SEROTONIN-S_2 RECEPTOR BINDING SITES AND FUNCTIONAL CORRELATES

J. E. LEYSEN, D. DE CHAFFOY DE COURCELLES, F. DE CLERCK, C. J. E. NIEMEGEERS and J. M. VAN NUETEN

Janssen Pharmaceutica Research Laboratories, B-2340 Beerse, Belgium

Summary—Serotonin-S_2 receptor binding sites have been labelled with several ligands such as [^{3}H]spiperone, [^{3}H]lysergic acid diethylamide (LSD), [^{3}H]mianserin and [^{3}H]ketanserin, the latter being the most selective ligand. The greatest concentration of the sites occurs in frontal cortical tissue, but they were also detected in striatum, nucleus accumbens and tuberculum olfactorium as well as on membranes of cat and human platelets. The serotonin-S_2 sites, labelled with different ligands in different tissues, revealed similar binding properties for drugs, but careful definition of specific binding is necessary because of marked variation in the non-specific binding of drugs in different tissues.

Serotonin-S_2 sites were demonstrated to have a role in serotonin-induced behavioural excitation in rodents and they probably mediate perception of subjective feelings elicited by serotonin mimetics. *In vivo* studies revealed involvement of the serotonin-S_2 sites in impairment of microcirculation by serotonin and in inflammation provoked by serotonin. On isolated tissues, serotonin-S_2 sites mediate serotonin-induced vasoconstriction in peripheral blood vessels (e.g. superficial, coronary, splanchnic vessels) and cerebral blood vessels from various species including man. Serotonin-S_2 sites also have a role in serotonin-induced contractions in tracheal smooth muscle and serotonin-induced bronchoconstriction *in vivo*, but the sites are unrelated to "M"- and "D"-serotonin receptor subtypes, which have been functionally characterized in gastrointestinal smooth muscle.

Platelets proved to be an integral model for studying serotonin-S_2 sites. Binding, measured on platelet membranes, was shown to be directly related to serotonin-induced platelet aggregation or changes in shape. Evidence was obtained that inositol-lipid turnover is a primary response following stimulation of serotonin-S_2 receptors in platelets. It is likely to be part of the signal-transducing system in which calcium is a second messenger candidate. Platelets are a promising tool for the study of alterations in serotonin-S_2 receptors in disease states and after treatment with drugs.

Key words: serotonin-S_2 receptor, radioligand binding, function, inositol-lipid turnover, brain, platelets.

The study of receptors by radioligand binding, using radioactively labelled drugs and tissue homogenates, has in ten years time developed to a new branch in biochemical pharmacology. The applied technique is fairly simple and rapid; drug binding to membranes appeared to be easily detectable and numerous data have been generated. However, inherent to the original definition of a receptor, binding of a substance to a receptor site is unseparably linked to eliciting or antagonizing a pharmacological or physiological effect (Langley, 1878). Moreover, binding studies themselves are prone to many artefacts (Leysen and Gommeren, 1981; Leysen, 1984). Therefore, it is of prime importance in radioligand binding studies to demonstrate the relationship between binding sites on membrane preparations and functions.

Purported serotonin receptors were first labelled in homogenates of rat brain using [^{3}H]serotonin and [^{3}H]LSD (Bennett and Snyder, 1976). Apparent differences between the drug-binding properties, labelled by the two ligands ([^{3}H]LSD binding was more potently inhibited by serotonin antagonists than by agonists and vice versa for [^{3}H]serotonin binding), prompted the hypothesis of an agonist and antagonist state of the receptor. Although the early study reported a thorough biochemical characterization of the binding sites, no attempt was made to find a functional correlate for the sites. In 1977 it was found that [^{3}H]spiperone, a neuroleptic used in dopamine receptor research labelled distinct specific binding sites in the frontal cortex of the rat revealing features of "serotonergic" receptors. Known serotonin antagonists showed nanomolar binding affinities for the sites, serotonin and serotonin-mimetics, bound at micromolar concentrations, but the serotonin binding affinity was at least 100-fold greater than that of various other biogenic amines. Most importantly, the binding affinities of drugs for the sites labelled by [^{3}H]spiperone in the frontal cortex, were found to correlate highly significantly with the potencies of the drugs to antagonize tryptamine-induced clonic seizures in rats (Leysen and Laduron, 1977; Leysen, Niemegeers, Tollenaere and Laduron, 1978). This represented the first demonstration of a functional correlate for serotonergic binding sites. In later studies Peroutka and Snyder (1979) designated the specific binding sites for [^{3}H]spiperone in the frontal cortex as 5-hydroxytryptamine-2 (5-HT_2) or serotonin-S_2 sites. The sites were distinguished from the 5-HT_1 or serotonin-S_1 sites labelled by

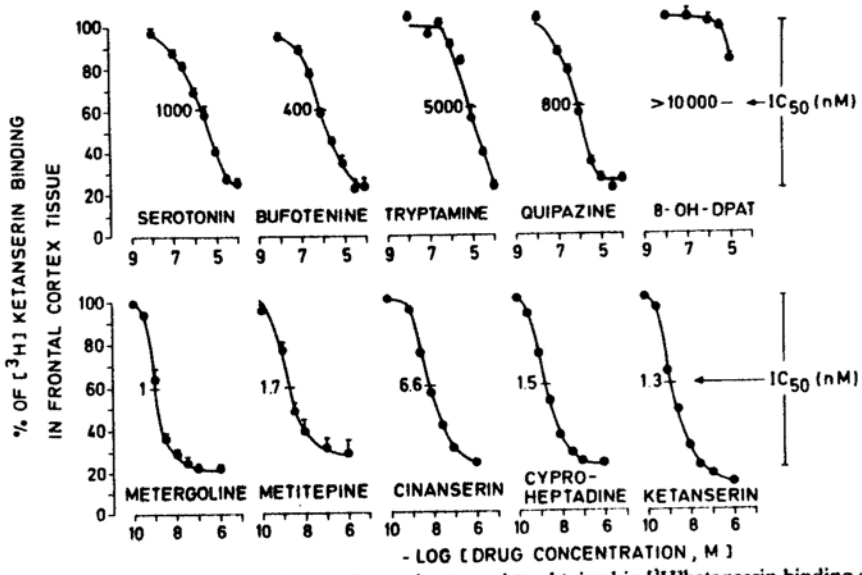

Fig. 1. Inhibition curves of serotonin agonists and antagonists obtained in [^{3}H]ketanserin binding assays, using frontal cortex membranes of rat. Assays were run at 37°C using 1 nM [^{3}H]ketanserin in Tris–HCl buffer pH 7.7. Specific binding was defined as the portion of binding inhibited by 1 μM methysergide (full methodological description in Leysen *et al.*, 1982).

