303P IMPLEMENTING TECHNOLOGY-BASED TEACHING AND LEARNING IN PHARMACOLOGY

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A consortium of pharmacologists was funded by the Teaching and Learning Technology Programme (TLTP) 2 to develop computer-assisted learning (CAL) packages. A major outcome was 40+ packages covering most areas of pharmacology of which 1200 copies have been supplied worldwide. However, staff reported that there were constraints to the implementation of such technology based teaching materials, such as lack of time to develop support materials and use these methods, and a culture which did not promote non-traditional approaches (Markham, Jones & Sutcliffe, 1997). In one study, student usage of CAL increased if it was integrated into the course and if it was assessed (Dewhurst & Hughes, 1999).

Funding has been obtained under TLTP3 to encourage implementation of such CAL packages into courses. The primary aim is to develop flexible Teaching and Learning Resource Packs (TLRPs) for selected CAL packages. A TLRP is a pack of courseware that provides staff with teaching materials and gives students tasks and exercises to do, based around one or more CAL packages. Thus, TLRPs may constitute sets of multiple choice questions, workbooks, problem-solving exercises, problem-based learning exercises and assessments. The intention is to make the work of staff easier by providing teaching materials, which can be readily customised for a variety of their courses, be useful for a number of student groups (science, medical, and para-medical), and which stimulate learning by students. In year 1, June 1998 to June 1999, 6 teams have developed and initially trialled 6 TLRPs. The topics covered in year 1 are: inflammation/asthma, practical exercise on agonists/ antagonists, recombinant DNA technology, pharmaco-epidemiology, drug targets/G-protein coupled receptors as drug targets, drug metabolism. Each TLRP will contain the teaching material, worked examples and tutor notes. These TLRPs are to be trialled and formally evaluated by members of the TLTP3 consortium in further Universities in year 2, June 1999 to June 2000, and will be generally available from September 2000. In addition, the software directory previously provided via the Education committee of the British Pharmacological Society will be updated and should be ready this September via the BPS web site (www.bphs.org.uk).

Markham, A., Jones, S.J. & Sutcliffe, M. (1997) *Br. J. Pharmacol.*, 120, 376P.
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304P CALCIUM CHANNELS

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High voltage activated (HVA) calcium channels are cornposed of the products of 4 genes coding for the α_1 subunit (7 genes), the β subunit (4 genes), the γ subunit (2 genes) and the α2δ subunit (1 gene). Low voltage activated (LVA) channels are composed of an unknown number of subunits. To date, three genes for an α_1 subunit have been identified.

We have cloned and expressed two additional genes for the α2δ subunit and three for the y subunit. Northern and in situ analysis indicated that the mRNA of the α2δ-2 and α2δ-3 genes are expressed predominantly in heart and brain, respectively. The mRNA of the α2δ-3 gene and that of the α_{1G} subunit (a brain LVA calcium channel gene) are not co-expressed in brain. Expression of the α_{1G} subunit in the absence or presence of the α2δ-1 or α2δ-3 suburiit in HEK293 cells yields T-type calcium currents, which are not affected by the presence of the α2δ subunit. The mRNA of the γ3, γ4 and γ5 subunit is predominantly found in brain and heart respectively.

The functional effect of the new γ3,4 and γ5 subunit on expressed HVA current are minimal. It is likely that the α2δ subunits are expressed only with the various α_1 subunits of the HVA channels, whereas the new γ subunits are part of the native LVA channel complex.

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305P FUNCTIONAL ROLE OF CFTR CHLORIDE CHANNELS IN AIRWAY AND GUT EPITHELIA.

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Mutations in the gene coding for CFTR give rise to the lethal, autosomal recessive disease cystic fibrosis. In the lecture I will discuss the consequences of mutations in the CF gene and describe studies in CF mice showing how function can be restored. Approaches used to restore function are either by gene therapy or by pharmacological means to circumvent the genetic lesion. Preliminary data from clinical trials will also be given.

306P PHARMACOLOGY OF P2X RECEPTORS

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Adenosine 5'-triphosphate and/or related nucleotides act at both ionotropic (P2X) and metabotropic (P2Y) receptors. P2X receptor subunits ($P2X_1$ — $P2X_7$) are encoded by distinct genes. They form ligand-gated cation channels, either as homomultimers or heteromultimers. Homomeric $P2X_1$ and $P2X_3$ receptors, and heteromeric receptors containing these subunits, are activated by α,β methyleneATP; others are not. However, one of the main difficulties in assigning physiological roles to extracellular ATP has been the paucity of antagonists.

In this presentation I shall review what is currently known of the selective actions of agonists and antagonists at heterologously expressed P2X receptors: homomeric $P2X_1$, $P2X_2$, $P2X_3$ and $P2X_4$, and heteromeric $P2X_2/P2X_3$ and $P2X_1/P2X_5$.

