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CORRELATION OF [^{3}H]5-HYDROXYTRYPTAMINE (5HT) BINDING TO BRAIN STEM PREPARATIONS AND THE PRODUCTION AND PREVENTION OF MYOCLONUS IN GUINEA PIG BY 5HT AGONISTS AND ANTAGONISTS

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In guinea pig brain stem preparations [^{3}H]5-hydroxytryptamine (5HT) bound specifically to both high and low affinity sites, but specific [^{3}H]spiperone binding was low and could not be consistently detected. This indicates a prevalence of 5HT-1 type receptors in this tissue. High affinity-specific [^{3}H]5HT binding was more potently displaced by indole-containing 5HT agonists than by piperazine-containing 5HT agonists. This agreed with the observation that indole-containing, but not piperazine-containing compounds induced dose-dependent myoclonus in guinea pigs which originates from brain stem. The capacity of indoleamine antagonists to displace [^{3}H]5HT-specific binding from guinea pig brain stem was similar to their reported potency in displacing [^{3}H]5HT from 5HT-1 receptors. The [^{3}H]5HT-labelled binding site in guinea pig brain stem is a 5HT-1 receptor and appears to be responsible for the induction of indoleamine-dependent myoclonus.

Myoclonus 5HT receptor subtypes Brain stem 5HT agonists 5HT antagonists Ligand binding

1. Introduction

The administration of L-5-hydroxytryptophan (5HTP) to guinea pigs induces myoclonic jerking due to stimulation of brain stem 5-hydroxytryptamine (5HT) receptors (Klawans et al., 1973; Chadwick et al., 1978). However, 5HT agonists differ markedly in their ability to induce myoclonus. 5HT agonists containing an indole nucleus induce dose-dependent myoclonus in guinea pigs (Luscombe et al., 1981, 1982a). However, 5HT agonists possessing a piperazine moiety induce only occasional jerking at toxic doses not specifically associated with 5HT agonist activity (Luscombe et al., 1981, 1982a). Other discrepancies in 5HT drug action are observed in this model. For example, the ability of centrally acting 5HT antagonists to inhibit 5HTP-induced myoclonus, and the ability of selective 5HT re-uptake blockers to potentiate the effects of a threshold dose of 5HTP, differ from their known potency in other models of central 5HT action (Luscombe et al., 1981).

This evidence suggests that a particular 5HT receptor population is responsible for indoleamine-dependent myoclonus. Brain 5HT receptors may be divided into distinct populations on the basis of the ligands labelling them and their regional distribution in brain. The 5HT receptors selectively labelled in vitro by [^{3}H]5HT, and showing preferential [^{3}H]5HT displacement by 5HT agonists, have been designated 5HT-1 receptors. They appear to be the dominant type of 5HT receptor in all rodent brain areas apart from fron-

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tal cortex. In contrast, the 5HT receptors specifically labelled in vitro by [^{3}H]spiperone, and exhibiting preferential displacement of [^{3}H]spiperone by 5HT antagonists, have been identified in rodent frontal cortex and are called 5HT-2 receptors (Peroutka and Snyder, 1979, 1981; Seeman et al., 1980; Leysen, 1981; Pedigo et al., 1981). At present all functional actions at 5HT receptors are associated with 5HT-2 receptors (Leysen, 1981; Peroutka et al., 1981; Green et al., 1983). The function of 5HT-1 receptors remains unknown despite the fact that they are selectively labelled by [^{3}H]5HT.

We have investigated the nature of 5HT receptors in the guinea pig brain stem. We examined the relationship between the ability of 5HT active compounds to induce or antagonise myoclonus in guinea pigs and their capacity to displace [^{3}H]5HT from specific binding sites in guinea pig brain stem preparations. We find evidence that 5HT-1 receptors are responsible for indoleamine-dependent myoclonus.

2. Materials and methods

2.1. Animals

Female Dunkin-Hartley guinea pigs (250-500 g; Little Lions Farm) were used in all studies. The animals were housed under standard conditions of lighting (12 h light/dark cycle) and temperature (22-24°C), and were allowed free access to food and water.

