255P A COMPUTER SIMULATION OF EXPERIMENTS DEMONSTRATING THE EFFECTS OF PHARMACOLOGICAL AGENTS ON THE CUTANEOUS INFLAMMATORY RESPONSE IN THE ANAESTHETISED RABBIT

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Computer programs, which simulate undergraduate pharmacological experiments, are now widely available and most undergraduate pharmacology courses in the UK employ examples of them in one form or another. Computer simulations such as that described here can be effective in presenting data in an interactive manner and encouraging students to use it to learn and practice data-handling, data-presentation, data-interpretation and report writing skills.

Here we demonstrate a computer simulation which simulates a range of experiments designed to demonstrate the action of inflammatory mediators and pharmacological agents on the *in vivo* inflammatory response in the anaesthetised rabbit. The program uses data obtained from actual experiments and is aimed at undergraduate students on courses in which pharmacology is a major component. It was developed using Macromedia Director (version 7) for PCs (minimum specification: Pentium PC, Windows 95/98/NT4, 16 Mb RAM, 10 Mb available HD space, 16 bit colour graphics).

'Introduction' and 'Methods' sections combine text and high-quality colour graphics to describe the animal preparation, the methods employed to measure oedema formation (extravascular accumulation of ^{125}I - albumin) and neutrophil accumulation, and to provide the student with the essential background information required to understand how the inflammatory response is triggered, and the mechanisms involved.
The 'Experiments' section allows the student to select, from a menu, to study the effects of the following agents on oedema formation (and, where appropriate, on neutrophil accumulation) in **normal rabbits**: (1) *a range of direct mediators of increased microvascular permeability* [histamine, bradykinin, platelet activating factor (PAF), substance P, leukotriene D_4], either alone (dose-response relation-ships), in the presence of a vasodilator (PGE_2) or with receptor antagonists; (2) *a range of agents which cause inflammation principally via neutrophil accumulation* [complement Factor C5a, cytokines interleukins IL-1 and IL-8, the bacterial peptide f-methyl-leucyl-phenylalanine (FMLP), leukotriene B_4, tumour necrosis factor (TNF_{alpha})], either alone (dose-response relationships) and in the presence of a vasodilator (PGE_2). The effects of neutrophil depletion and the importance of adhesion molecules are also covered; (3) *non-steroidal (local and systemic effects) and steroidal anti-inflammatory agents.*
A section describing the results of selected experiments using **sensitized rabbits** is also included and covers the IgG (Reverse Passive Arthus response) and IgE response.

The results are presented in graphical form either as bar-charts or line graphs. The program contains numerous self-assessment exercises which demand interpretation of experimental data presented to them, and an understanding of the underlying inflammatory mechanisms. These student-centred activities make the program useful for self-directed learning or, in the ideal situation, it would be incorporated into a structured teaching programme and used with a teacher-designed workbook.
It is envisaged that the program could be used in a number of ways: to better prepare students who will perform the practical at a later date; to debrief students after they have performed the practical; as a 'fallback' to provide data for students whose experiments were unsuccessful; as an alternative to the practical, though it should be remembered that different learning objectives may be achieved. It may be particularly useful as a student-centered alternative in those departments where there is a lack of equipment and/or technical expertise.

256P AN INTRODUCTION TO APOPTOSIS: A PICTURE IS WORTH A THOUSAND WORDS

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The term "apoptosis" was originally coined to describe a type of cell death distinct from classical necrosis. The first descriptions of apoptosis were entirely morphological in nature and applied to hepatic cells undergoing what was originally called "shrinkage necrosis", to prostate cells that die after withdrawal of androgen, to thymocytes exposed to corticosteroids, and to cells undergoing naturally-occurring developmental cell death. The morphology of apoptosis initially involves nuclear changes followed by cytoplasmic disintegration and rapid removal of cellular debris by phagocytosis not involving a significant inflammatory response. By contrast, classical necrosis initially involves cytoplasmic changes, distinct morphologies, and an inflammatory response.

From a functional perspective, the term apoptosis soon came to be thought of as a process of gene-directed cell suicide that occurs normally during development, and which can be triggered in adult cells by a variety of exogenous stimuli, e.g. corticosteroid- induced thymocyte death. This delayed process of gene-directed cell death is in contrast to the passive process by which an extrinsic stimulus directly and immediately kills a cell (necrosis).

An extraordinary degree of confusion is now evident in the literature regarding how to define and think about the terms "apoptosis" and "necrosis", whether they are definable entities, and whether apoptosis is synonymous with "programmed cell death (PCD)" (it is not, because apoptosis and necrosis are morphological terms whereas PCD refers to gene-directed cell death independent of morphology). Although this confusion about terminology may seem to be an unimportant semantic argument, the way in which these terms are used, and how we think about them, is of considerable pharmacological significance. For example, if we think about apoptosis as any cell death in which a biochemical process is involved, then virtually all brain cell death is apoptotic and undesireable. However, if the mechanism by which cancer cells die is also apoptosis, and desireable, then an "anti-apoptotic" compound developed to prevent brain cell degeneration would be expected to cause cancer. Or, perhaps, apoptosis is neither good nor bad, nor even a definable entity, and the carelessness with which terms are applied to biological processes requires impossible mental gymnastics.

This presentation will attempt to clarify these issues as they relate to neuronal degeneration and pharmacological intervention in particular. It will also attempt to undermine the audience members' confidence in their use of these confusing, misleading, and now impossible-to-define terms.

257P THE ROLE OF THE Bcl-2 FAMILY PROTEINS IN INTEGRATING SIGNALS GENERATED BY DRUG-INDUCED CELLULAR DAMAGE

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Proteins of the Bcl-2 family are principal participants in a cellular decision-making process regarding whether a cell in a specific context will live or die. This decision is presumably based upon the integration of incoming micro-environmental survival signals and signals emanating from cellular damage. The Bcl-2 family is comprised of both anti- anc pro- apoptotic proteins. Several mechanisms have been proposed that regulate the function of various Bcl-2 family members, including transcriptional control, protein-protein interactions, translocation, phosphorylation, proteolysis and conformational change. Cells survive in culture and *in vivo* with readily detectable constitutive levels of many pro-apoptotic proteins including Bak and Bax. This implies that death-inducing signals must activate their lethal function(s) and/or inactivate the protective mechanism(s) of anti- apoptotic proteins in order for a cell subsequently to undergo apoptosis.

