233P POSITIVE MODULATORS OF AMPA-RECEPTORS (AMPAKINES)

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Much of my research has been concerned with understanding the relationship between brain activity patterns, synaptic plasticity and learning. Thus, my work has mainly focused on the interaction between the hippocampal theta rhythm, LTP and memory formation. My goals are two-fold: first, to extract the key neurobiological variables that are essential for understanding how memories are stored, and second, to use this information for the development of therapeutic compounds directed at memory disorders associated with aging and disease.

In this symposium I will focus on aspects of my work that deal with the relationship between AMPA receptor modulation, LTP induction and memory formation. Over 80% of receptors in the brain are glutamatergic, roughly 2/3 of which are AMPA receptors, located both on principal cells and interneurons. The AMPA receptor mediates fast excitatory transmission in the forebrain and is crucial for transmission of information within and between brain regions. It is also an essential component of the machinery involved in the induction of long-term potentiation (LTP) under standard physiological conditions.

Specifically, high-frequency afferent stimulation used to induce LTP results in spatial and temporal summation of fast excitatory synaptic responses mediated by the AMPA receptor, the extent of which directly determines the degree of voltage-sensitive NMDA receptor activation and thereby the amount of LTP that occurs. I will describe a series of physiological and behavioral experiments that examined the effect of Ampakines, a class of compounds designed to cross the blood-brain barrier and selectively enhance AMPA receptor-mediated synaptic currents via allosteric modulation. We found that facilitation of AMPA receptor-mediated responses with Ampakines readily promotes LTP induction in hippocampal slices by reducing the amount of afferent stimulation needed to produce maximal LTP, and significantly increases the amount and duration of LTP in area CA1 of freely moving rats. We, as well as other laboratories, further found that Ampakines, by selectively enhancing the operation of the AMPA receptor, reliably promote learning across a broad range of behavioral paradigms in rats. Moreover, studies conducted in humans indicate that Ampakines are effective at enhancing recall in aged subjects and are able to improve memory in various cognitive tests.

234P DIVERSE SIGNALLING BY 5-HT RECEPTORS

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Fourteen different receptor subtypes might be regarded as a diversity that is sufficient to accommodate the wide-ranging physiological roles of 5-hydroxytryptamine (5-HT). However, it is becoming clear that, for 5-HT as for other neurotransmitters, the concept of a receptor as gatekeeper for a specific cellular process or event is too restrictive.

Multiple receptor-mediated biochemical cascades can be activated in cells in response to an agonist by a number of mechanisms. Whereas it is well established that different agonists do not necessarily elicit the same magnitude of response, they may probably also select between various possible signal transduction pathways. Receptor signalling may be diverse *via* a single receptor subtype as a consequence of specific agonist: receptor: G protein interactions.

5-HT receptors are even more heterogeneous when considering that the amino acid sequence of these receptor subtypes may vary from individual to individual, and the increasing number of receptor isoforms due to alternative splicing and RNA editing of 5-HT receptor transcripts. Activation, in particular the constitutive, agonist-independent one, for some of these receptor isoforms has been reported to be altered. This implies that ligands with similar binding affinities may display different pharmacological properties (partial agonist, antagonist or inverse agonist) *versus* these receptor isoforms dependent on their activation state. Therefore, intervention with receptor ligands to modify hampered neurotransmission pathways is a difficult task and needs to consider the growing evidence of diversity by G protein-coupled receptor signalling.

235P 5-HT AUTORECEPTOR ANTAGONISTS: THE NEXT GENERATION OF ANTIDEPRESSANTS ?

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A substantial body of evidence supports the hypothesis that elevation of CNS levels of 5-HT provides efficacious relief from the symptoms of depression. The current generation of antidepressants, the selective serotonin reuptake inhibitors (SSRIs), although diverse in structure, all have the common feature of potent 5-HT reuptake inhibition. These compounds are efficacious and are far superior in their side effect profile than more classical antidepressants. However, despite their considerable success (as with all other antidepressant medications) patients require 3-4 weeks of treatment with these drugs before their therapeutic effect is manifested. One of the neuroadaptive processes thought to be responsible for this latency to efficacy is desensitisation of 5-HT autoreceptors.