2.2. [^{3}H]5HT binding assay

Guinea pigs were sacrificed by cervical dislocation and decapitation. The brain was removed and dissected over ice. Following removal of the cerebellum, the brain stem was isolated by transections at the level of the superior colliculus and the upper cervical cord. The tissue was homogenised in 40 vol. of ice-cold 50 mM Tris buffer, pH 7.6, using a Polytron homogeniser (setting 7 for 15 s), and centrifuged at 37 500 × *g* for 10 min using a Sorvall RC5B centrifuge. The pellet was resuspended in 40 vol. of fresh buffer and preincubated at 37°C for 15 min to destroy endogenous 5HT (Nelson et al., 1978) prior to centrifugation and a further resuspension and centrifugation cycle. The final pellet was resuspended in 100 vol. of ice-cold 50 mM Tris buffer, pH 7.6 (containing 4 mM $CaCl_2$, 0.1% ascorbic acid and 10 μM pargyline) (Bennett and Snyder, 1976).

Aliquots of the membrane preparation (900 μl) containing approximately 9 mg tissue, were incubated for 10 min at 37°C in glass tubes containing 0.5-64 nM [^{3}H]5HT (specific activity 16.6 Ci/mmol; Amersham International) in a final volume of 1.0 ml of 50 mM Tris buffer, pH 7.6. Specific [^{3}H]5HT binding was defined as that displaced in the presence of 10^{-5} M 5HT added in 50 μl 0.1% ascorbic acid. When studied, competing drugs were dissolved in 0.1% ascorbic acid and 50 μl added to the cold homogenates 10 min prior to inclusion of 50 μl 4 nM [^{3}H]5HT. The concentration of ascorbic acid employed (0.1%) had no effect on the specific binding of [^{3}H]5HT to brain stem preparations. In any case the same quantity of 0.1% ascorbic acid was included into all samples. Incubations were terminated by rapidly filtering the samples under vacuum through Whatman GF/B glass fibre filters supported on a Millipore 1224 filtration manifold. The filters were rapidly rinsed with 3 × 5 ml of ice-cold 50 mM Tris buffer, transferred to vials and the radioactivity determined by liquid scintillation counting at an efficiency of approximately 40%.

2.3. [^{3}H]Spiperone binding assay

[^{3}H]Spiperone (specific activity 21 Ci/mmol; Amersham International) binding assays were performed using a similar protocol with the exception that the final washed tissue pellet was resuspended in 100 volumes of 50 mM ice-cold Tris buffer, pH 7.6 (containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline) (Leysen, 1981). The specific binding of [^{3}H]spiperone (0.25-32 nM) was defined using either 10^{-5} M 5HT or 10^{-6} M d-LSD (d-lysergic acid diethylamide tartrate).

2.4. Drugs used

The following classes of drugs were examined for their ability to displace [^{3}H]5HT from specific

binding sites in guinea pig brain stem. Each concentration of each drug was examined in duplicate.

1. The following indole-containing 5HT agonists were examined at 8 concentrations between 10^{-9} and 10^{-4} M: 5-hydroxytryptamine creatinine sulphate, N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, bufotenine monooxalate hydrate (Sigma Chemical Co.), d-LSD, psilocin, psilocybin (Sandoz Products Ltd.) and RU 24969 (5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole, succinate; (Roussel-UCLAF)).

2. The following piperazine-containing 5HT agonists were examined at 8 concentrations between 10^{-9} and 10^{-4} M: quipazine maleate (2-(1-piperazinyl)quinoline maleate; Miles Laboratories), MK 212 (6-chloro-2-(1-piperazinyl)pyrazine), 2-(1′-piperazinyl)quinoxaline hydrochloride (Merck, Sharpe and Dohme Ltd.), phenylpiperazine, 1-(m-trifluoromethylphenyl)piperazine, m-chlorophenylpiperazine dihydrochloride and p-chlorophenylpiperazine dihydrochloride (Aldrich Chemical Co.).