I will discuss how two pro-apoptotic proteins Bak and Bax are converted from innocuous latent forms to fully activated pro-apoptotic forms after disparate forms of cellular damage. A two-step model for the activation of Bak after cell damage signals is proposed. We reported exposure of an occluded N-terminal epitope in Bak after various types of cellular damage (Griffiths *et al,* 1999).

Using an antibody to the central BH-1 domair of Bak in CEM T lymphoma cells, a second damage-induced change in epitope availability occurred subsequently to that at the N-terminus. Exposure of both the N-terminal and BH1 epitopes occurred in the presence of the caspase inhibitor zVAD-fmk. Bak co-immunoprecipitated with Bcl-x_L in undamaged cells and after damage when the N-terminal epitope was exposed but the BH-I epitope remained occluded. A decrease in binding of Bak to Bcl-x_L correlated temporally with exposure of the Bak BH-I domain and the exposure of an occluded N-terminal epitope of Bax. Overexpression of Bcl-x_L did not effect the kinetics of exposure of the Bak N-terminal epitope but delayed exposure of the BH-I domain.

SH-EP1 and SH-SY-5Y human neuroblastoma cells, a pair of lines derived from a single precursor cell, differ greatly in their sensitivity to the tubulin polymer stabilising agent, paclitaxel, but show the same sensitivity to the DNA crosslinking agent, cisplatin. Both drugs, in both cell lines, induced exposure of a constitutively occluded N-terminal epitope of the pro-apoptotic protein Bax The exposure of the N-terminus of Bax was reversible and occurred before the translocation of cytosolic Bax to mitochondria. The N-terminal change in Bax and its subsequent movement to mitochondria were insufficient for commitment to death, occurring in the same proportion of cells that either maintained (SH-SY-5Y) or lost (SH- EP1) clonogenic survival after paclitaxel treatment. The N-terminus of Bax was cleaved by calpain, subsequent to exposure of the N-terminal epitope, but only in drug-sensitive cells.

These data suggest the existence of a second drug damage-induced signal that occurs following the translocation of Bax to mitochondria. This occurs prior to Bax cleavage. The activation step required for commitment to death is absent in paclitaxel resistant cells.

Griffiths DJ *et al.* 1999 J Cell Biol 144: 903-914

258P MOLECULAR ROLE OF CASPASES IN APOPTOTIC CELL SUICIDE AND HUMAN DISEASE PATHOGENESIS

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Cells commit suicide by apoptosis under both physiological and pathogenic circumstances. The caspases are a family of cysteine proteases which are necessary for both the initial signalling events and the downstream proteolytic cleavages that manifest the apoptotic phenotype.

Understanding the specific role of each of the currently known caspase family members has been aided by selective inhibitors, X-ray crystal structures, knock-out mice and the determination of precise substrate specificities using a combinatorial positional-scanning substrate library. Multiple pathways of caspase activation, subcellular compartmentalization and regulatory control of these enzymes by macromolecular inhibitors determine the circumstances under which apoptosis proceeds *in vivo.*

259P MECHANISM OF APOPTOSIS INDUCED BY THE HIV COAT PROTEIN, gp120, IN THE BRAIN OF RAT

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Cognitive impairment, postural disorders and tremor are among the most common symptoms encountered in patients suffering from *AIDS dementia complex* (ADC), a neurological syndrome described in some 80% of the patients suffering from AIDS (see Price & Perry, 1994).

Neuropathological features of the brain described at post *mortem* are myelin pallor, appearance of multinucleated giant cells, infiltration by blood-derived macrophages, astroglial cell reaction and brain cortical neuronal cell loss (see Everall *et al.*, 1991). The syndrome has been attributed to infection of the brain caused by the human immunodeficiency virus type I (HIV-I) because it is observed in patients free from opportunistic infections or concomitant cancer in the brain, though neuroinvasive strains of the HIV virus infect macrophages, microglial cells and multi-nucleated giant cells but not neurones (see Mucke *et al.*, 1995). Processing of the virus by cells of the myelo-monocytic lineage yields host and viral products known to initiate a complex network of events which may lead neurones to death and the development of ADC. In particular, the HIV-I coat protein gp120 has been proposed as a likely aetiologic agent of the described neuronal loss because it causes death of neurones in culture.

Using the TUNEL technique (Gavrieli *et al.*, 1992) we have shown the occurrence of DNA fragmentation in brain cortical tissue sections of adult rats receiving injections of the viral protein into one lateral cerebral ventricle (i.c.v.; Bagetta *et al*, 1995) suggesting the apoptotic nature of neuronal death; the latter deduction has been confirmed at the ultra-structural level (Bagetta *et al*, 1996). Recent immunohistochemical and western blotting data implicate the pro-inflammatory cytokine IL-lp in the mechanisms of apoptosis caused by gpl20 (Bagetta et *al.*, 1999). Furthermore, immunoprecipitation and immunoelectron microscopy data have established that gpl20 enhances IL-IP expression in the mitochondrial fraction of brain tissue preparations and this is paralleled by a large reduction of the mitochondrial pro-IL-1PPP 31 kDa band, suggesting that activation of interleukin converting enzyme (ICE) is implicated. In agreement with the latter deduction, combined treatment with gpl20 and the inhibitor II of ICE minimizes apoptotic cell death induced by the viral protein (Bagetta *et al.*, 1999). Interestingly, experimental evidence demonstrates that gpl20 induces the expression of COX- 2 (Bagetta *et al*, 1998), an inducible enzyme whose expression is enhanced by IL-l. The latter effect is paralleled by neocortical accumulation of PGE (Corasaniti *et al*, 2000), which may elevate synaptic glutamate to cause excitotoxic neuronal death (see Bezzi *et al*, 1998). In fact, pretreatment with NS398, a COX-2 inhibitor, with glutamate receptor antagonists or with U- 74389G, a free radical scavenger of the 21-aminosteroid family, reduced gpl20-induced apoptosis (Corasaniti *et al*, 2000). In conclusion, confirmation at the ultra-structural level of the occurrence of apoptosis in the brain cortex of AIDS patients (Petito & Roberts, 1995) and, indeed, of patients suffering from chronic neurodegenerative diseaseas such as Alzheimer's and Parkinson's disease, in which an important role for IL-lβ has been suggested (Griffin *et al*, 1989), will validate the usefulness of the rat model we have developed for the characterisation of the neuroprotective profile of drugs which interfere with mediators of neuroinflammation and crucial steps involved in the activation of the death programme.