[^{3}H]serotonin. Peroutka and Snyder provided evidence that serotonin-S_1 and serotonin-S_2 sites were distinct molecular entities, hence refuting the hypothesis of the two-state receptor model.

Various radioactive drugs were studied in serotonin receptor binding research, but many lacked specificity: [^{3}H]spiperone labelled both serotonin-S_2 and dopamine-D_2 sites (Leysen *et al.*, 1978; Leysen, 1979). [^{3}H]LSD labelled serotonin-S_1, serotonin-S_2 and dopamine receptor sites (Peroutka and Snyder, 1979; Leysen, 1981), [^{3}H]mianserin bound to serotonin-S_2 and histamine-H_1 sites (Peroutka and Snyder, 1981), [^{3}H]metergoline reportedly showed too high non-specific binding (Hamon, Mallat, Herbet, Nelson, Audinot, Pichat and Glowinski, 1981) and [^{3}H]metitepine was unsuitable because of high affinity binding to many different neurotransmitter receptor sites (Leysen, Awouters, Kennis, Laduron, Vandenberk and Janssen, 1981). The recently-introduced serotonin antagonist, [^{3}H]ketanserin, represented the first labelled ligand, which at nanomolar concentrations revealed marked specificity for serotonin-S_2 sites (Leysen, Niemegeers, Van Nueten and Laduron, 1982). Using this ligand, important progress was made in serotonin-S_2 binding research. The binding features of the serotonin-S_2 site in different areas of the brain and on blood platelets were investigated. Localization of the sites in mammalian brains, including man, and sensitivity of the sites to neuronal lesions was studied. Functional correlates of serotonin-S_2 sites in *in vivo* pharmacological studies, on isolated tissues and in functional and biochemical investigations on blood platelets were demonstrated. In this paper recently-acquired knowledge regarding serotonin-S_2 receptor binding sites will be discussed.

BINDING FEATURES OF SEROTONIN-S_2 SITES, USING VARIOUS ^{3}H-LIGANDS IN VARIOUS TISSUES

The frontal cortex is particularly enriched in serotonin-S_2 binding sites; this has been observed in various mammalian species including man (Leysen, Van Gompel, Verwimp and Niemegeers, 1983b; Schotte, Maloteaux and Laduron, 1983). [^{3}H]ketanserin binding in membrane preparations of rat frontal cortex is potently inhibited by various serotonin antagonists of different chemical classes, all showing nanomolar IC_{50}-values (drug concentration causing 50% inhibition of the specific binding of the labelled ligand). Serotonin, and its congeners bufotenine and tryptamine, show micromolar binding affinities, but the recently-introduced serotonin agonist 8-OH-DPAT [trivial name: 8-hydroxy-2 (di-*n*-propylamino) tetralin; chemical abstract name: 7-(dipropylamino)-5,6,7,8-tetrahydro-1-naphtolenol] (Hjörth, Carlsson, Lindberg, Sanchez, Wikström, Arvidsson, Hacksell and Nilsson, 1982) is virtually inactive on [^{3}H]ketanserin binding. Inhibition curves of the various compounds are presented in Fig. 1. Specific binding of [^{3}H]ketanserin in the membranes of rat frontal cortex, defined by the plateau values of the inhibition curves, represented about 70% of the total membrane labelling.

The same type of specific serotonin-S_2 receptor binding sites were detected with [^{3}H]ketanserin in various tissues (e.g. frontal cortex from rat, rabbit, guinea pig, dog, man; striatum from rat, guinea pig, dog; cat platelets) (Leysen *et al.*, 1983b; Leysen, Gommeren and De Clerck, 1983a; Schotte *et al.*, 1983), with [^{3}H]spiperone in frontal cortex of rat (Leysen *et al.*, 1982) and with [^{3}H]LSD on human platelets (Geaney, Schächter, Elliot and Grahame-

Table 1. K_i-values (nM) of compounds for serotonin-S_2 sites labelled with various ^{3}H-ligands in various tissues from different species

	[^{3}H]spiperone	[^{3}H]ketanserin				[^{3}H]LSD
	Rat frontal cortex*	Rat frontal cortex†	Rat striatum‡	Human frontal cortex§	Cat platelets‡	Human platelets‖
Serotonin antagonist						
Ketanserin	2.1	0.39	0.57	2.28	0.60	6.95
Cyproheptadine	6.5	0.44	1.0		2.0	12.2
Methysergide	12	0.94	2.3	5.2	7.1	15.8
LSD	8.2	2.5		2.0		0.77
Pipamperone	5.3	0.78	2.7	0.28	4.0	
Dopamine antagonists						
Spiperone	1.2	0.53	0.45	0.92	1.1	6.79
Haloperidol	48	22	20		32	580
Domperidone	327	78	100	24.2	54	
Histamine antagonist						
Mepyramine	2600	> 1000	1000	657	1140	1506
Adrenergic antagonist						
Prazosin		> 1000	1800	> 10,000	850	> 10,000
Amine reuptake blockers						
Amitriptyline	21	4.2	21		21	165
Desipramine		78	140		210	
Serotonin agonists						
Bufotenine	518	118	810		72	
Serotonin	1033	296	2870	1144	290	81.3
Tryptamine	5420	1482	8080		1070	
Various biogenic amines						
Dopamine	82,000	93,500	25,500	> 10,000	20,000	> 100,000
Norepinephrine		> 100,000	200,000	> 10,000	53,200	> 100,000
Histamine		> 100,000	102,000		42,500	> 100,000

Data from: *Leysen *et al.* (1978); Leysen (1981); †Leysen *et al.* (1982); ‡Leysen *et al.* (1983); §Schotte *et al.* (1983); ‖Geaney *et al.* (1984).