3. The following indoleamine antagonists were examined at 8 concentrations between 10^{-9} and 10^{-4} M: methergoline (Farmitalia), methysergide hydrogen maleate (Sandoz Products Ltd.), mianserin hydrochloride (Organon Labs.), cyproheptadine hydrochloride (Merck, Sharpe and Dohme Ltd.), cinanserin hydrochloride (Squibb) and BW 501C (α-anilino-N-2-m-chlorphenoxypropyl-acetamide hydrochloride; Burroughs Wellcome Ltd.).

4. The following 5HT precursors were examined at 3 concentrations between 10^{-6} and 10^{-4} M: L-tryptophan and L-5-hydroxytryptophan (Sigma Chemical Co.).

5. The following other neurotransmitters were examined at 8 concentrations between 10^{-9} and 10^{-3} M: tryptamine hydrochloride, dopamine hydrochloride and noradrenaline hydrochloride (Sigma Chemical Co.).

2.5. *Analysis of data*

Data were subjected to Scatchard analysis for determination of the number of binding sites (B_{max}) and the dissociation constant (K_D). Graphical analysis revealed two components of [^{3}H]5HT binding which were resolved using nonlinear regression analysis. Each ^{3}H-ligand binding assay was performed in triplicate at 8 ligand concentrations on at least 2 separate tissue pools. In displacement experiments linear regression analysis of the linear portion of the displacement curve was used to calculate IC_{50} values, that is, the concentration of displacing agent causing a half-maximal displacement of [^{3}H]5HT. The IC_{50} values for [^{3}H]5HT displacement by 5HT agonists and antagonists were determined on at least 3 separate tissue pools.

3. Results

3.1. *Specific binding of [^{3}H]5HT to guinea pig brain stem preparations*

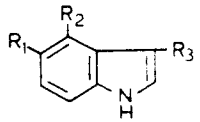
Scatchard analysis showed the specific binding of [^{3}H]5HT (0.5-64 nM) to be to 2 sites in guinea pig brain stem preparations (fig. 1). Resolution of the 2 components of ^{3}H-binding using nonlinear regression analysis revealed that the low affinity site had a B_{max} of 9.1 ± 3.4 pmol/g wet weight of tissue, K_D of 44.4 ± 17.6 nM, while the high affinity site had a B_{max} of 1.4 ± 0.2 pmol/g wet weight of tissue, K_D 2.8 ± 0.8 nM. A ligand concentration of 4 nM [^{3}H]5HT was chosen as suitable for displacement studies. Total binding of [^{3}H]5HT to brain stem preparations at a ligand concentration of 4 nM amounted to 481 ± 9 cpm. Approximately 30% of this total binding could be displaced by incorporation of 10^{-5} M 5HT giving a specific component of approximately 146 ± 9 cpm.

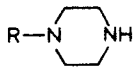
3.2. *Binding of [^{3}H]spiperone*

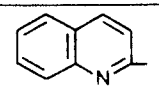
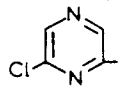
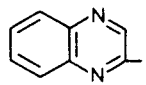
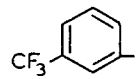
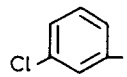
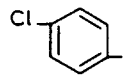
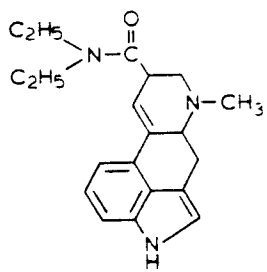
Specific binding of [^{3}H]spiperone (0.25-32 nM) to guinea pig brain stem preparations could not be reliably detected using either unlabelled 5HT (10^{-5} M) or d-LSD (10^{-6} M) as the displacing agent. Total binding of [^{3}H]spiperone at a concentration of 4 nM amounted to 2199 ± 86 cpm. In a range of experiments the amount of total [^{3}H]spiperone binding displaceable by 5HT varied between a 1% decrease and a 13% increase, while d-LSD caused a 6-9% increase in total binding of [^{3}H]spiperone.