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260P THE ROLE OF PROTEIN-PROTEIN INTERACTIONS IN THE TARGETING OF METABOTROPIC GLUTAMATE RECEPTORS

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The metabotropic glutamate receptors (mGluRs) are a family of seven transmembrane spanning, heterotrimeric G-protein coupled receptors. The mGluRs can be subdivided into three groups on the basis of their sequence and pharmacologies. Generally the Groupl mGluRs (mGluR1 and mGluR5) are postsynaptic, whilst members of Groups 2 and 3 can be found pre-synaptically.

Recently a family of proteins which interact with the C-terminal tail of the Groupl mGluRs has been identified, termed the Homers (Brakeman *et al*, 1997; Kato *et al*, 1998). In order to determine whether these have a role in the neuronal trafficking of the mGluRs we have examined the effects of expressing the Homer-la and Homer-1c on the cell surface expression in heterologous expression systems and the neuronal targeting of mGluR1 in primary cultures of rat cortical neurones. Expression of either Homer-la or Homer-1c with mGluRlα in HEK 293 cells caused a significant increase in the cell surface expression of the receptor as assessed by immunofluorescence staining of the cells and cell surface biotinylation of the membrane molecules. In these experiments, only Homer-1c was found at the plasma membrane and only when co-expressed with mGluR1α, suggesting that it may have a role in anchoring the receptor at the cell surface. This is also suggested by the reduction in the spontaneous recycling rate of cell surface mGluR1α when expressed with Homer-la. Expression of mGluR1α with Homer-1c also caused a dramatic increase in the dendritic trafficking of the receptor in rat cultured neurones.

These results indicate that these Homer-proteins facilitate the transport of mGluR1α to the cell surface and that Homer-lc acts to retain the receptor. Since the short splice variant of mGluR1, mGluR1β, is missing the Homer binding domain we have examined its intracellular targeting. Expression of this in cultured neurones results in the retention of the receptor in the cell soma. Transfer of the C-terminal tail of mGluRIβ to CD2, a single transmembrane molecule, also causes this to be retained in the cell soma, whereas native CD2 is readily transported throughout the axon and dendrites. Mutagenesis and truncation of the C-terminal tail of mGluR1β has identified a region of positively charged amino acids that cause both the somatic retention of the receptor in neurones, and reduce its cell surface expression in heterologous expression systems. The presence of the charged motif also results in the receptor being accumulated in the endoplasmic reticulum and having, even at the cell surface, immature endoglycosidase H sensitive carbohydrate groups.

Since, in the brain, the mGluR1β receptor is fully endoglycosidase H resistant, the data suggest that mGluR1β is differently trafficked in neurones. Together, these results suggest that the neuronal targeting of the mGluR1 splice variants may be regulated by different means.

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261P COMPETITIVE BINDING OF αCaMKII AND PSD-95 TO THE NR2A C-TERMINAL DOMAIN

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The N-Methyl-D-aspartate (NMDA) receptor complex, Ca^{2+}/ calmodulin-dependent protein kinase II (CaMKII) and PSD-95 are three major components of the hippocampal Post-Synaptic Density fraction (PSD). Both (αCaMKII and PSD-95 have been shown to directly bind NR2 subunits of the NMDA receptor complex, even if the nature and mechanisms of targeting to the NMDA receptor subunits are not completely understood.

Here we report that the C-terminal NR2A (S1389-V1464) sequence was sufficient to guarantee the association of both native and recombinant (αCaMKII and PSD-95. PSD-95 (54-256), containing the PDZ1 and PDZ2 domains, was able to compete in a concentration-dependent manner (1-50nM) with the binding of both native and recombinant (10 nM) (αCaMKII to NR2A(1244-1464); no competition was observed with the binding of αCaMKII to NR2A (1244-1461) lacking the tSDV domain.

Accordingly, recombinant (10 nM) αCaMKII(1-325) but not αCaMKII(315-478) competes with both native PSD-95 and native CaMKIl for the binding to NR2A(1244-1464). In addition, Ser/ Alal289 and Ser/Aspl289 point mutations on unique CaMKII phosphosite of NR2A did not significantly influence the binding of native aCaMKII and PSD-95 to the NR2A C-tail. Finally, the interaction between αCaMKII/PSD-95 and NMDA receptor complex was strongly modulated by chemically or electrically induced changes in synaptic plasticity in hippocampal slices. In particular, 15 min after either high frequency stimulation or after addition of glutamate/ glycine, αCaMKII was strongly targeted to NR2A/ B subunits and concomitantly PSD-95 was removed from the NMDA receptor complex; this biochemical effect was accompanied by an increased αCaMKII activity (+252.7±21.7%).

In conclusion, our data demonstrate that an αCaMKII/PSD-95 competition on the NR2A C-tail both *in vitro* and in hippocampal slices after LTP induction may account for the molecular changes in the postsynaptic compartment in activity-dependent synaptic plasticity.

262P GLYCINE RECEPTORS, $GABA_A$ RECEPTORS AND GEPHYRIN: PROTEIN INTERACTIONS AT INHIBITORY SYNAPSES

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Synapse formation and synaptic signalling require the selective assembly of membrane proteins, cytoskeletal components and signal transduction elements at specific sites of cell-cell contact.