Smith, 1984). Table 1 shows the binding affinities for a number of archetypical drugs measured in various serotonin-S_2 binding models, using different ^{3}H-ligands and different tissues. Highly significant correlations were found between the K_i-values of the drugs measured in the various models. Spearman rank correlation coefficients are presented in Table 2.

Although, for a particular drug the K_i-values [equilibrium inhibition constant, calculated according to Cheng and Prusoff (1973)], measured in the various binding models, were of the same order of magnitude, they appeared not to be identical. How can these apparent discrepancies be explained? Involvement of different receptor site subtypes seems highly unlikely because of the strong correlations between drug potencies in the various binding models. A first anomaly, is the somewhat lower apparent binding affinities of drugs for sites labelled by [^{3}H]ketanserin in rat striatal and cat platelet tissues, as compared to binding affinities in frontal cortex tissues of rat. This can probably be ascribed to differences in non-specific binding of the drugs in the different tissues. Figure 2 shows that serotonin antagonists, which are chemically unlike ketanserin (pipamperone and methysergide), show an intermediary plateau in their inhibition curves at 40–50% inhibition of the total [^{3}H]ketanserin binding to rat striatal membranes. However, unlabelled ketanserin shows a steep inhibition curve spanning 70% of the total binding. The structural congener of ketanserin, R 51 230 (see Fig. 2), which is a weak serotonin antagonist, does not show an intermediary plateau in the inhibition curve. It appears that in striatal membrane preparations, specific [^{3}H]ketanserin binding to serotonin-S_2 sites represents only 40–50% of the total binding, about 30% of the binding is to so-called displaceable non-specific binding sites and the remaining binding is due to non-displaceable adsorption. Also in platelet membranes from cat, [^{3}H]ketanserin displayed a similar pattern of binding

Table 2. Correlation between various radioligand binding models for serotonin-S_2 sites, using data from Table 1

	Spearman rank correlation coefficients					
	I	II	III	IV	V	VI
Serotonin-S_2 models						
(I) [^{3}H]spiperone, rat frontal cortex		$r_s = 0.960$ $P < 0.001$*	$r_s = 0.967$ $P < 0.001$*	$r_s = 0.924$ $P \ll 0.001$*	$r_s = 0.984$ $P < 0.001$*	$r_s = 0.907$ $P < 0.001$*
(II) [^{3}H]ketanserin, rat frontal cortex			$r_s = 0.949$ $P < 0.001$*	$r_s = 0.887$ $P < 0.001$*	$r_s = 0.979$ $P < 0.001$*	$r_s = 0.879$ $P < 0.001$*
(III) [^{3}H]ketanserin, rat striatum				$r_s = 0.890$ $P < 0.001$*	$r_s = 0.973$ $P < 0.001$*	$r_s = 0.893$ $P < 0.001$*
(IV) [^{3}H]ketanserin, human frontal cortex					$r_s = 0.890$ $P < 0.001$*	$r_s = 0.953$ $P < 0.001$*
(V) [^{3}H]ketanserin, cat platelets						$r_s = 0.958$ $P < 0.001$*
(VI) [^{3}H]LSD, human platelets						

*Significant.

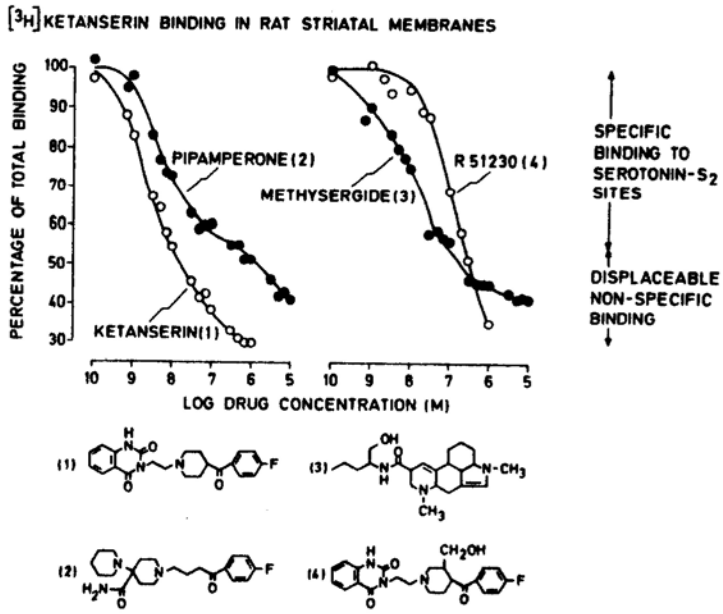

Fig. 2. Inhibition of [^{3}H]ketanserin (1 nM) binding in striatal membrane preparations of rat by drugs of various chemical structures, (1) (2) (3) are potent serotonin antagonists, (4) is a chemical congener of ketanserin with very weak serotonin antagonistic properties (experimental conditions were as described in Leysen *et al.*, 1982).