TABLE 1

The ability of 5HT indole-containing 5HT agonists, and piperazine-containing 5HT agonists to displace [^{3}H]5HT (4 nM) from specific binding sites in guinea pig brain stem preparations.





Name	R_1	R_2	R_3	IC_{50} (nM ± S.E.M.)	Name	R	IC_{50} (nM ± S.E.M.)
L-Tryptophan	H	H	$CH_2CH(NH_2)COOH$	$>10^{-6}$	Quipazine		7241 ± 2714
L-5HTP	OH	H	$CH_2CH(NH_2)COOH$	$>10^{-6}$			
5HT	OH	H	$CH_2CH_2NH_2$	6.1 ± 0.3	MK 212		17260 ± 9714
Tryptamine	H	H	$CH_2CH_2NH_2$	1391			
N,N-Dimethyl-tryptamine	H	H	$CH_2CH_2N(CH_3)_2$	280 ± 105	2-(1-Piperazinyl)-quinoxaline		42551 ± 21742
5-Methoxy-N,N-dimethyl-tryptamine	CH_3O	H	$CH_2CH_2N(CH_3)_2$	158 ± 24	1-(m-Trifluoromethyl-phenyl)piperazine		408 ± 124
Bufotenine	OH	H	$CH_2CH_2N(CH_3)_2$	23 ± 12			
Psilocin	H	OH	$CH_2CH_2N(CH_3)_2$	241 ± 81	m-Chlorophenylpiperazine		3261 ± 2738
Psilocybin	H	H_2PO_4	$CH_2CH_2N(CH_3)_2$	519 ± 82	p-Chlorophenylpiperazine		8729 ± 4303
RU 24969	CH_3O	H		30 ± 6	Phenylpiperazine		7476 ± 2725
d-LSD				3.4 ± 1.7			

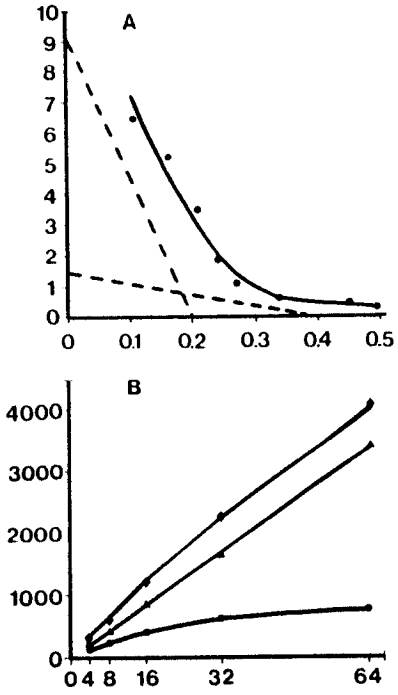


Fig. 1. Characteristics of [^{3}H]5HT binding to guinea pig brain stem preparations. A) Scatchard analysis of specific binding of [^{3}H]5HT (0.5-64 nM) using 10^{-5} M 5HT to define specific binding. The data were resolved into two components of binding using nonlinear regression analysis (interrupted lines). Ordinate: bound (pmol/g). Abscissa: bound/free (pmol/g) (nM). B) Total (◆), residual (▲) and specific binding (●) of [^{3}H]5HT (0.5-64 nM) as judged by displacement with 10^{-5} 5HT. Ordinate: [^{3}H]5HT binding (cpm). Abscissa: [^{3}H]5HT concentration (nM). N.B. For clarity, only data for higher concentrations of [^{3}H]5HT (4-64 nM) are shown.

A prevalence of [^{3}H]5HT-specific binding sites in guinea pig brain stem as compared to specific [^{3}H]spiperone binding sites has been previously reported (Peroutka and Snyder, 1981).