Recent data indicate an important role ofcytoskeleton-associated and lipid-anchored proteins in the formation and maintenance of inhibitory postsynaptic membrane specializations. Membrane apposition of the receptor-associated protein gephyrin constitutes an essential prerequisite for the recruitment of inhibitory glycine and $GABA_A$ receptors to developing postsynaptic sites. This process involves both microtubules and microfilaments, and appears to be controlled by both activity-dependent Ca^{2+} influx and trophic factors that regulate cytoskeletal dynamics and downstream signalling processes.

Newly disclosed interactions of gephyrin with exchange factors for G-proteins of the Rho/Rac- family, the translational regulator RAFT and actin binding proteins like profilin are postulated to integrate activity-dependent and hormonal signals at glycinergic and GABAergic postsynaptic sites.

We propose a membrane activation model for inhibitory neurotransmitter receptor complexes, in which receptor clustering and recruitment of subsynaptic signalling cascades depend on the activation of PIP-3 kinase.

263P THE PHARMACOLOGICAL SIGNIFICANCE OF PROTEIN-PROTEIN INTERACTIONS

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P harmacological intervention in the treatment of disease states typically involves selective targeting of drugs to specific protein receptors (e.g. ligand-gated ion channels [e.g. 5-HT_3 receptors], G-protein coupled seven transmembrane domain receptors [e.g. β-adrenoreceptors]). These established drug targets have generally been viewed as distinct entities functioning in isolation, with little or no direct interaction with other proteins (apart from direct interaction with transduction system proteins, e.g. G-proteins).

Recent work, much of which has been made possible with the advent of the yeast two hybrid technique and other methods allowing an assessment of direct interactions between proteins, has revealed that this is not the case. Receptor structure and function are in fact governed by complex interactions with other cytosolic proteins, many of which have been identified by cloning studies. These additional, accessory proteins have a number of functions. In some cases, they are an absolute requirement for the correct folding, in assembly and targeting of receptors to precise regions of the cell membrane. Alternatively, they may act to modulate receptor function or mediate downstream cell signalling processes.

Typical examples include the binding of gephyrin to a cytoplasmic domain of the inhibitory glycine receptor and PSD proteins with the C-terminus of excitatory ionotropic glutamate receptors. However, a great deal remains to be investigated. For instance, whether protein-protein interactions dysfunction in pathological states, either cause or effect, remains to determined. Indeed, given that the information concerning these protein-protein interactions is still limited, it may be too early to predict the potential benefits resulting from this knowledge. But the hope of being able to pharmacologically or genetically modulate physiologically relevant protein-protein interactions for therapeutic benefit provides a focus for this area of cell biology.

264P PRECLINICAL PHARMACOLOGY OF R121919, A CRFI RECEPTOR ANTAGONIST

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Corticotropin-releasing factor (CRF) is the major regulator of the hypothalamic pituitary-adrenal axis. This neuropeptide plays a key role in the coordination of the endocrine, autonomic and behavioral responses to stress. Several lines of evidence suggest that CRF is involved in the pathophysiology of affective disorders. Depressive patients have been found to exhibit elevated concentrations of CRF in cerebrospinal fluid.

Hypersecretion of endogenous CRF could also be implicated in anxiety-related disorders, since central administration of CRF produces anxiety-like behaviour in experimental animals. The effects of CRF are mediated by two G protein-coupled receptors named CRF1 and CRF2. Whilst both receptors are present in the central nervous system, the CRF1 receptor is the most abundant.

Based on the information reported above, CRF1 receptor antagonists may represent a novel class of drugs for the treatment of depression and/or anxiety disorders. The cloning and subsequent expression of human CRF receptors have enabled the discovery of different classes of non-peptide molecules that can specifically and selectively antagonise the function of the CRF1 receptor.

The compound R121919 is a pyrazolopyrimidine with high affinity for the CRF1 receptor (2 - 5 nM) and over 1000-fold weaker activity at the CRF2, CRF binding protein or 70 other receptor types. In *in vitro* assays, it potently inhibited CRF-stimulated cAMP accumulation from cells expressing the human CRF1 receptors and CRF-stimulated ACTH production from rat cultured pituitary cells. *In vivo* investigations have shown that R121919 dose-dependently occupied the central CRF1 receptors after oral aministration, and antagonized CRF- and stress-induced behavioural effects in rats and gerbils. Furthermore, R121919 attenuated the abnormal behviour in CRF-overexpressing transgenic mice. In addition, it has been shown active in several animal models of anxiety and antidepressant-like activity.

Altogether, these findings demonstrate that R121919 is a potent, orally active and selective CRFI receptor antagonist which could be beneficial in stress-related disorders.

265P EMERGING ROLE OF THE OREXIN LIGAND-RECEPTOR SYSTEM IN MODULATING AROUSAL AND BEHAVIOUR

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Orexin-A and orexin-B are novel neuropeptides initially isolated from hypothalamic extracts but now known to *be* present in fibres projecting to sites throughout the brain and spinal cord.

The orexins bind with high affinity and activate two G-protein coupled receptors, orexin-1 (OX_1) and orexin-2 (OX_2) (Sakurai *et al* 1998), which are also broadly distributed in the CNS thereby suggesting that the physiological role of the orexin ligand-receptor system is likely to be complex. Indeed, centrally administered orexins have been shown to evoke a number of behavioural changes including feeding, grooming, locomotion and arousal (Hagan *et al.*, 1999). In particular, there is growing evidence that the orexinergic system is an important modulator of the sleep-wake cycle of animals and man. For example, centrally administered orexin-A (1, 10 or 30 µg/rat icv) produces a dose-dependent increase in the time rats spend awake, when administered at the onset of the normal sleep period. The enhancement of arousal is accompanied by a marked reduction in paradoxical sleep and deep slow wave sleep at the highest dose (Piper *et al.*, 2000). In addition, prepro-orexin knockout mice exhibit a narcolepsy phenotype (Chemelli *et al.*, 1999) and the sleep disorder canine narcolepsy is caused by a mutation in the OX_2 receptor gene (Lin *et al.*, 1999).