to specific and displaceable non-specific binding sites (Leysen *et al.*, 1983a), whereas in frontal cortex of rat this was much less apparent (see Fig. 1). ^{3}H-ligand binding to displaceable non-specific binding sites is a commonly observed phenomenon in binding studies (Leysen, 1984), but it varies between different tissues. They represent mere recognition sites of a certain structural moiety of the ^{3}H-ligand and are unrelated to any pharmacological or physiological effect of the drugs. However, through a high degree of non-specific binding, the concentration of free drug in solution may be reduced and it is even possible that, through the non-specific binding, access of the drug to the specific binding sites is hindered. Direct information on the various types of non-specific binding is only obtained for radioactive drugs, but a similar problem exists for the non-labelled compounds. Therefore, slight variations in binding potencies of drugs between different tissues should not be regarded as an indication for the existence of different receptor subtypes. A second anomaly in the data presented in Table 1 are the higher K_i-values of drugs measured using [^{3}H]spiperone in frontal cortex or using [^{3}H]LSD in human platelets as, compared to the K_i-values derived from [^{3}H]ketanserin binding assays in the frontal cortex. This anomaly is probably due to wide variations in the dissociation rate of the ^{3}H-ligands from the receptor sites. Using a new filtration technique for measuring drug-receptor dissociation rates (equivalent to a dissociation method by infinite dilution) (Leysen and Gommeren, 1984), we recently measured the following half-lifes of dissociation at 25°C, ketanserin: $t_{1/2} = 11$ min, mianserin: $t_{1/2} = 15$ min, spiperone: $t_{1/2} = 30$ min, LSD: $t_{1/2} \gg 90$ min. The binding of the slowly-dissociating spiperone and LSD was found to be more difficult to inhibit, probably because binding equilibrium is not fully obtained during the relatively short incubation times which were applied (incubation of 15 min with [^{3}H]spiperone and 4 hr with [^{3}H]LSD). This again illustrates that binding data must be interpreted cautiously and several factors must be considered in order to avoid hypotheses based on artefactual data.

It can be concluded that similar serotonin-S_2 binding sites were detected with the various ^{3}H-ligands in the different brain tissues and on platelets.

ROLE OF SEROTONIN-S_2 SITES IN SEROTONERGIC EFFECTS *IN VIVO*

Administration of serotonin-mimetic agents or precursors of serotonin to rodents causes behavioural excitation. This has been described as the serotonin syndrome (head twitches, forepaw treading, cyanosis, hindlimb abduction, gastric lesions, dead), observed following administration in rats or mice of large doses of 5-hydroxytryptophan plus a monoamine oxidase inhibitor (Jacobs, 1976). Head twitches are elicited by administration of 5-hydroxytryptophan to rats and guinea pigs, or by mescaline in rats and mice (Niemegeers, Colpaert, Leysen, Awouters and Janssen, 1983). Forepaw treading is seen following administration of tryptamine to rats (Leysen *et al.*, 1978). Ample evidence was provided by several investigators that in large series of drugs, their potencies to antagonize the above behavioural effects, induced by serotonin-mimetics, are highly

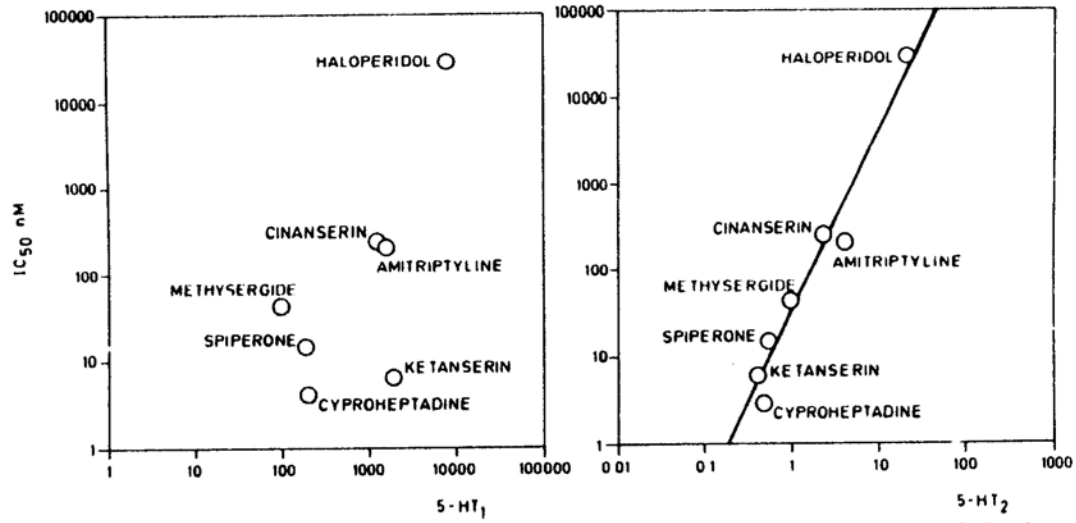

Fig. 3. Correlation between the potencies of drugs (IC_{50}-values, nM) to suppress the contraction induced by 3×10^{-7} M serotonin in canine basilar arteries and the binding affinities (K_i-values, nM) of the drugs for 5-HT_1 sites (labelled with [^{3}H]serotonin in rat hippocampal tissue) or 5-HT_2 sites (labelled with [^{3}H]ketanserin in rat frontal cortex tissue). Spearman rank correlation coefficients are for relationship with 5-HT_1 site binding $r = 0.393$, $P = 0.19$; for relationship with 5-HT_2 site binding $r = 0.929$, $P < 0.001$ (from van Nueten *et al.*, 1984, by permission).

significantly correlated with the binding affinities of the drugs to serotonin-S_2 sites (reviewed in Leysen and Tollenaere, 1982; Leysen, 1984). Similar highly significant correlations were found between serotonin-S_2 binding affinities of drugs and potencies to antagonize the development of gastric lesions due to release of serotonin from mast cells after administration of compound 48/80 (Awouters, Leysen, De Clerck and Van Nueten, 1982) or potencies of drugs to antagonize serotonin-induced paw oedema (Ortmann, Bischoff, Radeke, Buech and Delini-Stula, 1982).

From these observations it was concluded that serotonin-S_2 sites have a role in behavioural excitation induced by serotonin-mimetics, in impairment of microcirculation by serotonin and the provocation of inflammation by serotonin.

Studies of the discriminative stimulus produced by various serotonin-mimetics (LSD, quipazine, 5-hydroxytryptophan) in rodents lead some authors to suggest that the feelings of anxiety, tension and neurotic depression, perceived by the animals, were mediated by serotonin-S_2 sites (Colpaert, Niemegeers and Janssen, 1982; Glennon, Young and Rosecrans, 1983; Friedman, Barrett and Sanders-Bush, 1984).