3.3. Drug displacement of specific [^{3}H]5HT binding

3.3.1. Monoamine neurotransmitters and 5HT precursors

Specific [^{3}H]5HT binding was potently displaced by 5HT ($IC_{50} = 6.1 \pm 0.3$ nM), but not by noradrenaline ($IC_{50} = 62\,600$ nM) or dopamine ($IC_{50} = 159\,000$ nM). Tryptamine showed moderate activity ($IC_{50} = 1391$ nM) in displacing [^{3}H]5HT. The 5HT precursors, L-tryptophan ($IC_{50} = 2671$ μM) and 5HTP ($IC_{50} = 1216$ μM) were virtually inactive (table 1).

3.3.2. 5HT agonists

5HT agonists differed markedly in their ability to displace specific [^{3}H]5HT binding from guinea pig brain stem preparations (table 1, fig. 2). Thus [^{3}H]5HT was potently displaced by indole-containing 5HT agonists (range of $IC_{50} = 3.4$-519 nM) but only weakly displaced by piperazine-containing 5HT agonists (range of $IC_{50} = 408$-42 551 nM).

All indole-containing 5HT agonists displaced total [^{3}H]5HT binding to within ±10% of the amount displaced by an excess concentration of cold 5HT (10^{-5} M). A similar result was obtained with piperazine-containing 5HT agonists except that even at the highest concentration (10^{-4} M) MK212 and 2-(1′-piperazinyl)quinoxaline respectively displaced only 88.5% and 84.2% of the amount of total [^{3}H]5HT binding displaced by 10^{-5} M 5HT.

Within the series of indole-containing 5HT agonists the presence of a 5-hydroxy group in the

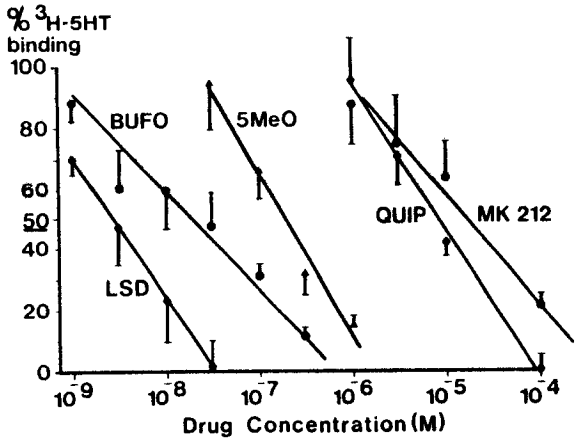


Fig. 2. Displacement of [^{3}H]5HT (4 nM) from guinea pig brain stem preparations by 5HT agonists. LSD, d-lysergic acid diethylamide; BUFO, bufotenine monoxalate hydrate; 5MeO, 5-methoxy-N,N-dimethyltryptamine; QUIP, quipazine (2-(1-piperazinyl)quinoline maleate); MK 212 (6-chloro-2-(1-piperazinyl)pyrazine). Points indicate mean: vertical bars indicate S.E.M. Ordinate: % specific binding of [^{3}H]5HT (defined by 10^{-5} M 5HT) Abscissa: concentration of displacing agent (M).

indole nucleus enhanced [^{3}H]5HT displacement (compare 5HT and bufotenine with tryptamine, N,N-dimethyltryptamine, 5-methoxy-N,N-dimethyltryptamine, psilocin and psilocybin) (table 1). The presence of a 4-hydroxy group did not potentiate displacement as evidenced by the equipotency of psilocin and N,N-dimethyltryptamine. Activity was increased by replacement of the dimethylated side-chain of 5-methoxy-N,N-dimethyltryptamine with the hydrogenated pyridinyl ring of RU 24969 (5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole, succinate) (table 1).

Phenylpiperazine derivatives were the most potent displacers of specific [^{3}H]5HT binding within the piperazine-containing 5HT agonists. The presence of a pyrazine ring greatly decreased efficacy (see MK 212 (6-chloro-2-(1-piperazinyl)pyrazine) and 2-(1′-piperazinyl)quinoxaline) (table 1). Addition of a trifluoromethyl group in the m-position of the phenyl ring enhanced displacing potency compared to phenylpiperazine. Chloro-substitutions in the m- but not p-position of the phenyl ring also increased [^{3}H]5HT displacement but was less effective than the m-CF_3 group (table 1).