An alternative approach to enhancing serotonergic transmission could be to directly antagonise 5-HT autoreceptors. If such antagonists produce an immediate elevation in extracellular 5-HT levels, a rapid onset to clinical effect would be predicted. SB-224289 is a potent and selective 5-HT_{1B} receptor antagonist, readily bioavailable after oral administration and hence an ideal tool to measure the effect of terminal autoreceptor blockade. In guinea-pig, acute administration of paroxetine has little effect on 5-HT levels in the dentate gyrus, as measured by microdialysis. However after 3 weeks treatment with paroxetine, a subsequent dose produces a marked elevation in 5-HT levels, consistent with autoreceptor desensitisation. SB-224289 produced a similar increase in 5-HT levels in the dentate gyrus on acute administration. This effect was brain region specific, SB-224289 having no effect in the frontal cortex. At this time it is unclear which brain area requires elevated 5-HT to produce an antidepressant response, although several studies link the dentate gyrus and hippocampus with depression.

Acute treatment of guinea-pigs with a mixed 5-$HT_{1B/1D}$ receptor antagonist, GR127935, results in a decrease in 5-HT levels in the frontal cortex. This effect was reversed into an elevation of 5-HT when animals were concomitantly dosed with a 5-HT receptor anatgonist WAY100635, the latter given alone had no effect. A combination of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptor antagonism therefore offers a second approach to increasing extracellular 5-HT levels.

Finally, there is considerable evidence, both at a preclinical and clinical level, that a combination of SSRI activity together with 5-HT_{1A} receptor antagonism, can produce an immediate increase in extracellular 5-HT and a rapid antidepressant effect. A single compound with both properties, though a very challenging target for drug discovery, offers the third approach in this quest for the next generation of antidepressants.

236P THE 5-HT_{2C} RECEPTOR, A TARGET FOR PANACEAS

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The 5-HT_{2C} receptor has two features that are currently unique amongst 5-HT_{2C} receptor subtypes. Firstly, the 5-HT_{2C} receptor is not thought to be functionally expressed outside the central nervous system (CNS). However, within the CNS, the 5-HT_{2C} site is widely distributed with a particularly high density in the choroid plexus and lower densities in the hippocampus, striatum, nucleus accumbens, cortex, amygdala and hypothalamic nuclei (Hoyer *et al.*, 1994). Secondly, the receptor undergoes mRNA editing where adenosine nucleotides can be transformed to inosine nucleotides at three positions in the 2nd intracellular loop. This has functional consequences, as the potency of agonists is markedly reduced at the fully edited (VGV) receptor isoform compared with the unedited (INI) receptor isoform (Niswender *et al.*, 1999). Interestingly, a differential distribution of these mRNA isoforms has been reported in brain tissues. Thus, it is conceivable that 5-HT_{2C} receptor agonists could cause regionally selective activation of the receptor. A third interesting feature of the receptor is that in addition to causing inositol phosphate hydrolysis it may, like the 5-HT_{2A} receptor, also be coupled to arachidonic acid metabolism (Stout *et al.*, 1997).

Studies on the functions of the 5-HT_{2C} receptor have been greatly facilitated by the availability of agonist tools, such as m-chlorophenylpiperazine (mCPP) and the more selective 5-HT_2 agonist, Ro 60, 0175 (Martin *et al.*, 1998). Selective antagonists, such as SB-242084 (Kennett *et al.*, 1997) have also been characterised. Furthermore, the ethical acceptability of administration of mCPP (as a metabolite of the antidepressant, trazodone,) to humans has had a major impact on clinical research. Results from preclinical and clinical studies suggest that the 5-HT_{2C} receptor may play an important role in anxiety, depression, reward, feeding behaviour, epilepsy, motor control and migraine.

