Trends in Pharmacological Sciences
including TOXICOLOGICAL SCIENCES

5-HT receptor subtype-specific drugs and the cardiovascular system

THE SYSTEM for classification of 5-HT receptors set up by Bradley *et al.*[1,2] in 1986 has formed a basis for subsequent 5-HT receptor identification. They proposed the existence of three main types of 5-HT receptors: 5-HT_2 and 5-HT_3, defined pharmacologically using selective antagonists; and 5-HT_1-like, defined with the help of selective agonists and non-selective antagonists. 5-HT_1-like binding sites have since been further classified into four distinct subtypes, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}, but these sites do not always correlate with the functional heterogeneity of 5-HT_1-like receptors[3].

However, at a recent symposium* on the multiple effects exerted on the cardiovascular system by 5-HT, it became clear that some of the

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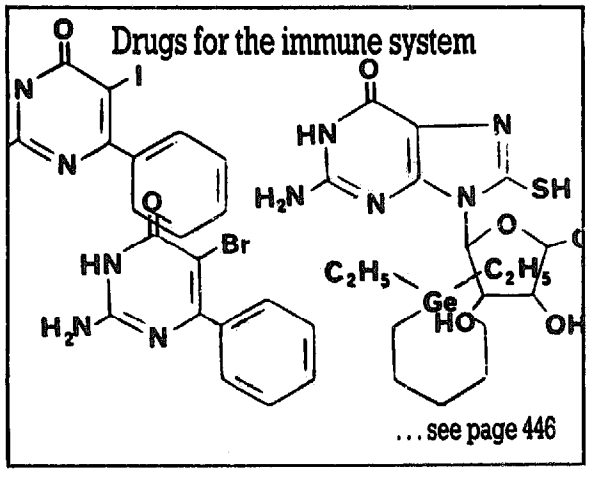

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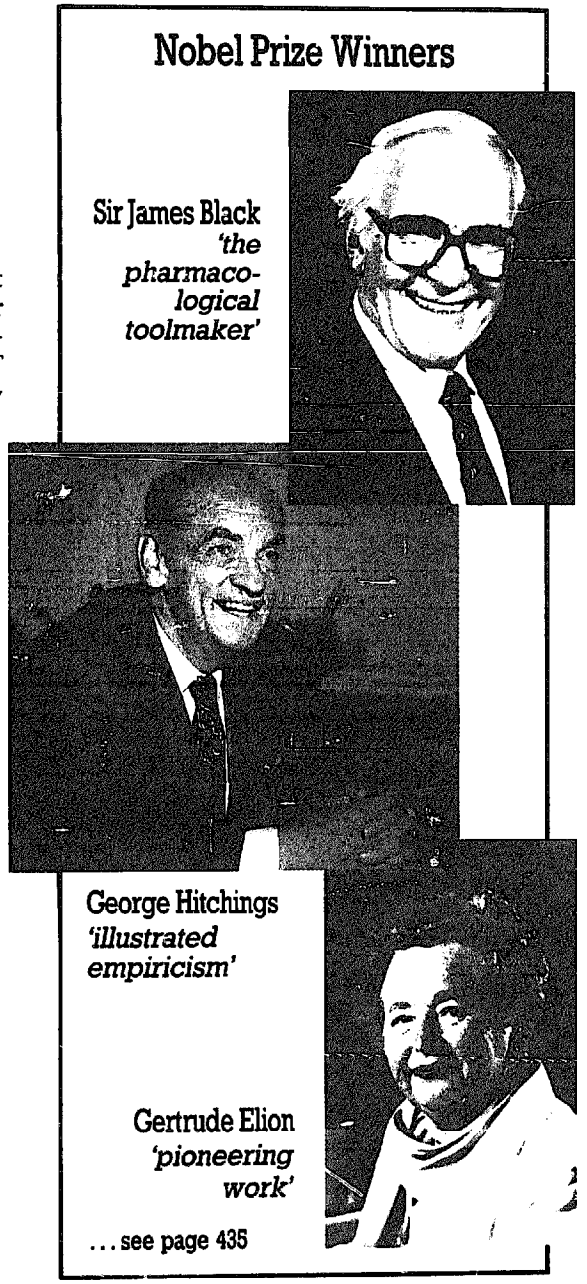

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receptors involved do not fit into this classification. Such receptors have been described by the term 'orphan receptor'.

It was also recognized that many of the effects of 5-HT on the cardiovascular system vary from species to species. For example, 5-HT induces bradycardia by activation of 5-HT_3 receptors on afferent nerves in the cardiac muscle. But when this bradycardia is inoperative, 5-HT may produce tachycardia – which involves 5-HT_3 receptors in the isolated rabbit heart preparation, 5-HT_2 receptors in the ganglion-blocked rat and 5-HT_1 receptors in the spinal-vagotomized cat (Cohen, Indianapolis). The receptor responsible for the tachycardiac response in the pig cannot even be classified: a number of drugs that antagonize the different types of 5-HT receptors do not attenuate the chronotropic response to 5-HT; neither do antagonists at the receptors for adrenaline, acetylcholine, histamine and dopamine (Bom and Verdouw, Rotterdam). It is thus an example of an orphan receptor.