The potency ranking for disaplacement of specific [^{3}H]5HT binding within each group of 5HT agonists agrees well with previous reports for both indole-containing 5HT agonists (Bennett and Snyder, 1976; Fillion et al., 1978; Whitaker and Seeman, 1978) and piperazine-containing 5HT agonists (Fuller et al., 1980; Nelson et al., 1980) in preparations of rat and bovine brains.

3.3.3. Indoleamine antagonists

Indoleamine antagonists showed a differential ability to displace specific [^{3}H]5HT binding (table 2). Thus methergoline and methysergide potently displaced specific [^{3}H]5HT binding while cinanserin and BW 501C were only weakly effective; mianserin and cyproheptadine displayed modest potency (table 2, column a). Ergot derivatives were thus the most effective displacing agents, in agreement with previous studies (Seeman et al., 1980; Leysen, 1981; Peroutka et al., 1981).

The capacity of indoleamine antagonists to displace [^{3}H]5HT from guinea pig brain stem preparations closely parallels their ability to displace [^{3}H]5HT from known 5HT-1 receptors but not [^{3}H]spiperone from 5HT-2 receptors (Leysen, 1981; Peroutka et al., 1981) (table 2).

4. Discussion

The different post-synaptic 5HT receptors present in brain are identified by different ^{3}H-ligands. The 5HT-1 receptors are labelled selectively by [^{3}H]5HT while [^{3}H]spiperone identifies 5HT-2 receptors (Peroutka and Snyder, 1979, 1981; Seeman et al., 1980; Leysen, 1981; Pedigo et al., 1981). The finding of a saturable binding site for [^{3}H]5HT but not for [^{3}H]spiperone, in guinea pig brain stem preparations suggests that 5HT-1 sites predominate in this area.

Indole-containing 5HT agonists potently displaced [^{3}H]5HT from its specific binding site in

TABLE 2

Comparison of the ability of indoleamine antagonists to displace ^{3}H-ligands from rodent brain 5HT-1 and 5HT-2 receptors. a) This study; guinea pig brain stem ([^{3}H]5HT). b) Peroutka et al. (1981); rat cortex ([^{3}H]5HT and [^{3}H]spiperone). c) Leysen (1981); rat hippocampus ([^{3}H]5HT) and frontal cortex ([^{3}H]spiperone).

Indoleamine antagonist	[^{3}H]5HT (5HT-1)			IC_{50} (nM)	[^{3}H]Spiperone (5HT-2)	
	a	b	c		b	c
Methergoline	7.1 ± 1.5	9.9	25		2.1	4
Methysergide	8.9 ± 2.5	150	126		3.1	35
Mianserin	499 ± 99	820	1259		4.7	40
Cyproheptamine	607 ± 501	1110	794		2.4	20
Cinanserin	3377 ± 870	1800	3981		15	126

TABLE 3

Comparison of the ability of 5HT agonists to displace specific [^{3}H]5HT binding from guinea pig brain stem and to induce brain stem mediated myoclonus in guinea pigs. * The myoclonus score of each guinea pig was assessed using a 0-4 observer rating system reflecting both the frequency and intensity of the myoclonic jerking. ** The mean duration of myoclonus was that observed for the dose of each compound causing the maximum myoclonus score shown. *** Seizures also were evoked at the dose indicated. Behavioural data from Luscombe et al. (1982a).

