Biochemical Pharmacology, Vol. 33, No. 15, pp. 2349–2353, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

COMMENTARY

VASCULAR SEROTONIN RECEPTORS

CORRELATION WITH 5-HT_1 AND 5-HT_2 BINDING SITES

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Serotonin (5-hydroxytryptamine; 5-HT) is a potent vasoactive agent that was first isolated from serum in 1948 [1]. During the past three decades, the constrictor actions of 5-HT have been studied extensively using *in vitro* vascular smooth muscle preparations. The physiologic effects of 5-HT, however, vary greatly depending upon the specific vessel under investigation. For example, the concentration of 5-HT necessary to cause a half-maximal vessel contraction ($K_{ED_{50}}$) ranges from 6.3 nM in the canine basilar artery [2] to 6000 nM in the rat aorta [3]. In addition, the ability of serotonergic antagonists to inhibit 5-HT-induced vascular contractions is extremely variable. While nanomolar concentrations of ketanserin or spiperone will block 5-HT-induced contractions of the rat caudal artery [4–6], micromolar concentrations of these drugs have no effect on 5-HT-induced contractions of the canine saphenous vein [7]. These pharmacologic differences have led a number of investigators to conclude that at least two types of 5-HT receptors must exist in vascular smooth muscle [8–17]. However, unlike the "M" and "D" receptors described in intestinal smooth muscle [18], a classification system for the effects of 5-HT on vascular smooth muscle has not yet been proposed.

Serotonergic receptors have also been studied extensively in the central nervous system. Using brain membrane homogenates, radioligand binding studies have differentiated two 5-HT receptors based on the distinctive pharmacologic characteristics of the ^{3}H-ligand binding sites [19]. 5-HT_1 sites appear to correlate with stimulation of adenylate cyclase, activation of serotonergic autoreceptors, and contractions of the canine basilar artery. In contrast, the 5-HT_2 receptor appears to mediate 5-hydroxytryptophan- and mescaline-induced head twitches, tryptamine-induced seizures, and 5-HT-induced contractions of the rabbit aorta and rat aorta, jugular vein and caudal artery [20–22].

The correlation of radioligand binding data with physiologic actions can provide important information concerning the pharmacological properties of receptors. Although ^{3}H-ligand binding techniques provide a rapid and reliable measure of drug potency, binding studies cannot differentiate the agonist versus antagonist properties of drugs, relative efficacy of agents, or drug interactions with other receptor sites involved in a specific physiologic response. Therefore, establishment of a correlation between radioligand studies and physiologic systems requires an extensive analysis of both the absolute and relative potencies of a series of drugs. As a result, physiologic studies that have utilized only one or two drugs are of limited value in attempts to correlate pharmacologic actions with ^{3}H-ligand binding data. In the present report, data obtained from *in vitro* contraction studies of 5-HT in circular or helical strips of vascular smooth muscle will be reviewed. The data show that vascular responses to 5-HT can be divided into two distinct groups on the basis of vessel response to 5-HT, pattern of drug inhibition, and antagonist potencies. Each group appears to correlate with either 5-HT_1 or 5-HT_2 receptors as defined by radioligand binding studies.

Pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites

Serotonergic agents interact with a number of brain membrane binding sites that can be labeled by ^{3}H-ligands [21]. Disparities in drug affinities for the various serotonergic ligands led to the differentiation of 5-HT_1 and 5-HT_2 receptors [19]. Thus, 5-HT_1 binding sites can be labeled with either [^{3}H]5-HT or [^{3}H]lysergic acid diethylamide (LSD). As initially described, serotonergic agonists have high affinity for this site while "classical" antagonists such as cyproheptadine and methysergide have a much lower affinity. On the other hand, [^{3}H]spiperone, [^{3}H]LSD, [^{3}H]mianserin, [^{3}H]ketanserin and [I^{125}]LSD can be used to label 5-HT_2 binding sites. In contrast to 5-HT_1 sites, serotonergic antagonists have high affinity for this site while 5-HT and related tryptamines are markedly less potent.