Even more strikingly, two independent groups of researchers have now shown a dramatic 85-95% loss of orexins in the hypothalamus and associated projection areas of narcoleptic subjects (Peyron *et al.*, 2000; Thannickal *et al.*, 2000). Thesefindings are consistent with an earlier study demonstrating markedly reduced CSF levels of orexin-A in 7 out of 9 narcolepsy patients(Nishino *et al.*, 2000).

Taken as a whole, available data suggest that deficient orexin neurotransmission, possibly caused by a degenerative process (van den Pol 2000), may be a key factor leading to narcolepsy.

Studies *in vivo* with the recently identified selective OX_1 receptor antagonist l-(2-methylbenzoxazol-6-yl)-3-U ,5]naphthyridin-4-yl urea hydrochloride, SB-334867-A (OX_1 pKb = 7.4; OX_1 pKb = 5.7), indicate that this particular receptor subtype also plays a role in mediating the behavioural arousal evoked by the orexins (Duxon *et al.*, 2000; Jones *et al.*, 2000).

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266P FUNCTIONAL ANALYSIS OF THE KALLIKREIN-KININ SYSTEM IN TRANSGENIC ANIMAL MODELS.

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Kinins are important mediators in cardiovascular homeostasis, inflammation and nociception. In order to clarify the cardiovascular actions of these peptides we generated a transgenic rat model expressing the human tissue kallikrein gene in the whole body. The abundance of kinins in this model leads to alterations in blood pressure control and protects the heart from hypertrophic and ischemic damage.

Two kinin receptors have been described, Bl and B2. TheB2 receptor is constitutively expressed and its targeted disruption leads to salt-sensitive hypertension and altered nociception. The Bl receptor is a heptahelical receptor distinct from the B2 receptor in that it is highly inducible by inflammatory mediators like bacterial lipopolysaccharide and interleukins.

In order to clarify its physiological function, we have generated mice with a targeted deletion of the gene for the Bl receptor. Bl-receptor deficient animals are healthy, fertile, and normotensive. In these mice bacterial lipopolysaccharide-induced hypotension is blunted and there is a reduced accumulation of polymorphonuclear leukocytes in inflamed tissue. Moreover, under normal non-inflamed conditions they are analgesic in behavioral tests of chemical and thermal nociception.

Using whole cell patch clamp recordings, we showed that the Bl receptor is not necessary for regulating the noxious heat sensitivity of isolated nociceptors. However, using an *in vitro* preparation we could show for the first time that functional Bl receptors are present in the spinal cord, and their activation can facilitate a nociceptive reflex. Furthermore, in Bl-receptor deficient mice we observed a reduction in the activity-dependent facilitation (wind-up) of a nociceptive spinal reflex. Thus, the kinin-BI receptor plays an essential physiological role in the initiation of inflammatory responses and in the modulation of spinal cord plasticity that underlies the central component of pain. These results from our transgenic animal models with alterations in the kallikrein-kinin system led us to conclude that kinin receptors represent suitable pharmacological targets for the treatment of cardiovascular diseases as well as of inflammatory disorders and pain.

267P THE ROLE OF CGRP AND CGRP ANTAGONISTS IN MIGRAINE

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The 37-amino acid peptide calcitonin gene-related peptide (CGRP) is widely distributed throughout the body, where it is mainly localised in neurones of the central and the peripheral nervous system. CGRP is co-localised with substance P in primary afferent neurones, playing an important role in the transmission and modulation of nociceptive input. One of the most pronounced effects of CGRP, however, is its vasodilatory action on a wide range of blood vessels. CGRP, once released from nerve endings associated with the blood vessel wall, is believed to be amongst the most potent endogenous vasodilators.

Migraine is one of the most common neurological disorders, involving periodical attacks of headache, nausea as well as a plethora of other symptoms. Although considerable progress has been made, the pathophysiology of migraine is still not understood. However, several observations point to an involvement of CGRP. Migraine headache involves the activation of the trigeminal system and dilatation of cranial vessels. CGRP is localised to neurones in the trigeminal ganglia and is released into the blood after stimulation of the trigeminal ganglia in man. Most importantly, CGRP levels in the cranial circulation are increased during a migraine attack, possibly causing the vasodilatation observed. Further research into the physiological role played by CGRP in migraine has, however, in the past been hampered by the lack of good pharmacological tools. Nevertheless it is conceivable that inhibition of CGRP-evoked dilatation of the cranial vessels may provide a novel treatment for migraine headache.

We have therefore aimed to develop a non-peptide CGRP antagonist to probe this hypothesis.The prototypical compound, BIBN 4096BS, shows sub-nanomolar affinity for CGRP receptors on human SK-N-MC neuroblastoma cells, which express functional CGRP-1 receptors. Furthermore, BIBN4096BS is a potent antagonist (pKB~11) for CGRP-induced cAMP stimulation in SK-N-MC cells. In functional experiments on cultured human microvascular endothelial cells, BIBN4096 inhibits CGRP stimulated cAMP production with a pKB value of 10.9. The radioanalogue, [^{3}H]-BIBN4096 labels a single class of high affinity binding sites on SK-N-MC cells as well as various marmoset brain regions and dura mater. Most importantly, in the marmoset, BIBN4096BS potently inhibits facial skin flow evoked by electrical stimulation of the trigeminal ganglion. It is, furthermore, interesting to note that BIBN4096 is highly selective for the primate versus rodent CGRP I receptor.

These data underline that we have developed a high-affinity, selective CGRP antagonist, which will be a useful tool to investigate whether antagonising the effects of CGRP in migraine may be a novel therapeutic concept.

268P A D1-RECEPTOR/PKA/DARPP-32/PPI PATHWAY REGULATES AMPA RECEPTOR PHOSPHORYLATION AND CONDUCTANCE IN THE NEOSTRIATUM

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Dopamine (DA) plays a critical role in regulating the glutamate excitability of medium spiny neurons of the neostriatum. One mechanism by which DA modulates neuronal excitability is through activation of D1-type receptors linked to stimulation of the cAMP-dependent protein kinase (PKA) and phosphorylation of DARPP-32 (dopamine and cAMP-dependent phosphoprotein, Mr32kD), an inhibitor of protein phosphatase I (PPI). We examined the possible role of the DARPP-32/PPI signalling cascade in regulating the AMPA-type glutamate receptors using mice lacking the gene for DARPP-32.