Name	IC_{50} (nM ± S.E.M.)	Threshold myoclonic dose (mg/kg i.p.)	Maximum myoclonus score ± S.E.M. (dose, mg/kg) *	Mean duration of myoclonus (min) **
Indole-containing agonists				
d-LSD	3.4 ± 1.7	0.25	3.9 ± 0.1 (8.0)	60
Bufotenine	23 ± 12	80	1.9 ± 0.6 (160)	25
RU 24969	30 ± 6	40	3.0 ± 0.0 (80)	270
5-Methoxy-N,N-dimethyltryptamine	158 ± 24	2.5	3.8 ± 0.2 (20)	30
Psilocin	241 ± 81	10	3.0 ± 0.0 (160)	85
Dimethyltryptamine	280 ± 105	20	3.4 ± 0.2 (160)	110
Psilocybin	519 ± 82	10	2.5 ± 0.5 (180)	50
Piperazine-containing agonists				
1-(m-Trifluoromethyl-phenyl)piperazine	408 ± 124	40	2.0 ± 1.0 (120) ***	55
m-Chlorophenylpiperazine	3261 ± 2738	80	1.7 ± 0.5 (160) ***	5
Quipazine	7241 ± 2714	30	2.3 ± 0.5 (160) ***	10
Phenylpiperazine	7476 ± 2725	> 160	0 (160) ***	
p-Chlorophenylpiperazine	8729 ± 4303	> 160	0 (160) ***	
MK 212	17260 ± 9714	40	2.0 ± 0.0 (80) ***	1
2-(1-Piperazinyl) quinoxaline	42551 ± 21742	5	0.6 ± 0.3 (10)	10

TABLE 4

Comparison of the ability of indoleamine antagonists to displace specific [^{3}H]5HT binding from guinea pig brain stem and to inhibit myoclonus induced in guinea pigs by either 5HTP (plus carbidopa) or tryptamine (plus pargyline). * 5HTP-induced myoclonus in guinea pigs. Indoleamine receptor antagonists were intraperitoneally administered immediately prior to 5HTP (75 mg/kg s.c.) plus carbidopa (25 mg/kg i.p. 60 min previously). This combination generally produced continuous, rhythmic myoclonus (average score 3.8 ± 0.1) and the table shows the % reduction of the myoclonus score by the antagonist, assessed 90 min after 5HTP treatment (Luscombe et al., 1981, 1982b). ** Tryptamine-induced myoclonus in guinea pigs. Indoleamine receptor antagonists were intraperitoneally administered either before or after tryptamine (40 mg/kg i.p.) plus pargyline (75 mg/kg i.p. 60 min previously). This combination usually induced myoclonus (average score = 2.2 ± 0.3) and the table shows the % reduction of the myoclonus score by the antagonist, assessed 30 min after tryptamine administration (Luscombe et al., 1982b). N.T., not tested.

Drug (mg/kg)	IC_{50} (nM ± S.E.M.)	% Reduction of myoclonus score induced in guinea pigs by	
		5HTP *	Tryptamine **
Methergoline (5)	7.1 ± 1.5	65	68
Methysergide (10)	8.9 ± 2.5	15	55
Mianserin (10)	499 ± 99	48	55
Cyproheptadine (10)	607 ± 501	92	36
Cinanserin (10)	3377 ± 870	12	18
BW 501C (10)	3898 ± 590	0	N.T.

guinea pig brain stem preparations whereas piperazine-containing 5HT agonists were markedly less active. This agrees with the greater capacity of indole-containing 5HT agonists to induce 5HT-dependent brain stem-mediated myoclonus in guinea pigs since piperazine-containing 5HT agonists evoked occasional myoclonus only at toxic doses (see table 3) (Luscombe et al., 1982a). This apparent agreement of in vivo/in vitro efficacy suggests that the brain stem receptor protein responsible for the induction of myoclonus may possess a distinct physical conformation rendering it accessible to 5HT agonists with an indole nucleus but not those with a piperazine group. No absolute correlation between the activity of 5HT agonists in displacing [^{3}H]5HT and the induction of myoclonus was observed. This is perhaps not surprising since pharmacokinetic factors are not taken into account by the receptor binding assay. For example, bufotenine potently inhibited [^{3}H]5HT binding but only weakly induced myoclonus due to its poor cerebral penetration (Sanders and Bush, 1967). Similarly, the potent displacement of [^{3}H]5HT by methysergide would not be predicted from its modest antagonism of indoleamine-induced myoclonus in guinea pigs (see table 4), but methysergide also is poorly accumulated in brain (Doepfner, 1962).