The marked differences in drug affinities for these two binding sites allow for a simple and rational classification system for peripheral serotonergic receptors. For example, 5-HT has an apparent affinity (K_i) value of 2.7 nM for 5-HT_1 sites (Table 1). However, its apparent affinity for 5-HT_2 sites is 2700 nM. As a result, 5-HT exhibits a thousand-fold difference in affinity between the two binding sites. The inverse situation exists with certain serotonergic antagonists. For example, ketanserin, cyproheptadine and spiperone are significantly more potent at 5-HT_2 than 5-HT_1 sites. The relative affinities can be expressed

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Table 1. Drug affinities for 5-HT_1 and 5-HT_2 receptors

Drug	K_i (nM)		Ratio 5-HT_1/5-HT_2
	5-HT_1	5-HT_2	
5-Hydroxytrytamine	2.7	2700	0.001
Methysergide	88	2.6	30
Ketanserin	860	4.8	200
Cyproheptadine	1500	2.0	800
Spiperone	730	0.51	1000

as a potency ratio at 5-HT_1 versus 5-HT_2 binding sites. Thus, 5-HT has a ratio of 0.001 or a three order of magnitude preference for 5-HT_1 over 5-HT_2 receptors. Spiperone is at the other extreme of relative potencies with a ratio of 1000. This million-fold range of ratios should allow for a classification of the vascular effects of 5-HT using a minimal number of selective drugs.

Agonist interactions with vascular 5-HT receptors

Depending on the specific vessel studied, the concentration of 5-HT needed to produce a half-maximal contraction varies from nanomolar to millimolar. However, certain patterns have been noted. For example, a number of investigators have observed that intracranial vasculature is more sensitive to 5-HT than peripheral vasculature [10, 12, 14, 17, 23–27]. The $K_{ED_{50}}$ of 5-HT in the intracranial circulation has consistently been noted to be less than 100 nM. The initial studies of Toda and Fujita [24] obtained half-maximal stimulation of canine intracranial vessels at 43 nM 5-HT while other laboratories have reported $K_{ED_{50}}$ values as low as 3.0 nM in similar systems [14]. Canine basilar artery segments can be contracted by 5-HT with $K_{ED_{50}}$ values ranging from 5.0 to 36 nM [2, 4, 27–32]. Similarly, human intracranial vessels such as the basilar artery, middle cerebral artery, and pial vessels contract in response to relatively low concentrations of 5-HT with half-maximal contraction occurring at 6.5 to 57 nM [10, 12, 14, 17, 31]. Although less extensively studied, potent actions of 5-HT on intracranial vasculature have also been reported in the cat and rabbit [10, 12, 14].

The interaction of 5-HT with peripheral vessels has produced more variable results. Only a single extracranial vessel, the canine saphenous vein, has been shown consistently to have a $K_{ED_{50}}$ value of less than 100 nM in response to 5-HT [11, 13, 33, 34]. In all other peripheral vessels thus far tested, 5-HT is a much weaker agonist. For example, the $K_{ED_{50}}$ of 5-HT in the rabbit aorta is 150–170 nM [35, 36]. The rat aorta, on the other hand, is one of the least responsive vessels to 5-HT with $K_{ED_{50}}$ values ranging from 3800 to 6000 nM [3, 17]. Other vessels in this category are the rat caudal artery ($K_{ED_{50}}$ = 190 nM) [4] and the canine femoral artery ($K_{ED_{50}}$ = 200–420 nM) [13, 27]. For purposes of this review, vascular smooth muscle will be divided into two major groups (Table 2): Group 1 contains vessels with 5-HT $K_{ED_{50}}$ values less than 100 nM while Group 2 includes vessels with $K_{ED_{50}}$ values greater than 100 nM.

In addition, certain laboratories have reported that methysergide occasionally displays agonistic actions on vascular smooth muscle. Allen *et al.* [2] showed that methysergide in the canine basilar artery produced 58% of the maximal contraction obtained using 5-HT and had a $K_{ED_{50}}$ value of 90 nM. Agonist effects of methysergide in canine cerebral arteries

Table 2. Competitive (C) versus-non competitive (N-C) drug antagonism of 5-HT-induced vascular contractions

Vessel	5-HT $K_{ED_{50}}$ (nM)	Methysergide	Cyproheptadine	Spiperone	Ketanserin	Refs.
Group 1						
Canine basilar artery	5–36	N-C (agonist)	N-C	N-C	N-C	[2, 4, 27–32, 37, 38]
Human basilar artery	7–57	N-C (agonist)	N-C			[17, 31]
Human apial vessels	7–11	N-C	N-C			[10, 12]
Canine saphenous vein	31–71	N-C (agonist)	N-C		N-C	[4, 8, 9, 11, 13, 33, 34]
Group 2						
Rabbit aorta	150–170	C	C	C	C	[6, 7, 36, 39]
Rat aorta	3800–6000	C	C	C	C	[3, 6, 17]
Rat caudal artery	190	C	C		C	[4, 6]
Canine femoral artery	200–420	C	C			[13]