Using whole-cell patch clamp techniques, activation of Dl receptors with SKF81297 (10 pM) was found to enhance kainate-induced AMPA currents in striatal neurons from wildtype mice. This effect was abolished in striatal neurons from DARPP-32 knockout mice. We then examined the possibility that Dl receptor activation enhanced AMPA currents via direct PKA-mediated phosphorylation of the AMPA receptor modulated by the DARPP-32/PPI pathway. In striatal slices from wildtype mice, DA (100 pM) increased phosphorylation of the receptor on the GluR1 subunit at Ser845, a PKA-dependent residue that controls channel open time probability of the AMPA receptor. In contrast, DA-stimulated phosphorylation of GluR1 at Ser845 was absent in slices prepared from DARPP-32 knockout mice.

These data support the idea that direct phosphorylation of the AMPA receptor, mediated by a pathway involving PKA, DARPP-32, and PPI is necessary for Dl receptor regulation of AMPA currents. In addition, we sought to determine whether physiological activation of DA neurotransmission during psychostimulant drug treatment resulted in DARPP-32-dependent phosphorylation of the AMPA receptor. Wildtype and DARPP-32 knockout mice were injected with cocaine (10mg/kg) and sacrificed by focused microwave irradiation, a technique that preserves the state of phosphorylation of brain phosphoproteins. Phosphorylation of GluR1 at Ser845 was increased severalfold after cocaine treatment in the striatum of wildtype, but not DARPP-32 knockout mice.

Taken together, these data indicate that a PKA/DARPP-32/PPI pathway mediates phosphorylation-dependent activation of AMPA receptors *in vivo*.

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269P STIMULATION OF DI/PKA/DARPP-32/PP-1 PATHWAY IS REQUIRED FORCORTICOSTRIATAL LTD AND LTP

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Dopamine (DA) receptor activation represents a critical requirement for the induction of both corticostriatal LTD and LTP and modulates the activity of PKA and DARPP-32, a potent inhibitor of protein phosphatase-I (PP-I).

To test whether the stimulation DI/PKA/DARPP-32/PP-1 pathway is required for striatal synaptic plasticity, we performed intracellular recordings from mouse and rat corticostriatal slices. Both LTD and LTP were absent in slices obtained from mice lacking DARPP-32. In these animals, however, these forms of synaptic plasticity were restored by okadaic acid (100 nM) or calyculin A (100 nM), two PP-I inhibitors. In control animals, bath application of either SCH 23990 (10 pM), a Dl-like DA receptor antagonist, or H89 (l0pM), a PKA inhibitor, were equally effective in preventing both LTD and LTP, whereas, when H89 (100 pM) was applied intracellularly through the recording electrode only LTP was blocked.

These data confirm the requirement of the Dl-like receptor/PKA pathway for corticostriatal LTD and LTP induction, but suggest that for LTD this pathway is not activated post-synaptically on spiny neurons but presumably on NOS-positive intemeurons. Accordingly, the NO donor SNAP and zaprinast, a cGMP-phosphodiesterase inhibitor were able to induce LTD in control mice. These pharmacological agents, however, failed to induce LTD in DARPP-32 lacking mice suggesting that DARPP-32 is activated by PKA to induce LTP and by PKG, stimulated by NO released from interneurons, to induce LTD. Moreover, SCH 23990 failed to prevent the induction of SNAP- and zaprinast-induced LTD suggesting that the stimulation of Dl-like receptors, required for the induction of post-tetanic LTD, lies upstream of NO activation.

270P THE DOPAMINERGIC MODULATION OF STRIATAL EXCITABILITY IS STATE-DEPENDENT: NEW INSIGHTS INTO OLD PARADOXES

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Traditional approaches have failed to resolve a number of apparent paradoxes concerning dopaminergic regulation of striatal excitability. For example, how is it that dopamine can excite neurons in one situation and inhibit them in others? Is this attributable to receptor heterogeneity, complex circuit behavior or some other phenomenon?

Approaches that view dopamine as a neuromodulator, rather than a classical transmitter that can be classified as inhibitory or excitatory, are beginning to malce significant progress toward unraveling this mystery. Through coordinated modulation of several distinct ionic conductances, D_1 and D_2 receptors regulate excitability in a state-dependent fashion. This state dependence arises from the tendency of striatal medium spiny neurons to move from hyperpolarized "down" states to depolarized "up" states where they discharge.

D_1 receptor stimulation stabilizes the hyperpolarized down-state and makes the transition to the up-state less probable. However, once the up-state is achieved, discharge probability is enhanced. By modulating a complementary set of conductances, D_2 receptors destabilize the up-state, bringing about a session of firing in response to cortical input. This state-dependent behavior has fundamental implications for our understanding of how dopamine shapes striatal information processing.

My talk will: (1) briefly summarize the evidence that D, receptors modulate ion channels governing up- and down-states in medium spiny neurons; and (2) present new data showing that the D_2 receptor modulation of Na^+ and Ca^{2+} channels regulating the up-state in medium spiny neurons relies upon a novel signalling linkage.

271P DOPAMINE D_3 RECEPTORS: PHARMACOLOGY AND POTENTIAL THERAPEUTIC APPLICATIONS

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Dopamine D_3 receptor mRNA has been reported in the ventral striatum, nucleus accumbens, dentate gyrus and cortical areas of rat and human brain, and autoradiographic studies with a variety of ligands confirm this distribution. Although there are a number of hypotheses relating to the roles of D3 receptor functions clarification of these roles has been hampered by a lack of selective ligands. SB-277011-A is a high affinity antagonist at cloned human dopamine D_3 receptors (pKi= 7.95) with 80 fold selectivity over hD_2 receptors.