It has also been proposed that serotonin-S_2 sites may have a role in the etiology of depressive illnesses. This assumption was derived from the observation that serotonin-S_2 sites are apparently down-regulated following the chronic treatment of animals with some antidepressant drugs (e.g. the serotonin re-uptake blocker amitriptyline). The slow development of down-regulation of receptors was thought to correspond to the slow onset of the therapeutic action of the drugs (Peroutka and Snyder, 1980). However, down-regulation of serotonin-S_2 sites is not a common property of potent blockers of serotonin re-uptake (Anderson, 1983). The mechanism of the down-regulation of receptors is poorly understood and is probably not only a consequence of enhanced concentrations of serotonin at the receptor. The role of serotonin-S_2 sites in depression is still an open question.

Several other functions observed *in vivo* have been ascribed to serotonin: such as the role of serotonin in pain perception, sleep and sexual behaviour. Studies in these areas mostly involved too limited a number of drugs in order to allow characterization of the receptor sites mediating these effects.

ROLES OF SEROTONIN-S_2 SITES IN SEROTONIN-INDUCED EFFECTS ON ISOLATED TISSUES

The earliest studies concerning serotonin receptor sites were conducted using isolated tissues. Based on investigations of serotonin-induced contractions in gastrointestinal smooth muscle, Gaddum and Picarelli (1957) proposed a subclassification of serotonin receptors as M- and D-receptors. M-receptors were predominantly found in ileum, morphine was described as a typical antagonist at these receptor sites. D-Receptors were detected in the ileum and the fundus, and stimulation of these sites was reportedly antagonized by phenoxybenzamine. Thus far, a radioligand binding model corresponding to M- or D-receptor sites has not been found. Later studies concerning effects of serotonin on vascular smooth muscle, revealed that serotonin-induced contractions in various blood vessels (e.g. rat caudal artery and rabbit femoral artery, canine vessels) were potently antagonized by known antagonists of serotonin such as ketanserin, cyproheptadine and methysergide (Van

Nueten, Janssen, Van Beek, Xhonneux, Verbeuren and Vanhoutte, 1981). It appeared that the concentration of serotonin to produce half-maximal contraction of vessels ($ED_{50} = 100$ nM in caudal artery, $ED_{50} = 500$ nM in rabbit femoral artery) corresponded to the binding affinity of serotonin for serotonin-S_2 binding sites ($K_i = 300$ nM for [^{3}H]ketanserin binding, frontal cortex). Also potencies of drugs to antagonize serotonin-induced vasoconstriction matched the binding affinities of drugs for serotonin-S_2 sites. This supplied ample evidence for a role of serotonin-S_2 sites in serotonin-induced vasoconstriction (Leysen, 1981; Leysen *et al.*, 1982).

Serotonin was also reported to contract segments of the dog basilar artery. Based on an apparent correlation between binding affinities of drugs at serotonin-S_1 sites and potencies of drugs to suppress the serotonin-induced contraction of the basilar artery, Peroutka, Noguchi, Tolner and Allen (1983) suggested that this phenomenon was mediated through serotonin-S_1 sites. However, Van Nueten, Leysen, De Clerck and Vanhoutte (1984) showed that antagonism of the pharmacological effect was complex and not of a full competitive nature. It was observed that antagonists, in various manners, shifted the dose–response curve for serotonin to larger concentrations and suppressed the maximal response. This suggests a dual type of antagonism. When evaluating the suppression of the response at a submaximal concentration of serotonin, it was found that IC_{50}-values (drug concentrations producing 50% suppression) of drugs correlated highly significantly with the drug binding affinities for serotonin-S_2 sites and less so with binding affinities for serotonin-S_1 sites. This is illustrated in Fig. 3. Also Müller-Schweinitzer and Engel (1983) reported that the potencies of a series of indole-like serotonin agonists to induce contractions of the basilar artery, corresponded to the binding affinities of the compounds for serotonin-S_2 sites. Similarly, the potencies of a large series of antagonists to suppress the serotonin-induced contractions of the basilar artery were highly significantly correlated with serotonin-S_2 site binding affinity. Hence, although in the basilar artery the mutual interactions between serotonin and antagonists are not of a full competitive nature, involvement of serotonin-S_2 sites seems more likely than involvement of serotonin-S_1 sites. However, involvement of multiple receptor sites cannot be excluded at this stage.

It was further observed that serotonin can induce relaxation of contracted vessels, e.g. in the dog coronary artery, metitepine is one of the few antagonists, however, this compound acts potently on many different receptor sites. The receptor involved in relaxation of vessels by serotonin is as yet not characterized; but involvement of serotonin-S_2 sites can be excluded because of the inactivity of ketanserin (Van Nueten *et al.*, 1984).

Finally, it was shown that serotonin-S_2 sites have a role in serotonin-induced contraction of tracheal smooth muscle from guinea pigs and in serotonin-induced bronchoconstriction *in vivo* (Van Nueten, Leysen, Vanhoutte and Janssen, 1982).

THE PLATELET, AN INTEGRAL MODEL FOR CHARACTERIZATION OF SEROTONIN-S_2 RECEPTORS: STUDY OF BINDING SITES, FUNCTIONAL RESPONSE AND RECEPTOR-COUPLED SIGNAL TRANSDUCING SYSTEM

