebrate brains and ganglia. The neuronal αBgtBPs have a nicotinic pharmacology but their function is unknown. αBgtBPs that were purified by αBgt affinity chromatography were reported to be composed of 2-4 subunits, and the N terminal protein sequence of one subunit was reported (Conti Tronconi et al., 1985, 82, 5208-5212)).

We have now identified cDNA clones encoding two subunits of chicken brain αBgtBPs. The deduced protein sequences identify them as members of the ligand gated cation gene family. Subunit specific antisera and monoclonal antibodies were raised against bacterially expressed αBgtBP cDNA fragments and used to define two subtypes of αBgtBP. With the obtained cDNAs and mAbs we have now the tools to address the functional role of neuronal αBgtBPs.

CP 211 IMMUNOCHEMICAL STUDY OF THE GLUTAMATE- AND ARGIOPININ-BINDING RECEPTOR PROTEIN, Tatyana M. Volkova, Naira A. Avetisyan, Andrei B. Kudelin and Eugene V. Grishin, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR

Low-molecular-mass compounds from the *A. lobata* spider venom (argiopinins) are the antagonists of glutamate receptors. The receptor complex containing protein components with molecular masses 68, 54, 45, and 40 kD was isolated from bovine cortex membranes by two-stage biospecific chromatography on immobilized glutamate and argiopinins. The homogeneous 40 kD protein was shown to possess one type of glutamate-binding sites (k_d 0.18 μM and B_{max} 43 nmol/mg of protein). The 40 kD protein tryptic fragments were sequenced. The peptide (19 amino acid residues) corresponding to a fragment of the 40 kD protein was synthesized, monospecific antibodies (mα2C) against it were obtained. The antibodies interact not only with the 40 kD protein but also with 68, 54 and 45 kD proteins. This experiment confirms that either the 40 kD protein is a fragment of a larger protein or the receptor complex subunits are of high homology. By immunoaffinity chromatography on immobilized mα2C the preparation containing only the 68 kD component and having the glutamate binding sites (k_d 0.18 μM) was obtained.

CP 212 STABLE EXPRESSION IN MOUSE FIBROBLASTS OF THE MAJOR SUBTYPE OF BRAIN NICOTINE ACETYLCHOLINE RECEPTORS,

[1]Paul Whiting, [1]Tony Priestley, [2]R. Schoepfer and [2]J. Lindstrom, [1]Merck Sharp and Dohme Laboratories, Eastwick Road, Harlow, Essex, England; [2]Salk Institute for Biological Studies, San Diego, U.S.A.

It is now becoming clear that a family of neuronal nicotinic acetylcholine receptor (nAChR) genes exists, encoding various subtypes of nAChRs. The major brain nAChR subtype consists of an ACh binding subunit (cDNA $\alpha 4$), and a structural subunit (cDNA $\beta 2$). cDNAs encoding chicken $\alpha 4$ and $\beta 2$ subunits were inserted into the eukaryotic expression vector pMSG neo, which contains the dexamethasone inducible MMTV promoter. Mouse L cells were contrasfected, selected for neomycin resistance, and 6 cell lines established. One of these expressed both $\alpha 4$ and $\beta 2$ mRNA, and high affinity ^{3}H nicotine binding sites. These AChRs had a pharmacology essentially identical to the native chicken brain AChR. Expression was induced 1500 fold by dexamethasone, to ~ 10,000 nAChR molecules/cell. Electrophysiological studies confirmed that these nAChRs were functional. Whole cell patch clamp experiments demonstrated inwardly rectifying currents which were activated by 30μM ACh and blocked by 100μM hexamethonium. These studies are further evidence that the major brain nAChR subtype is composed of 2 types of subunits, and are an ideal starting point for further studies of nAChR function, expression and regulation.