307P INVOLVEMENT OF ACETYLCHOLINE RECEPTORS IN AUTOIMMUNE AND GENETIC DISORDERS

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The acetylcholine receptor (AChR) is one of the best studied ligand gated ion channels and is the target for both autoimmune and genetic disorders. The adult isoform of the AChR consists of α, β, δ and ε subunits; the ε replaces the γ that is present during fetal development. Each subunit has a large extracellular domain, four transmembrane domains (Ml-M4) and a cytoplasmic loop between M3 and M4 which contains phosphorylation sites. Mutations in the AChR genes have been detected in many families with congenital myasthenic syndromes. The syndromes present neonatally or later in life and result in muscle weakness, fatigue, and in some cases wasting. The functional effects of the mutations can be studied in *Xenopus* oocytes or in HEK293 cells expressing the mutant cRNAs. Mutations in the ε subunit (that defines the adult isoform) are present in >60% of cases of "AChR deficiency". In six such families that we have studied, a deletion in the cytoplasmic loop can be partially rescued by exon misplicing that leads to inclusion of intron 11 and returns the open reading frame. The "slow channel syndrome" is due to mutations that affect AChR ion channel activations, either by prolonging single openings or by prolonging bursts of normal openings. Many of these mutations are in the M2 domain that lines the pore of the AChR channel, but the primary effects of others is on the ACh binding site resulting in an increase in the duration of ACh binding. Rare mutations in the α or ε subunits lead to a "fast channel syndrome" in which the ACh-induced events are abnormally short.

The main antibody-mediated disease involving AChRs is myasthenia gravis in which antibodies lead to loss of AChR and destruction of the postsynaptic membrane. The patients become weak but can be treated by immunotherapies. The antibodies are measured by immunoprecipitation of ^{125}I-α-bungarotoxin-labelled human AChRs, and many are directed to sites on the c submits (separate from the α-BuTx-binding sites). About 15% of myasthenia gravis patients do not have detectable antibodies against the AChR. Nevertheless, application of sera from these patients to muscle cell lines leads to AChR phosphorylation and a reversible reduction in AChR function. It is proposed, therefore, that these antibodies bind to another muscle surface receptor and activate a second messenger cascade that leads to AChR phosphorylation and increased AChR desensitisation. Antibodies to AChRs in some mothers with myasthenia, and even in some healthy mothers, can inhibit the function of the fetal AChR isoform; these antibodies can cross the placenta during pregnancy and paralyse the fetus causing a severe developmental condition, known as arthrogryposis multiplex congenita, that often leads to neonatal death.

The approaches used to define the role of antibodies to AChRs in myasthenia gravis have also been used to demonstrate the presence and pathogenic role of antibodies to voltage-gated calcium channels in the Lambert Eaton myasthenic syndrome and to voltage-gated potassium channels in acquired neuromyotonia.

Reviews:

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Engel AG, Ohno K, Sine SM 1999 Congenital myasthenic syndromes: Recent Advances. *Arch Neurol* 56:163-167.

308P MAPPING OF THE LIGAND BINDING DOMAIN OF THE HUMAN TACHYKININ NK2 RECEPTOR

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The tachykinin receptors belong to the rhodopsin-like GPCRs. To date three mammalian tachykinin receptor subtypes have been cloned, namely the NK1, NK2 and NK3 receptors. Their preferred physiological peptide agonists (substance P, neurokinin A (NKA) and neurokinin B) consist of a variable N-terminal region and a conserved C-terminal motif, the latter being the most essential for ligand binding.

To define this ligand-binding site more accurately, putative ligand binding residues in the extracellular and transmembrane regions were targeted for site-directed mutagenesis. Asp-79, Asn-86, Asn-90 (helix 2), Gln-109 (helix 3), Ile-202 (helix 5), His-267 Gly-273 (helix 6) and Met-297 (helix 7) appear to be important for NKA binding but not for the binding of the non-peptide antagonist SR 48968. His-198 on the other hand plays a role in the binding of both NKA and SR 48968 [2 and unpublished data from this laboratory]. To differentiate receptor sites which specifically interact with NKA from residues which influence binding site conformations, the binding and covalent attachment of Cys-containing NKA analogues to Cys-substituted receptors were assessed. These data reveal the interaction of Met-297 in helix 7 with Leu-9 in NKA.

These data also infer that the agonist and antagonist binding determinants in the NK2 receptor may vary. The affinities of two NK2 receptor peptide antagonists (GR 100679 and MEN 10207) and two non-peptide antagonists (SR 48968 and GR 149861) for nineteen NK2 receptor mutants were determined. The mutation Tyr-266-Phe (helix 6) selectively reduced the affinity for GR 149861. Using six related compounds this interaction was suggested to be a hydrogen bond between the piperidinol moiety of GR†149861 and Tyr-266 in the receptor (unpublished data from this laboratory). Ser-274 (helix 6) and Leu-292 (helix 7) also influence binding of this antagonist. Several residues implicated in NKA binding (Gln-109, His-198, Ile-202, Gly-273 and Leu-292) were found to be important for receptor interaction of the peptide antagonists.

In conclusion, our data suggest that subsets of NK2 receptor selective ligands interact with overlapping NK2 receptor binding sites. Part of the binding site has common elements with the small ligand receptors such as rhodopsin. However, peptide ligands appear to make more interactions with the receptor than their smaller non-peptidic counterparts particularly with side chains exposed at the extracellular surface.

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