In microdialysis experiments *in vivo,* the D_2/D_3 receptor agonist quinelorane reduced dopamine efflux in nucleus accumbens and striatum and pre-treatment with SB-277011-A dose-dependently antagonised this effect of quinelorane in the nuc. accumbens but not in the striatum. Even at high doses, SB-277011-A (2.5-79 mg/kg p.o.) did not induce catalepsy and did not increase serum prolactin levels. The effect of acute and repeated administration of SB-277011-A was studied on the activity of spontaneously active midbrain dopamine (DA) neurons in anaesthetized, male Sprague-Dawley rats, using m *vivo* extracellular single unit recording. Repeated administration (once daily for 21 consecutive days) of SB-277011-A (1, 3 and 10 mg/kg p.o.) produced a significant decrease only in the number of spontaneously active DA neurons in *the* ventral tegmental area.

As previously reported, repeated administration of the non-selective dopamine D_2/D_3 receptor antagonist haloperidol produced a significant decrease in the number of spontaneously active DA cells in lx)th the substantia nigra pars compacta and ventral tegmental area compared to vehicle controls.

These data suggest that dopamine D_3 receptors may play a role in regulating the functions of dopaminergic neurons and that SB-277011-A will be a valuable tool in further understanding the role of dopamine D_3 receptors in regulating physiological and behavioural functions.

272P FROM A SCIENTIFIC FINDING TO AN INDUSTRIAL PROJECT

Cesare R. Sirtori, Professor of Clinical Pharmacology, University of Milan, Italy.

Novel opportunities for collaboration between Academia and Industry may stem from occasional discoveries in experimental work and in the clinic, leading to molecules of potential therapeutic interest. A significant case in point has been the discovery of the apolipoprotein AI_{Milano} (AIM) mutation. This mutation of a major lipid-carrying molecule results, phenotypically, in markedly reduced highdensity lipoprotein (HDL) cholesterolaemia and, paradoxically, apparent protection from cardiovascular disease. This paradox has become a cult item for the media.

Biochemical and structural analyses of the mutant AIM (a Cys for Arg substitution at amino acid 173) uncovered a remarkable potential for tissue cholesterol removal *versus* wild type Al. This unique property is particularly evident with the dimeric form of AIM (AIM dimer) that also displays a prolonged elimination half life. Molecular cloning and production of the AIM dimer by engineered *E.coli* was successfully achieved by Kabi Pharmacia in Sweden. Post-merger, Pharmacia-Upjohn decided not to proceed with AIM development.

A number of significant data had, however, been collected in animal models. Among the major properties were the capacity for direct removal of diffuse atheromatous plaques in apo E KO mice with single injections of bolus doses, a marked reduction of arterial restenosis after perivascular manipulation in rabbits, dramatically reduced intra-arterial proliferation after coronary stenting in pigs (at very low doses) and, finally, direct real-time removal of atheromatous plaques from a rabbit model of carotid stenosis. A new company was, therefore, started in collaboration with the former Parke Davis research group responsible, among others, for the development of atorvastatin, now the number one drug on the world market.

Esperion Therapeutics opened in 1998 and went public on August 2000. Current market values average around US $300-320 million and cash availabilities around US $75 million. The company has 48 employees and 5 molecules at various stages of development: l»esides AIM, two synthetic molecules for raising HDL cholesterol levels (thus enhancing the reverse cholesterol transport system), large unilamellar phospholipid vesicles for plaque removal, and wild type pro apoA-1, to be associated with apheretic therapy for severe hypercholesterolaemia.

The start of Esperion also made it possible to achieve significant funding for ongoing research on the molecule at the University of Milan. The project had been intermittently and generally modestly financed for the past 20 years. Esperion provides another example of a start up company giving benefit to research and eventually, to investors, in the face of the severe crisis of large pharmaceuticals, unlikely to reach financial expectations in the years to come and likely actors in a series of mergers (James, SCRIP Magazine, Sept. 2000).

Companies of this type may offer a potential opening for pharmacologists plagued, on the one side, by the upcoming crisis of pharmaceutical industry and, on the other, by the questionable fame of getting substantial benefits from industry and, thus, not entitled to receive adequate public support. Will pharmacologists go back to discovering drlug treatments?

273P NEW SAFE MEDICINES FASTER: A EUFEPS-LED PROPOSAL FOR EU 6th FRAMEWORK PROGRAMME

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A targeted effort to speed up the development of safe, new medicines is sorely needed in Europe. Stronger links between industry, academia and regulatory authorities, more efficient use of modern technology, new methods of drug exploration and targeted training are all vital elements of a streamlining process that cries out to be set in motion. Without it, the European pharmaceutical industry is in danger of losing important ground on global markets - a situation detrimental both to European economies and the patients seeking relief from illness and disease.

Despite being the fifth strongest industry in Europe, generally the pharmaceutical industry is severely hampered by an approach to drug development and approval that is ill equipped to exploit the huge opportunities presented by modern drug discovery. Growing demands regarding safety, efficacy and quality documentation consume vast amounts of research and development expenditure. But, at the same time, the average number of years spent or getting a new drug through development and onto the market has not changed materially.

In 1999, the European Federation for Pharmaceutical Sciences (EUFEPS), European Federation of Pharmaceutical Industries and Associations (EFPIA) and the Danish Medicines Agency took the initiative to set the ball of change rolling. A key action entitled "New safe medicines faster" was proposed for the EU's forthcoming 6th RTD framework programme.

The proposed key action has three main objectives:

- to seek new technology capable of more effective selection of potential drug candidates for innovative medicines while accommodating safety demands,
- to use such technology to increase the capacity as well as speed up the pharmaceutical development process and eliminate bottlenecks,
- to cultivate a pan-European interdisciplinary network that bridges the gap between industry, academia and regulatory authorities.

Significant strides have been made in moving this important research-led initiative forward through various discussions together with an EU supported meeting in Brussels, bringing together industrialists, academics and regulators. Progress to date will be reported.

274P THE BIOMEDICAL SCIENCE PARK; A STIMULATING ENVIRONMENT IN WHICH TO FOSTER INTERACTIONS BETWEEN ACADEMIA AND INDUSTRY

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In 1996, the Schering-Plough Institute established a research unit focussed on neuroscience at the San Raffaele Science Park in Milan, within a multi-tenant building located next to a large and renowned teaching hospital. Here there are a number of research groups funded by the San Raffaele Foundation, public and private universities, the National Research Council and various charities. In addition, like Schering Plough, other companies have set up research groups of 50 or less. This is the case for Roche (immunology), Bayer (biotechnology) and Bracco (diagnostics) to name but a few.