Serotonin is known to affect platelet function. In some species (such as human) this is reflected in a mild response (reversible platelet shape change), whereas in other species (such as the cat) stimulation of platelets by serotonin produces an avid platelet aggregation, which becomes irreversible when larger concentrations of serotonin are used (De Clerck and Herman, 1983; De Clerck, Xhonneux, Leysen and Janssen, 1984a, b). The finding that ketanserin was particularly effective in inhibiting serotonin-induced platelet function, tentatively suggested a role for serotonin-S_2 sites (De Clerck and Herman, 1983). The first successes to demonstrate serotonin-S_2 receptor binding sites on platelet membranes with radioligand binding techniques were achieved using [^{3}H]ketanserin and platelet membrane preparations of cat (Leysen *et al.*, 1983a) and shortly thereafter using [^{3}H]LSD and human platelet membranes (Geaney *et al.*, 1984). As described above, the serotonin-S_2 binding sites on the platelet membranes display the same binding properties as the serotonin-S_2 binding sites characterized in brain tissue. Several lines of evidence were obtained demonstrating that serotonin-induced functional responses in platelets were mediated by the serotonin-S_2 receptor sites. Studies using cat platelets showed that the potency of serotonin to induce platelet aggregation, expressed by its K_m-value = 600 nM (calculated using the initial slope of aggregation induced by serotonin), matched the K_i-value = 300 nM of serotonin for inhibition of [^{3}H]ketanserin binding to the platelet membranes of cat. Moreover, as illustrated in Fig. 4, the potencies of antagonists to inhibit the serotonin-induced aggregation closely correspond to the serotonin-S_2 binding affinities of the drugs (De Clerck *et al.*, 1984a, b). The fact that drugs belonging to other pharmacological classes (selective dopamine antagonists, histamine antagonists, α_1-adrenergic receptor blockers) were poorly or not active, corroborated the specificity of the serotonin-S_2 binding sites and the aggregation response to serotonin. The inactivity of selective uptake blockers of serotonin in these tests demonstrated that the effects were unrelated to the uptake of serotonin in platelets (Leysen *et al.*, 1983a; De Clerck *et al.*, 1984a). It was similarly found that binding affinities of drugs for serotonin-S_2 sites, detected with [^{3}H]LSD in human platelet membranes, were highly significantly correlated with the potencies of the compounds to antagonize serotonin-induced shape change in human platelets (Geaney *et al.*, 1984).

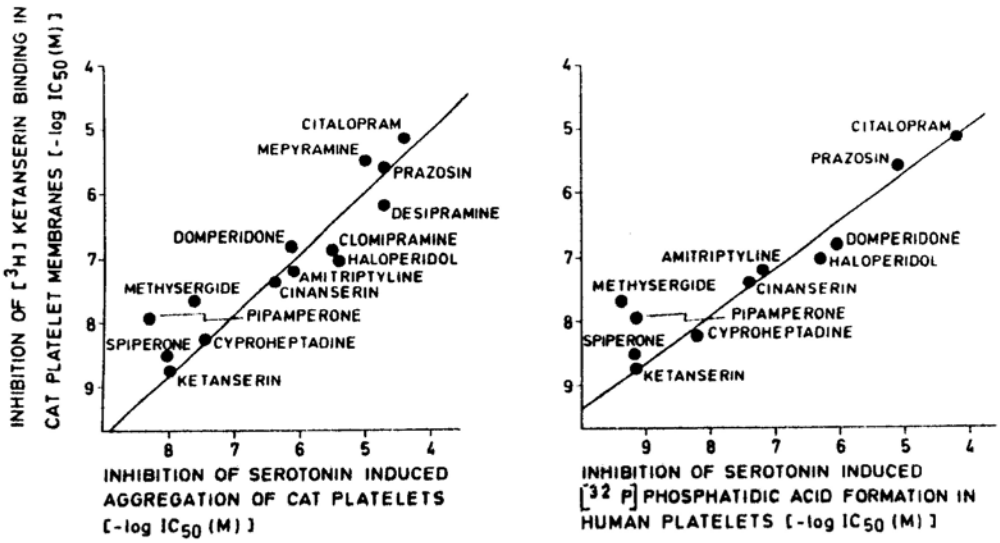

Fig. 4. Correlation between measured binding affinities *in vitro* of drugs for serotonin-S_2 sites on platelet membranes from cat (Leysen *et al.*, 1983a) and the drug potencies to inhibit serotonin-induced aggregation of platelets from cat (De Clerck *et al.*, 1984a) or to inhibit serotonin-induced formation of [^{32}P]phosphatidic acid in human platelets (de Chaffoy de Courcelles *et al.*, unpublished).

Besides the fact that the platelet model allowed investigation of the relationship between binding and function in the same tissue, it also proved to be suitable for the study of the biochemical response coupled to activation of serotonin receptors. Recently, it was found that stimulation of intact human platelets with serotonin caused a rapid, initially linear, increase in ^{32}P-incorporation in phosphatidic acid in the platelet membranes (de Chaffoy de Courcelles, De Clerck, Leysen, Van Belle and Janssen, unpublished). This biochemical response has become a tool for measuring the activation of phospholipase *C*, a signal transducing step for receptors which use Ca^{2+} as a second messenger. Serotonin concentration-dependent changes in formation of [^{32}P]phosphatidic acid ran entirely parallel with changes in the slope of serotonin-induced platelet aggregation. The concentration of serotonin producing half-maximal stimulation of [^{32}P]phosphatidic acid formation (=200 nM) again matched the K_i-value (=300 nM) of serotonin for binding to serotonin-S_2 receptor sites. Antagonists revealed the same potencies for inhibition of the biochemical response (serotonin stimulated [^{32}P]phosphatidic acid formation), the functional test (serotonin stimulated platelet shape change) and ^{3}H-ligand binding to serotonin-S_2 receptor sites ([^{3}H]ketanserin binding in rat brain tissue or cat platelets, [^{3}H]LSD binding in human platelets...) (de Chaffoy de Courcelles *et al.*, unpublished; De Clerck *et al.*, 1984a, b; Geaney *et al.*, 1984). The highly significant intercorrelations between potencies of drugs, measured in the various tests are illustrated in Fig. 4. Hence, ample evidence was obtained, indicating that the activation of phospholipase *C* yielding diacylglycerol that is subsequently phosphorylated to phosphatidic acid, is a primary response directly coupled to stimulation of serotonin-S_2 receptors in human platelets.

A second step in the event of signal transmission through phospholipid turnover is the activation of the protein kinase *C* (Nishizuka, 1983). On stimulation of human platelets with serotonin, this enzyme is triggered by the transient accumulation of diacylglycerol. The inhibitory potency of ketanserin on protein phosphorylation matched the one of formation of [^{32}P]phosphatidic acid, thus providing evidence for the direct link in the serotonin-S_2 receptor signal transduction (de Chaffoy de Courcelles, Roevens and Van Belle, 1984).