Table 3. Drug potencies (pA_2 values) in group 2 vessels

Vessel	pA_2 values				Refs.
	Methysergide	Cyproheptadine	Spiperone	Ketanserin	
Rabbit aorta	8.5	8.7		8.7	[6]
	8.5	8.7	8.9	8.7	[39]
			9.1		[36]
Rat aorta	8.0	8.8			[17]
		9.0		8.4	[6]
			9.7		[3]
Rat caudal artery	10.0	8.7		8.8	[6]
				9.1	[4]
Canine femoral artery	8.5	8.7			[13]

have been observed to occur at nanomolar concentrations by Toda *et al.* [40] and Muller-Schweinitzer [15]. Additionally, this agent mimics the action of 5-HT in the human basilar artery [17] and canine saphenous vein [8, 9, 11, 13]. On the other hand, methysergide potentiates the effects of 5-HT in human pial vessels [10, 12]. In all Group 2 vessels, methysergide acts as an antagonist at nanomolar concentrations and no agonist activity has ever been noted at less than micromolar concentrations (Table 3).

Competitive versus non-competitive antagonism

Antagonists of 5-HT can provide important information concerning the nature of the 5-HT receptor. Based on the hypothesis that 5-HT and its antagonists compete for receptors according to mass law equations [41], antagonist affinity for the 5-HT receptor can be derived by drug competition experiments. Two classical types of drug antagonism have been recognized: competitive and non-competitive. In competitive inhibition, agonists such as 5-HT are able to overcome drug antagonism. The agonist dose–response curve is shifted to the right and is parallel to the baseline response. Furthermore, the theoretical affinity of the antagonist for the receptor can be derived from a Schild plot and is called the pA_2 value [42]. In non-competitive inhibition, on the other hand, drug antagonism is insurmountable. The dose–response curve is not shifted but the maximal contraction is decreased. Schild plot analysis, therefore, cannot be performed. Instead, the concentration of an antagonist needed to decrease the maximal response by 50% has been suggested as a useful measure of drug potency and has been designated the pA_h [41] or pD_2 [43] value.

As shown in Table 2, a comparison of the types of 5-HT antagonism observed in Group 1 and 2 vessels reveals a striking pattern. In Group 1 vessels (i.e. those with relatively high affinity for 5-HT), non-competitive antagonism has consistently been documented by a variety of laboratories. For example, methysergide and cyproheptadine do not cause parallel shifts of the 5-HT dose–response curve in the canine basilar artery [4, 30, 37, 38], human basilar artery [17], human pial vessels [10, 12], or canine saphenous vein [9, 13] (Table 2). Instead, the $K_{ED_{50}}$ of 5-HT is essentially unaffected while the maximal response to 5-HT is reduced. Similarly, ketanserin is an insurmountable antagonist of 5-HT in the canine basilar artery [4, 30] and the saphenous vein [4]. Although studied only in canine basilar artery, spiperone also produces non-competitive antagonism of 5-HT-induced contractions [30].

The pattern of inhibition in Group 2 vessels, on the other hand, is almost exclusively competitive (Table 3). In the rabbit aorta, for example, methysergide, cyproheptadine, spiperone and ketanserin all cause parallel shifts of the 5-HT dose–response curve [6, 7, 36, 39]. The same pattern of competitive antagonism by these four drugs occurs in the rat aorta [3, 6, 17]. In the rat caudal artery, inhibition of 5-HT-induced contractions by methysergide, cyproheptadine and ketanserin has been reported to be competitive [6] although another laboratory has shown non-competitive inhibition by methysergide in this vessel [4]. Finally, both methysergide and cyproheptadine are competitive antagonists of 5-HT in the canine femoral artery [13].

Antagonist drug potencies at 5-HT vascular receptors

Because drug antagonism in Group 1 vessels is non-competitive, Schild plot analysis cannot be used to determine drug potencies. Instead, the concentration of inhibitor drug which reduces the C_{max} of the agonist to 50% of its baseline value can be used as a measure of drug activity [41, 43]. Using this method in the canine basilar artery, methysergide was the most potent antagonist with a 50% blockade of the 5-HT-induced contraction occurring at 380 nM [30]. Spiperone and ketanserin were less potent, non-competitively blocking 5-HT contractions at micromolar concentrations [30, 44]. In the canine saphenous vein, spiperone and ketanserin were without effect at micromolar concentrations [7]. In another study of five extracranial vessels, ketanserin was weakest in the canine saphenous vein where approximately 50% of C_{max} could still be obtained in the presence of 10 μM ketanserin [4]. As a result, 5-HT is markedly more potent than each of the antagonists thus far tested in Group 1 vessels.

