This phosphatase only dephosphorylates extremely acidic phosphopeptides; its real physiological role is probably deactivation of phosphatidyl-3,4,5-triphosphate (PIP_3) by removal of the 3-phosphate group. One naturally occurring oncogenic point mutant, Gly129→Glu, turns out to retain protein kinase activity, but not lipid kinase activity. In further support of this role, PTEN transfection into U87MG glioblastoma cells leads to deactivation of PKB and prevention of BAD (a pro-apoptotic member of the BCL-2 family) phosphorylation, both steps known to be survival-promoting, and downstream of PI3K. Transfection of the wildtype PTEN into LnCAP cells led to apoptosis, but the Gly129→Glu mutant did not. The structural basis for the selective ablation of lipid kinase activity awaits next year's conference.

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Imidazol receptors – an enlightening experience

For many pharmacologists, like myself, who trained more than a decade ago, the emergence of imidazoline receptors has been an enigma, and even those pharmacologists who have attempted to rectify their ignorance by reference to standard textbooks have been thwarted, as these receptors rarely, if ever, receive a mention. I was therefore extremely pleased to be able to attend a state-of-the-art symposium entitled *Identification, Characterisation and Controversies on the Role of Imidazol Receptors* during the recent International Congress of Pharmacology (München, Germany), in which six speakers actively working in the field presented a concise overview of the subject and its therapeutic potentials.

Historical perspective

The first speaker, Pascal Bousquet (Faculte de Medecine, Strasbourg, France), outlined the history of the imidazoline receptor. Initially it was known that the imidazoline compound clonidine, known to be an agonist of α_2-adrenoceptors, was able to act in the brainstem to induce hypotension. It then became apparent that clonidine was binding to receptors other than the α_2-adrenoceptors. These receptors were named imidazoline receptors (I-receptors). Bousquet defined such receptors as being 'receptors that are sensitive to imidazolines, but non-sensitive to catecholamines'. As early as 1992 it was recognized that there are several subgroups of imidazol receptors, the first classification being based on the relative affinities for clonidine and idazoxan (an α_2-adrenoceptor antagonist). The I_1-receptors have affinity for both clonidine and idazoxan, whilst I_2-receptors have affinity for idazoxan alone. More recently it has been postulated that both I_1- and I_2-receptors are subdivided into high- and low-affinity subtypes.

The high-affinity I_1-receptor has now been purified and has been shown to be distinct from any other known receptor. It has been identified in human brain stem and has been shown to be involved in the cardiovascular effects of clonidine-like drugs. There is, however, dispute about whether α_2-adrenoceptors are also involved in the cardiovascular effects of clonidine. Using the novel I_1-selective agonist LNP509 (patent pending), it has been shown in anaesthetized rabbits that both LNP509 and α_2-adrenoceptor agonists have only small effects on blood pressure, but a combination of the two agents results in a large reduction in pressure. This is taken to indicate that α_2-adrenoceptors are required for the effects of LNP509.

Imidazoline analogues and hypertension

Rilmenidine is one of a new class of drugs that are analogues of imidazoline and have been developed for the treatment of hypertension. In humans, rilmendine has been shown to be effective in the relief of all forms of hypertension, whilst being free from the common side effects of sedation, drowsiness, dry mouth and weight change. Furthermore, this compound has been shown to be effective in preventing bicuculline-induced arrhythmias in anaesthetized rabbits, at sub-hypotensive doses. The results therefore suggest that rilmenidine may be a useful antihypertensive and/or anti-arrhythmic agent, relatively free from adverse effects.

The controversy within the field was then highlighted by the presentation of Bela Szabo (Universität Freiburg, Germany). Using conscious rabbits he has shown that the effects of rilmenidine and moxonidine (both I_1-receptor agonists) and UK14304 (an α_2-adrenoceptor agonist) can all be reversed by yohimbine (an α_2-adrenoceptor antagonist). Similar results were found using the α_2-adrenoceptor antagonist SKF86466. These results suggest that rilmenidine and moxonidine both produce their effects via α_2-adrenoceptors.