Various hypotheses have been put forward regarding the sequence of events taking place in membranes where inositol-lipid turnover seems to be coupled to receptor stimulation. For the serotonin-S_2 receptor system, de Chaffoy de Courcelles *et al.* (unpublished) suggested that the initiating factor, following occupation of serotonin receptor, is activation of phosphatidylinositol-4′,5′-biphosphate phosphomonoesterase. This enzyme hydrolyses TPI (phosphatidylinositol-4,5-biphosphate) (which is an extremely strong Ca-chelator and is likely to be a storage site for Ca within the membrane) into DPI (phosphatidylinositol-4′-phosphate). Through that reaction sufficient Ca is released (Erne, Bühler, Affolter and Bürgisser, 1983), necessary for the activation of phospholipase *C*. The latter enzyme generates diacylglycerol and this reaction product triggers, in its turn, protein kinase *C* activity. Activation of this enzyme has been proposed as a key event in transmission of receptor stimuli (Nishizuka, 1983). Current research is directed towards providing experimental proof for the sequence of events involved in inositol-lipid turnover-coupled signal transduction

and to examine whether Ca possibly fulfils the role of second messenger in these reactions. The platelet model represented the first tissue in which a major part of the signal transducing system, coupled to serotonin-S_2 receptor sites, was identified. It must now be confirmed whether the same coupling, between serotonin-S_2 receptor sites and inositol-lipid turnover, exists in brain areas and various peripheral tissues where the receptor sites were found to have a role.

Finally apart from the importance of platelets for the fundamental research on receptor-coupled events, platelets represent an easily accessible and promising tool for the investigation of the role of serotonin-S_2 receptor sites in disease states in humans. For example, the supposed role of serotonin-S_2 receptor sites in depressive illnesses, and also alteration in receptors upon chronic treatment with drugs affecting the serotonergic system in man can now be closely followed. This should eventually lead to a better understanding of the hypothesized adaptive mechanism of serotonin receptors. A whole new field of human clinical research remains to be explored.

Acknowledgements—Part of this work was supported by a grant from the Instituut tot Aanmoediging van Wetenschappelijk Onderzoek in Nijverheid en Landbouw. Part of this paper was reported in a chapter on "Characterization of serotonin receptor binding sites" to be published in *Neuropharmacology of Serotonin* (Green A. R., Ed.). Oxford University Press, 1984.

REFERENCES

Anderson J. L. (1983) Serotonin receptor after chronic antidepressant treatments: ligand binding, electrophysiological, and behavioural studies. *Life Sci.* **32:** 1791–1801.

Awouters F., Leysen J. E., De Clerck F. and Van Nueten J. M. (1982) General pharmacological profile of ketanserin (R 41 468), a selective 5-HT_2 receptor antagonist. In: *5-Hydroxytryptamine in Peripheral Reactions* (De Clerck F. and Vanhoutte P. M., Eds), pp. 193–197. Raven Press, New York.

Bennett J. P. Jr and Snyder S. H. (1976) Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to post-synaptic serotonin receptors. *Molec. Pharmac.* **12:** 373–389.

de Chaffoy de Courcelles D., Roevens P. and Van Belle H. (1984) Stimulation by serotonin of 40 kDa and 20 kDa protein phosphorylation in human platelets. *FEBS Lett.* **171:** 289–292.

Cheng Y. C. and Prusoff W. H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmac.* **22:** 3099–3108.

Colpaert F. C., Niemegeers C. J. E. and Janssen P. A. J. (1982) A drug discrimination analysis of lysergic acid diethylamide (LSD): *In vivo* agonist and antagonist effects of purported 5-hydroxytryptamine antagonist and of pirenperone, a LSD-antagonist. *J. Pharmac. exp. Ther.* **221:** 206–214.

De Clerck F. F. and Herman A. G. (1983) 5-Hydroxytryptamine and platelet aggregation. *Fedn Proc. Fedn Am. Socs exp. Biol.* **42:** 228–232.

De Clerck F., Xhonneux B., Leysen J. and Janssen P. A. J. (1984a) The involvement of 5-HT_2-receptor sites in the activation of cat platelets. *Thromb. Res.* **33:** 305–321.

De Clerck F., Xhonneux B., Leysen J. and Janssen P. A. J. (1984b) Evidence for function 5-HT_2 receptor sites on human blood platelets. *Biochem. Pharmac.* In press.

Erne P., Bühler F. R., Affolter H. and Bürgisser E. (1983) Excitatory and inhibitory modulation of intracellular free calcium in human platelets by hormones and drugs. *Eur. J. Pharmac.* **91:** 331–332.

Friedman R. L., Barrett R. J. and Sanders-Bush E. (1984) Discriminative stimulus properties of quipazine: mediation by serotonin$_2$ binding sites. *J. Pharmac. exp. Ther.* **228:** 628–635.

Gaddum J. H. and Piccarelli Z. P. (1957) Two kinds of tryptamine receptors. *Br. J. Pharmac.* **12:** 323–328.

Geaney D. P., Schächter M., Elliott J. M. and Grahame-Smith D. G. (1984) Characterisation of [^{3}H]lysergic acid diethylamide binding to a 5-hydroxytryptamine receptor on human platelet membranes. *Eur. J. Pharmac.* **97:** 87–93.

Glennon R. A., Young R. and Rosecrans J. A. (1983) Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT_2 antagonists. *Eur. J. Pharmac.* **91:** 197–206.

Hamon M., Mallat M., Herbet A., Nelson D. L., Audinot M., Pichat L. and Glowinski J. (1981) ^{3}H-Metergoline: A new ligand of 5-HT receptors in rat brain. *J. Neurochem.* **36:** 613–626.