Recent results from studies of the role of 5-HT_{2C} receptors in feeding behaviour are of particular interest. MCPP was first reported to reduce rat food consumption in 1979 by Samanin *et al.* This has subsequently been shown not only to be 5-HT_{2C} receptor mediated (Kennett & Curzon, 1991), but also a specific action on satiety (Kitchener & Dourish, 1994). Recently, the action of the clinically effective anorectic agent, d-fenfluramine, was reported to be attenuated in mutant mice lacking the 5-HT_{2C} receptor (Vickers *et al.*, 1999) and in rodents is prevented by selective 5-HT_{2C} receptor antagonists (Vickers & Kennett, 1999). The site of action of 5-HT_{2C} receptor mediated hypo-phagia is unclear with both the paraventricular nucleus of the hypo-thalamus and the parabrachial nucleus being implicated (Kaplan *et al.*, 1998). However, it is of interest that whilst tolerance to the hypo-locomotor and anxiogenic-like actions of mCPP is rapid (Fone *et al.*, 1998, Vickers *et al.*, 2000, Goetghebeur *et al.*, 2000), the hypophagic response is maintained for up to 28 days (Vickers et al., 2000). This suggests that responses induced by activation of the 5-HT_{2C} receptor may undergo differential tolerance on repeated activation and this phenomenon could conceivably be related either to the existence of mRNA edited isoforms or regionally specific linkage to alternative secondary messenger systems, as suggested by the results of Stout *et al.* (1997).

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237P THE 5-HT_3 RECEPTOR: ONE RECEPTOR, TWO SUBUNITS, MANY FUNCTIONS

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With the appreciation that Gaddum's 5-hydroxytryptamine (5-HT) M receptor ('antagonised' by morphine) equates to the 5-HT_3 receptor, responses mediated via this receptor have been documented for over half a century. However, the current availability of selective 5-HT_3 receptor ligands (e.g. the antagonists granisetron and ondansetron), now facilitates pharmacological classification of this receptor. In addition to the recognition site for 5-HT, the 5-HT_3 receptor possesses sites which mediate allosteric modulation of the receptor complex (e.g. by alcohols and anaesthetic agents). Whether these latter sites will provide therapeutic targets remains a topic for investigation.

The 5-HT_3 receptor is associated with neurones in both the CNS and the PNS. Within the CNS, highest levels of receptor expression are associated with the dorsal vagal complex in the brainstem. This region is intimately involved in the vomiting reflex and antagonism of these receptors is likely to contribute to the antiemetic action of 5-HT_3 receptor antagonists. 5-HT_3 receptor expression in the forebrain is also evident (e.g. hippocampus) and these receptors mediate alterations in animal behaviour (e.g. behaviours relevant to anxiety, cognition, reward) as well as a number of well documented neurochemical and electrophysiological responses (e.g. mediation of fast synaptic transmission, modulation of neurotransmitter release). Unfortunately, except for the anti-emetic action, most of the clinical reports do not substantiate the therapeutic efficacy of 5-HT_3 receptor antagonists predicted from the preclinical investigations.

At the molecular level, the 5-HT_3 receptor is a cys-cys loop ligand-gated ion channel (primarily a non-selective monovalent cation channel) such that this receptor shares a common ancestry with the other members of this superfamily (e.g. nicotinic, $GABA_A$ and glycine receptors) rather than the other 5-HT receptors which are all G-protein coupled receptors. The 5-HT_3 receptor complex is comprised of multiple subunits, two of which have been identified to date (5-HT_{3A} and 5-HT_{3B} receptor subunits); although the presence of further proteins associated with the native receptor complex remains likely. Consequently, the occurrence of structurally distinct 5-HT_3 receptor complexes may provide an opportunity to pharmacologically manipulate different populations of native 5-HT_3 receptors.

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238P THE 5-ht6 RECEPTOR: POTENTIAL CNS FUNCTIONS

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The 5-ht6 receptor has a typical G-protein coupled topography and is positively linked to adenylyl cyclase. 5-ht6 receptor mRNA is highly expressed in limbic and cortical regions but not present in the periphery, offering the potential to develop treatments for CNS disorders with limited peripheral side effects. Initially, due to the absence of selective 5-ht6 ligands, antibodies were produced against the N-terminus of the rat 5-ht6 receptor protein to establish the precise anatomical localisation and phenotype of neurones expressing this receptor. In addition, central injection of an antisense oligonucleotide (A.O.) directed against the 5-ht6 receptor was utilised to evaluate CNS function. In later studies, the first selective, CNS penetrant, 5-ht6 receptor antagonist (4-amino-N-(2,6 bis-methylamino-pyrimidin-4-yl)-benzene sulfonamide was evaluated in rat paradigms examining locomotion, memory and attention.