The aim of this talk is to illustrate our experiences within this new environment. Strengths and weaknesses will be critically examined in an attempt to provide useful information to other initiatives which largely rely on efficient interaction between academia and private enterprise.

We had a great advantage in recruiting high quality researchers. A location within a highly scientific environment with good standing within the scientific community is critical in building up a group of research excellence: scientists of high calibre have a higher propensity to accept positions in a nrivate biotech or pharma companv if the environment allows optimal exchange with the scientific community. Ultimately, scientists share a pride in being part of the science park in addition to their individual group. Moreover, the presence of young, talented scientists is also critical in building up a high-quality group. PhD students and post-doctoral fellows are more likely to work within private enterprise when a 'university/campus' atmosphere is present. Their presence and spirit are certainly an important addition to the group.

Another obvious advantage is the so-called 'cross-fertilisation' among scientists within the science park community. This accelerates the acquisition and maintenance of an updated knowledge base and state-of-the-art technology.

Regarding research projects, our experience is that the group should maintain a maximum degree of freedom whilst paying attention primarily to expertise available locally. This is a delicate issue: on the one hand, the need to identify collaborations within the same community is obvious; however, the project itself deserves the highest priority and should guide the identification of the possible partners, regardless of their location.

Our experience is that the science park can provide several competitive advantages for small research groups (either small-medium enterprises or research groups of large companies). Working in close proximity affords significant mutual benefits to both private and public research groups.

275P LEVERAGING TECHNOLOGY THROUGH ACADEMIC-INDUSTRIAL COLLABORATION

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One of the key drivers for the recent spate of mergers and acquisitions in the pharmaceutical industry has been the spiralling cost of Research & Development. Pharmaceutical companies must continually seek new ideas and information, as well as maximising its own knowledge base, in order to gain and/or maintain a competitive advantage. Increasingly, companies are looking to gain leverage of science and technology through partnerships. Potential partners for industry include other companies, including biotechnology and university spinoff companies, universities and research institutes, the national and European funding councils and the research charities. The primary objective of these research partnerships is to share the costs and risk of the research and to maximise the potential for shared rewards from exploitation of the science.

This paper focuses on a variety of collaborative mechanisms, citing specific examples, where the output of the collaboration is greater than the sum of the parts.

Consortia have been initiated to address certain scientific problems too large for any single company to tackle in a timeframe that will lead to competitive advantage. For example the Single Nucleotide Polymorphism (SNP) Consortium was initiated in April 1999 to identify 300,000 and to map 150,000 SNPs for subsequent use in association studies. This £30 million project has a two-year time span and is funded by The Wellcome Trust and twelve companies. All of the information generated from this project is released into the public domain and is managed by a board with an independent chairman. This model is currently being used to assess whether it is possible to initiate a similar scheme where companies are aiming to determine X-ray crystal structures of hundreds of proteins.

Smaller consortia have been put together to address the specialised training needs of industry through sponsorship of Masters' training packages. This training will often involve areas of technology where the major advances have been driven by industry rather than academia, and collaboration is essential if we are to train our next cohort of recruits in "State of the Art" techniques.

The large pharmaceutical companies have an excellent track record of working with university research groups to nurture research ideas and technologies. This may be through a number of national or internationally driven schemes where additional funds may be leveraged to support basic underpinning research. Examples may range from a CASE studentship or LINK agreement to an EU Marie Curie Fellowship or Framework programme grant. This paper will provide several examples of "win-win" collaborations demonstrat ing the value of a well-managed portfolio of research collaborations.

276P THE CONSORZIO ROBERTO LEPETIT FOR THE DEVELOPMENT OF BIOTECHNOLOGY: NEW IMPULSE TO ACADEMIA AND INDUSTRY COLLABORATION

Rolando Lorenzetti, Consorzio Roberto Lepetit, via R. Lepetit 34, 21040 Gerenzano (VA), Italy

The Consorzio Roberto Lepetit was founded in February 1998 to promote research and training activities in the field of biotechnology. Participating in this Consortium, named in remembrance of Roberto Lepetit, are the Universities of Bologna and Palermo, among the major Italian Universities, and Biosearch Italia SpA, a biotechnology company established in 1997 through a management buy-out process of the former Gerenzano Research Center of Aventis Pharma and recently quoted on "Nuovo Mercato". Biosearch Italia is dedicated to the research, development, production and commercialization of new antibiotics active against infections sustained by microorganisms resistant to the drugs currently in clinical use. By using an integrated biotechnological approach, the Company looks for new natural molecules produced by microorganisms, of which it owns one of the largest collections in the world.

To fulfil its mission, the Consortium has established a form of facilitated participation by other industries and academic insititutions as well as single investigators from Universities or other public research institutions. The Consortium can avail itself of the up-to-date facilities present at Biosearch Italia including, among others, a pilot plant for microbial fermentation, chemical synthesis, protein and natural products purification and automated systems for high throughput screening, as well as the most advanced analytical instrumentation.

The Consortium aims to promote the development of biotecnology through the management of research projects which, because of the complexity of the required facilities, technology and competences, are hardly feasible in a single location, either academic or private. It is a way to put into practice those initiatives of stimulation of national research through a more direct contact between the academic and industrial worlds: a contact finalized to the development of products and processes of particular applicative interest. This initiative will allow us to carry out a continuous osmosis of ideas and personnel for the realization of research projects in a field as critical for the Italian economy as biotechnology, with the possibility of immediate transfer of results and know-how to the productive world.

Along this line, the Consortium is also devoting part of its activities to the management of training courses in order to allow young investigators to develop from good academic researchers into excellent industrial researchers. In line with most recent governmental initiatives for the promotion of employment in research, The Consortium will contribute to the formation of these young scientists, allowing them to develop specific competences; and also through practical experiences carried out in an industrial environment, which will facilitate their positioning on the work market.

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