5-HT_2 receptors have been identified as being responsible for vascular smooth muscle contraction in a variety of preparations. Currently, this receptor is classified on the basis of antagonism by ketanserin or cyproheptadine; 5-HT_3 antagonists such as MDL-72222 must be inactive. Analysis of the pA_2 or pK_d values in a number of vascular and other smooth muscle preparations has revealed some variations, but these do not as yet form a consistent enough pattern to constitute receptor heterogeneity (Mylecharane, Sydney).

Some of the complications arise from the noncompetitive blockade of the effects of 5-HT by, for instance, ritanserin, methysergide or LY-53857 in the calf coronary and rat tail arteries. This phenomenon can be explained either by allosteric modulation of the 5-HT_2 receptors (Kaumann, Welwyn) or by pseudoirreversible inhibition (Clarke, Houston).

5-HT-induced contraction can also be mediated by 5-HT_1-like receptors such as those in the dog saphenous vein. The actions of the novel and highly selective 5-HT_1-like receptor agonist GR-43175 make it an important new tool for studying this class of receptor. It is a potent agonist at the 5-HT_1-like receptors that mediate vascular smooth muscle contraction and inhibit the release of sympathetic neurotransmitter. It is also devoid of agonist or antagonist activity in a range of isolated tissues containing 5-HT_2 and/or 5-HT_3 receptors (e.g. pig and greyhound coronary artery, dog femoral artery and rat vagus nerve) and even certain 5-HT_1-like receptors (e.g. those responsible for vascular or intestinal relaxation) (Feniuk and Humphrey, Ware). The finding that GR-43175 contracts cranial arteries (human basilary artery and feline arteriovenous anastomoses) might be of particular interest to the proposed clinical use of GR-43175 in the treatment of acute migraine (Tfelt-Hansen, Copenhagen).

5-HT and hypertension

5-HT_2 receptor antagonists are used clinically to treat hypertension. Ketanserin is one of the best known, although it is recognized that its anti-hypertensive action is at least partly due to its α_1-adrenoceptor antagonist activity – to which the 5-HT_2 receptor antagonism may contribute. Both ketanserin and the more selective 5-HT_2 antagonist ritanserin antagonize the arterial and venous constrictor responses to 5-HT in the human forearm (Blauw and van Brummelen, Leiden).

Another action of ketanserin is to counteract 5-HT-induced platelet aggregation and amplification of vasoconstrictor responses to other substances (van Nueten, Beerse). The amplification phenomenon seems to be more marked in pathophysiological circumstances like chronic hypertension, atherosclerosis and vasospasm. Another proposed mechanism of action of ketanserin was the reduction of a particular, apparently easily realizable pool of monoamines in peripheral tissues. In hypertensive and senescent animals the pool becomes larger in certain vascular tissues and spleen (Leysen, Beerse).

Reduction of blood pressure can also be achieved via central activation of 5-HT_{1A} receptors. The potent and selective 5-HT_{1A} agonists flesinoxan and 8-OH-DPAT lower blood pressure and heart rate in all species studied so far (Wouters, Weesp; Mir, Basel). The first studies with flesinoxan in hypertensive patients also show that this compound lowers blood pressure in man (de Voogd, Weesp).

5-HT and migraine

Our current understanding of the pathogenesis of migraine is still incomplete, the two hypotheses being that the headache is of cardiovascular or neurogenic origin.

The craniovascular pharmacology of 5-HT supports the first view, but does not necessarily rule out the second. Migraine is associated with a rapid release from platelets and excretion in urine of 5-HT. The resulting reduction in blood 5-HT may cause a combination of arterial dilation, arteriolar constriction and opening of arteriovenous anastomoses. Several drugs effective during migraine attacks, including ergotamine and GR-43175, constrict arteriovenous anastomoses and thereby may restore the blood flow through the cranial circulation (Saxena, Rotterdam).

According to the neurogenic hypothesis of migraine, the activation of the sensory innervation of the cranial vasculature leads to vasodilation via an axonal reflex mechanism which may involve 5-HT_3 receptors located on the trigeminal nerve endings. This hypothesis is primarily based on the positive results reported with the 5-HT_3 receptor antagonist MDL-72222 in the acute management of migraine (Fozard, Basel). However, Lataste (Basel) reported that under placebo-controlled double-blind conditions ICS 205-930, another potent and selective 5-HT_3 receptor antagonist, is ineffective in aborting migraine. He argued, though, that migraine may result from a cascade of events following the initial release of 5-HT from platelets, and that treatment after onset of an attack may be too late to have an effect. Clinical trials to explore possible prophylactic effectiveness of continuous 5-HT_3 receptor antagonism with ICS 205-930 are in progress.