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Paradoxically the selective I_1-receptor antagonist efaroxan was able to antagonize the hypotensive effects of rilmenidine, moxonidine and α-methyl dopa (an α_2-adrenoceptor agonist). These results therefore question the selectivity of rilmenidine and moxonidine for I_1-receptors when producing their antihypertensive effects, but evidence was also presented to indicate that the effects of these drugs may be peripheral. The final conclusion that was drawn by the speaker was that there was little evidence of rilmenidine and moxonidine producing their effects via central I_1-receptors. This conclusion was fiercely challenged by several members of the audience and no consensus was achieved, the findings did, however, reiterate the complex relationship between I_1-receptors and α_2-adrenoceptors in the control of blood pressure.

Conflicting results

Paul Ernsberger (Case Western School of Medicine, Cleveland, OH, USA) then addressed the question of the signalling pathways of imidazoline receptors, but this was not before he reopened the debate of the role of imidazoline receptors in the control of hypertension. Using spontaneously hypertensive rats, his data indicate that the effects of moxonidine can be blocked by the selective I_1-receptor antagonist efaroxan, but not by α_2-adrenoceptor antagonists. These data contradict that of Bela Szado, although it was obtained in a different animal model. A later presentation by Richard Hear (CSIRO, Adelaide, Australia) attempted to reconcile the views of workers in the field of hypertension by suggesting that the older imidazoline agents acted mainly on α_2-adrenoceptors, while the newer agents, such as rilmenidine and moxonidine, act predominantly on I_1-receptors. The actions of these receptors, however, were dependent on post-synaptic α_2-adrenoceptors, thus the imidazoline receptors and the α_2-adrenoceptors appeared to act in series. Whether these findings are able to explain all of the (previously presented) contradictory findings remains to be seen.

The main subject of Ernsberger's presentation, however, was the demonstration that the actions of moxonidine and other imidazoline agonists were mediated by production of prostaglandins and stimulation of phospholipase C that resulted in production of diacylglycerol, but not inositol triphosphate, as would be expected for most stimulants of phospholipase C. Moxonidine has also been shown to stimulate phosphokinase C.

Elusive endogenous ligand

Donald Reis (Cornell University, New York, USA) described the search for the endogenous ligand for the imidazoline receptors. A clonidine-displacing substance was first identified in 1984. This substance has a molecular weight of 580, is non-peptide and non-catecholamine and also displaces other imidazolines; its structure is unknown. Another clonidine-displacing substance has been identified more recently, this is agmatine. Agmatine is a small amine formed from arginine by arginine decarboxylase and is found in bacteria, plants and humans; it is able to displace clonidine from α_2-adrenoceptors and both I_1- and I_2-receptors. It has now been shown to fulfil most of the usual criteria for classification as a neurotransmitter and has been found in the hypothalamus, brainstem and hippocampus, with lowest concentrations in the cortex. There is also reuptake of agmatine into synaptosomes.

Interestingly, however, agmatine is not selective for the imadozoline-like receptors as it has been shown to block the NMDA glutamate ion channel and to inhibit nitric oxide synthase. This factor mitigates against agmatine as being the primary endogenous ligand for the imidazoline receptors, but as yet there are no better qualified candidates.

Imidazoline receptors in depression

An interesting presentation from Jesus Garcia-Sevilla (University of the Balearic Islands, Palma de Mallorca, Spain) concerned the putative role of imidazoline receptors in depressive disorders. Garcia-Sevilla presented evidence to suggest that the brains and platelets of depressive suicide victims possess decreased amounts of a 29 kDa protein and increased amounts of a 45 kDa protein. In depressed patients receiving antidepressant therapy the platelet levels of the the 45 kDa protein level fell towards normal values.

The evidence was presented to suggest that the 45 kDa protein represents the I_1-receptor, whilst the lower molecular weight protein represents the I_2-receptor. It is therefore suggested that depressive illness involves an increase in I_1-receptors and a decrease in I_2-receptors. It was postulated that it may be possible to treat depression by use of an I_1-receptor agonist, which would be expected to induce a down-regulation of I_1-receptors, or possibly to use of an I_2-receptor antagonist to up-regulate theI_2-receptor population.

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In short…

The Automation Partnership (Royston, UK) has recently developed the fourth generation robotic system for cell culture – Cellmate™ – which enables scale-up through automation of labour intensive procedures without the need for process change and provides consistent, reliable and reproducible data. Cellmates are used in the production of viral vaccines, therapeutic proteins, gene therapy and also for producing cells for high-throughput screening assays in drug discovery.