Extensive 5-ht6 immunopositive neurones were located in the rat; cerebral cortex and olfactory tubercles > dentate gyrus, hippocampal CA1, endopyriform nucleus, cerebellar lobes and several other areas, but absent following pre-incubation with antigenic peptide. Dual labelling immunohistochemistry showed only a low level (<20%) of co-existense between choline acetyltransferase- and 5-ht6- positive cells in a few regions, such as the caudate nucleus, nucleus accumbens and some septal nuclei. In contrast, extensive neuronal co-localisation between 5-ht6- and glutamic acid decarboxylase-immunoreactivity occurred in 33 out of 41 regions; being >50% in 16 areas. Both the 5-ht6 A.O. and Ro 04-6790 (3, 10 and 30 mg/kg i.p.) induce a stretching behaviour in the conscious rat, which was antagonised by scopolamine but not haloperidol pre-treatment. Scopolamine and raclopride both decreased the ability of rats to discriminate a novel from a familiar object. Ro 04-6790 (10 mg/kg i.p.) completely reversed the deficit in novel object discrimination produced by scopolamine (0.5 mg/kg i.p.) but had no effect on the abolition produced by raclopride (0.5 mg/kg i.p.). In separate experiments, 5-ht6 A.O. (1.5μg x 2 for 6 days i.c.v.) or Ro 04-6790 (10 or 30 mg/kg i.p.) did not affect acquisition but enhanced retention of the learnt platform position in the Morris water maze. Rats spent significantly longer searching the trained position in a probe test one day after the final trial in A.O. and 7 and 11 days after in Ro 04-6790 treated animals.

Collectively, this data suggests that attenuation of 5-ht6 receptor function activates cholinergic neuronal pathways involved in stretching behaviour and may enhance; retention of spatial learning, working memory and/or attention. This interaction with cholinergic neurones may be indirect by disinhibition of GABAergic neurones.

239P 5-HT_7 RECEPTORS: PHARMACOLOGY AND POTENTIAL THERAPEUTIC APPLICATIONS

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5-HT_7 receptor mRNA has been reported in a variety of tissues including the brain, spinal cord, vasculature, GI tract and sympathetic ganglia. Semi-quantitative RT-PCR analysis using Taqman™ confirmed that, within the CNS, expression levels are highest in hypothalamus, amygdala, thalamus and hippocampus. This distribution supports hypotheses, derived from preliminary pharmacological studies, that selective 5-HT_7 receptor ligands may find therapeutic application in several diseases, including depression, anxiety, sleep and circadian abnormalities and migraine. Investigations of 5-HT_7 receptor function have been hampered by a lack of selective agonists and antagonists.

We have recently reported on a novel 5-HT_7 receptor antagonist, SB-269970-A which has high affinity for recombinant 5-$HT_{7(a)}$ receptors (pK_i 8.9) and 5-HT_7 receptors from a variety of other species including guinea-pig (pK_i 8.3). SB-269970 is >100 fold selective over a range of receptors and enzymes except 5-$ht_{5(a)}$ (50 fold selective). The compound is a competitive antagonist at human recombinant 5-$HT_{7(a)}$ receptors (pA_2 8.5) and 5-HT_7 receptors in guinea pig hippocampus (pK_B 8.3). The hypothermic response to 5-CT in guinea pigs was reversed by SB-269970-A (1-30 mg kg^{-1} i.p.) suggesting that this response is mediated by 5-HT_7 receptor stimulation.

When administered prior to the sleep phase in conscious rats, SB-269970-A (30 mg kg^{-1} i.p.) reduced the overall time spent in Paradoxical Sleep (PS) during the first 3 hours of EEG recording. Thus, 5-HT_7 receptors may play a role in regulating sleep. Further studies on the functions of the 5-HT_7 receptor will also be discussed. SB-269970-A will be a valuable tool in defining these functions.