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The receptor pharmacology of 5-HT is still at a developing stage where certain ideas have yet to be crystallized. For example, 5-HT_1-like receptors are often identified by their resistance to 5-HT_2 and 5-HT_3 receptor antagonists and susceptibility to blockade by the

non-selective antagonist methiothepin. Martin (Beckenham) has analysed the interactions of tryptamines with 5-HT receptors and produced 'fingerprints' consisting of affinity and, in the case of agonists, efficacy estimates, and has proposed this approach as a method to extend and improve the current classification scheme. More positive identification of the receptors requires the development of more selective drug tools.

Pharmacological analysis of receptors is, however, only one way to look at the effects of a substance. The isolation and cloning of 5-HT receptors has already revealed a remarkable resemblance between the 5-HT_{1C} receptor and the 5-HT_2 receptor, and between the β-adrenergic receptor and the 5-HT_{1A} receptor (Hartig, Baltimore). This multidisciplinary interest in the 5-HT receptor will ultimately lead to a better understanding of the cardiovascular effects of 5-HT.

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• Proceedings of the symposium will be published by Kluwer Academic Publishers.

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3 Humphrey, P. P. A. and Richardson, B. P. in *Serotonin* (Mylecharane, E. J., *et al.*, eds), MacMillan Press, in press

GR-43175: 3-[2-(dimethylamino)ethyl]-*N*-methyl-1*H*-indole-5-methane sulphonamide
ICS 205-930: (3α-tropanyl)-1*H*-indole-3-carboxylic acid ester
MDL-72222: 1α*H*, 3α, (5α*H*-tropan-3-yl)3,5-dichlorobenzoate
LY 53857: 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)4,6,6a, 7,8,9,10,10a-octahydroindolo-(4,3-*fg*)quinoline maleate.

CURRENT AWARENESS

Hirudins and the role of thrombin: lessons from leeches

One of the most important tools in biological research is a pure preparation of a potent and specific inhibitor of the enzyme or receptor of interest. In the 1950s Markwardt described the properties of a component of leech saliva that appeared to be a highly potent and specific inhibitor of the coagulation protease thrombin. He named it hirudin – a reference to its source, *Hirudo medicinalis*, the European medicinal leech (Fig. 1)[1]. Hirudin is mixed with the blood of the victim as it is ingested and presumably maintains fluidity in the gut so that the leech can remain mobile during its subsequent retirement whilst digestion takes place.

The importance of hirudin has been recognized since its original discovery but its low yield and the recent scarcity of the leech have limited its use. However, expression of a recombinant gene for hirudin has now been achieved by several groups in both yeast and bacteria with high yield[2,3].

The molecule and its specificity

Hirudin is an acidic polypeptide of molecular weight about 7000. A number of variants with amino acid sequence differences are known, but it appears that they have similar properties and potency[4,5]. Recombinant hirudins differ from natural hirudins by the lack of a sulphate group on Tyr63. This group is added post-translationally in the leech and increases the inhibition constant (K_i) for thrombin from 2×10^{-13} M (recombinant desulphatohirudin variant 1) to 2×10^{-12} M (natural hirudin variant 1)[6], although the desulphatohirudin is still obviously very potent.

These compounds also appear to be highly selective thrombin inhibitors. Thus for example factor Xa, kallikrein, plasmin, tissue plasminogen activator, the digestive enzymes trypsin and chymotrypsin, and the complement enzymes are not inhibited by desulphatohirudin, even at micromolar concentrations[7,8].

No other known molecule has such a specificity for thrombin. Other anticoagulants such as the heparins and related anionic polysaccharides and coumarin act indirectly. Desulphatohirudin thus provides a unique opportunity to examine the physiological and pathological roles of thrombin.

Antithrombotic properties

What is the effect of this specific thrombin inhibitor on thrombus generation? The pathways that lead to thrombosis are complex and highly dependent on the inducing stimulus and the flow rate of the blood at the site (Table I). The final common events are the accumulation of platelets, which is most marked in high flow situations (e.g. in arteries), and the polymerization and precipitation of fibrin, which is more marked in

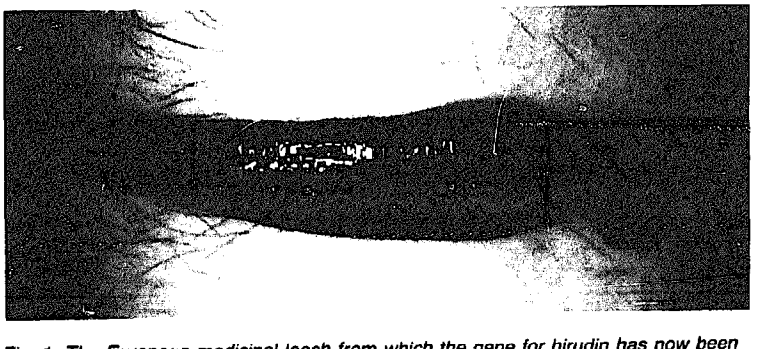

Fig. 1. The European medicinal leech from which the gene for hirudin has now been expressed in yeast.