By contrast, Schild plots can be appropriately used to calculate pA_2 values in Group 2 vessels. The pA_2 is defined as the concentration of inhibitor which causes a 2-fold increase in the $K_{ED_{50}}$ of the agonist. As shown in Table 3, there is little variation in the pA_2 values for each antagonist among the four vessels. That is, methysergide ($pA_2 = 8.0$ to 10.0) and cyproheptadine ($pA_2 = 8.6$ to 9.0) have been

reported by a variety of laboratories to have a similar potency in each of the four Group 2 vessels studied [6, 13, 17, 39]. The pA_2 values of spiperone (8.9 to 9.7) against the rabbit and rat aorta vary by less than an order of magnitude despite the fact that the data were obtained from three different laboratories [3, 36, 39]. Ketanserin ($pA_2 = 8.4$ to 9.1) is essentially equipotent in its antagonism of 5-HT-induced contractions of the rabbit aorta, rat aorta and rat caudal artery [4, 6, 39].

The finding that antagonists have equal potencies against the same agonist under different tissue conditions suggests that the receptor mediating contraction in these four vessels is the same [41]. Of note is the fact that each of the antagonists has nanomolar potency in vessels in which the 5-HT $K_{ED_{50}}$ is greater than 100 nM. Although less extensively studied, a similar pattern of drug interactions has been noted in the rat jugular vein [3, 45], rabbit femoral artery [31, 46] and canine carotid and external lingual arteries [13]. Furthermore, a similarity between the pattern of inhibition at these receptor sites and classical "D" receptors [18] has been noted [39].

Conclusion

Vascular smooth muscle can be contracted by nanomolar to micromolar concentrations of 5-HT. However, the effects of 5-HT, related agonists, and antagonists vary markedly depending on the specific vessel under study. Although at least two distinct vascular 5-HT receptors have been hypothesized by a number of investigators, no systematic classification system has yet been proposed. A review of *in vitro* vascular smooth muscle contraction studies differentiates two distinct types of responses to serotonergic agents. In Group 1 vessels, 5-HT is relatively potent (less than 100 nM $K_{ED_{50}}$), may be mimicked by methysergide and, most importantly, is only weakly inhibited by classical 5-HT antagonists in a non-competitive manner. In marked contrast, Group 2 vessels are characterized by a relatively low affinity for 5-HT ($K_{ED_{50}}$ greater than 100 nM), methysergide invariably acts as an antagonist, and other classical antagonists produce extremely potent, competitive inhibition.

The major finding of the present report is that these two groups of vascular smooth muscle receptors appear to correspond to the 5-HT_1 and 5-HT_2 binding sites identified in brain membranes. Indeed, 5-HT_1 sites are characterized by high affinity for 5-HT and low affinity for classical antagonists. The same characteristics are present in Group 1 vessels where 5-HT is approximately two to three orders of magnitude more potent than the classical 5-HT antagonists. On the other hand, 5-HT_2 binding sites have high affinity for serotonergic antagonists but are unaffected by 5-HT at similar concentrations. The situation is also found in Group 2 vessels where the classical antagonists are two to three orders of magnitude more potent than 5-HT.

The correlation of drug effects at brain membrane binding sites with physiologic data has important implications. First of all, ^{3}H-ligand studies label membrane recognition sites. The finding that these binding sites mediate physiologic actions such as contraction of vascular smooth muscle allows them to be designated "receptors". Second, an understanding of the specific receptor mediating contraction may provide useful information concerning the molecular pharmacology and physiology of vascular receptor function. For example, the differentiation of competitive versus non-competitive inhibition at specific receptor sites implies a fundamental difference in receptor activation mechanisms. Finally, knowledge of receptor subtype localization in vasculature may elucidate the functional role of this neurotransmitter in vascular smooth muscle and thus generate useful clinical information. For example, if 5-HT_1 receptors do predominate in in intracranial vasculature, as suggested above, then a specific pharmacologic blockade of cerebral vasoconstriction may be beneficial in many vascular neurological diseases such as migraine, vasospasm, and ischemia.

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