Hjörth S., Carlsson A., Lindberg P., Sanchez D., Wikström H., Arvidsson L.-E., Hacksell U. and Nilsson J. L. G. (1982) 8-Hydroxy-2-(di-n-propylamino)tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *J. Neural Trans.* **55:** 169–188.

Jacobs B. L. (1976) An animal behavior model for studying central serotonergic synapses. *Life Sci.* **19:** 777–786.

Langley (1878) On the physiology of the salivary secretion. *J. Physiol.* **1:** 339–369.

Leysen J. E. (1979) Different neuroleptic receptors in various rat brain areas. In: *Catecholamines: Basic and Clinical Frontiers* (Usdin F., Kopin I. J. and Barchas J., Eds), pp. 556–558. Pergamon Press, New York.

Leysen J. E. (1981) Serotoninergic receptors in brain tissue: Properties and identification of various [^{3}H]ligand binding sites *in vitro*. *J. Physiol.* **77:** 351–362.

Leysen J. E. (1984) Problems in *in vitro* receptor binding studies and identification and role of serotonin receptor sites. *Neuropharmacology* **23:** 247–254.

Leysen J. E., Awouters F., Kennis L., Laduron P. M., Vandenberk J. and Janssen P. A. J. (1981) Receptor binding profile of R 41 468, A novel antagonist at 5-HT_2 receptors. *Life Sci.* **28:** 1015–1022.

Leysen J. E. and Gommeren W. (1981) Optimal conditions for [^{3}H]apomorphine binding and anomalous equilibrium binding of [^{3}H]apomorphine and [^{3}H]spiperone to rat striatal membranes: Involvement of surface phenomena versus multiple binding sites. *J. Neurochem.* **36:** 201–219.

Leysen J. E. and Gommeren W. (1984) The dissociation rate of unlabelled dopamine antagonists and agonists from the dopamine-D_2 receptor, application of an original filter method. *J. Recept. Res.* In press.

Leysen J. E., Gommeren W. and De Clerck F. (1983a) Demonstration of S_2-receptor binding sites on cat blood platelets using [^{3}H]ketanserin. *Eur. J. Pharmac.* **88:** 125–130.

Leysen J. E. and Laduron P. M. (1977) A serotonergic component of neuroleptic receptors. *Archs int. Pharmacodyn. Ther.* **230:** 337–339.

Leysen J. E., Niemegeers C. J. E., Tollenaere J. P. and Laduron P. M. (1978) Serotonergic component of neuroleptic receptors. *Nature* **272:** 168–171.

Leysen J. E., Niemegeers C. J. E., Van Nueten J. M. and Laduron P. M. (1982) [^{3}H]Ketanserin (R 41 468), a selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. *Molec. Pharmac.* **21:** 301–314.

Leysen J. E. and Tollenaere J. P. (1982) Chapter 1. Bio-

chemical models for serotonin receptors. *A. med. chem. Rep.* **17:** 1–10.

Leysen J. E., Van Gompel P., Verwimp M. and Niemegeers C. J. E. (1983) Role and localization of serotonin$_2$ (S_2)-receptor-binding sites: Effects of neuronal lesions. In: *CNS Receptors. From Molecular Pharmacology to Behavior* (Mandel P. and DeFeudis F. V., Eds), pp. 373–383. Raven Press, New York.

Müller-Schweinitzer E. and Engel G. (1983) Evidence for mediation by 5-HT_2 receptors of 5-hydroxytryptamine-induced contraction of canine basilar artery. *Naunyn-Schmiedebergs Arch. Pharmac.* **324:** 287–292.

Niemegeers C. J. E., Colpaert F. C., Leysen J. E., Awouters F. and Janssen P. A. J. (1983) Mescaline-induced head-twitches in the rat: An *in vivo* method to evaluate serotonin-S_2 antagonists. *Drug Dev. Res.* **3:** 123–135.

Nishizuka Y. (1983) Phospholipid degradation and signal translation for protein phosphorylation. *Trends Biochem. Sci.* **8:** 13–16.

Ortmann R., Bischoff S., Radeke E., Buech O. and Delini-Stula A. (1982) Correlations between different measures of antiserotonin activity of drugs. Study with neuroleptics and serotonin receptor blockers. *Naunyn-Schmiedebergs Arch. Pharmac.* **321:** 265–270.

Peroutka S. J., Noguchi M., Tolner D. J. and Allen G. S. (1983) Serotonin-induced contraction of canine basilar artery: Mediation by 5-HT_1 receptors. *Brain Res.* **259:** 327–330.

Peroutka S. J. and Snyder S. H. (1979) Multiple serotonin receptors differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]lysergic and diethylamide, [^{3}H]spiroperidol. *Molec. Pharmac.* **16:** 687–699.

Peroutka S. J. and Snyder S. H. (1980) Regulation of serotonin$_2$ (5-HT_2) receptors labeled with [^{3}H]spiroperidol by chronic treatment with the antidepressant amitriptyline. *J. Pharmac. exp. Ther.* **215:** 582–587.

Peroutka S. J. and Snyder S. H. (1981) [^{3}H]-Mianserin: Differential labeling of serotonin$_2$ and histamine$_1$ receptors in rat brain. *J. Pharmac. exp. Ther.* **216:** 142–148.

Schotte A., Maloteaux J. M. and Laduron P. M. (1983) Characterization and regional distribution of serotonin S_2-receptors in human brain. *Brain Res.* **276:** 231–235.

Van Nueten J. M., Janssen P. A. J., Van Beek J., Xhonneux R., Verbeuren T. J. and Vanhoutte P. M. (1981) Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT_2 serotonergic receptors. *J. Pharmac. exp. Ther.* **218:** 217–230.

Van Nueten J. M., Leysen J. E., De Clerck F. and Vanhoutte P. M. (1984) Serotoninergic receptor subtypes and vascular reactivity. *J. cardiovasc. Pharmac.* In press.

Van Nueten J. M., Leysen J. E., Vanhoutte P. M. and Janssen P. A. J. (1982) Serotonergic responses in vascular and non-vascular tissues. *Archs int. Pharmacodyn. Ther.* **256:** 331–334.