How does the 5HT receptor responsible for the induction of myoclonus relate to previously reported post-synaptic 5HT receptor populations? Using ligand binding studies post-synaptic 5HT receptors are divided into 5HT-1 and 5HT-2, each with a differing regional distribution (Peroutka and Snyder, 1979, 1981; Seeman et al., 1980; Leysen, 1981; Pedigo et al., 1981). 5HT-1 receptors are specifically labelled in vitro by [^{3}H]5HT which is preferentially displaced by 5HT agonists. 5HT-2 receptors are specifically labelled in vitro by [^{3}H]spiperone which is preferentially displaced by 5HT antagonists (Peroutka and Snyder, 1979; Leysen, 1981; Peroutka et al., 1981). Since guinea pig brain stem preparations possess specific binding sites for [^{3}H]5HT but do not reliably demonstrate specific [^{3}H]spiperone binding, it appears that the brain stem 5HT receptor involved in the production of myoclonus is of the 5HT-1 type.

Association of the 5HT-1 receptor with behavioural change contrasts, however, with reports that inhibition of 5HTP-induced head twitching in mice and of tryptamine-induced forepaw clonus in rats by indoleamine receptor antagonists correlates with their ability to displace specific [^{3}H]spiperone binding from 5HT-2 receptors in rat frontal cortex preparations (Leysen, 1981; Peroutka et al., 1981). However, head twitching and clonus are both brain stem-mediated responses while the ligand binding studies were performed in a forebrain region. The present study in guinea pigs, however, has investigated [^{3}H]5HT binding in the brain area reponsible for the production of myoclonus, that is, the brain stem (Chadwick et al., 1978).

Another feature of previous studies was that antagonism of indoleamine-induced behaviour was studied rather than the ability of 5HT agonists to induce behaviour. Perhaps agonists and antagonists act through related but distinct 5HT sites.

One other explanation might lie in the relative involvement of 5HT and tryptamine receptors. Previously we and others have demonstrated that the differential inhibition of 5HT-dependent and tryptamine-induced responses by indoleamine antagonists indicates the presence of pharmacologically distinct 5HT and tryptamine receptors (Clineschmidt and Lotti, 1974; Cox et al., 1981; Luscombe et al., 1982b). Displacement of specific [^{3}H]5HT binding to guinea pig brain stem preparations by indoleamine antagonists has been compared to their differential ability to inhibit myoclonic jerking evoked in guinea pigs by either 5HTP (plus carbidopa) or tryptamine (plus pargyline) (Luscombe et al., 1981, 1982b) (table 4). The [^{3}H]5HT displacement efficacy of indoleamine antagonists appears more closely related to their capacity to inhibit tryptamine-induced myoclonus rather than 5HTP-induced myoclonus (table 4). This might suggest that [^{3}H]5HT binds to tryptamine receptors in guinea pig brain stem. This seems unlikely since a recent study of specific [^{3}H]tryptamine binding to rat cortical membranes reported that the ligand was potently displaced by unlabelled tryptamine but not displaced by d-LSD (Kellar and Cascio, 1982); these are not the properties of the [^{3}H]5HT binding site reported herein. Indeed the potency of [^{3}H]5HT displacement from guinea pig brain stem preparations by indoleamine

antagonists closely parallels their efficacy of [^{3}H]5HT displacement from known 5HT-1 receptors, but not [^{3}H]spiperone from 5HT-2 receptors (Seeman et al., 1980; Leysen, 1981; Peroutka et al., 1981) (table 2).

In conclusion, we have demonstrated that guinea pig brain stem preparations possess specific binding sites for [^{3}H]5HT but not for [^{3}H]spiperone, suggesting the presence of 5HT-1 receptors. In addition, indoleamine antagonists possess IC_{50} values for [^{3}H]5HT displacement from guinea pig brain stem which are typical of those reported for interaction with 5HT-1 rather than 5HT-2 receptors. The notably more potent displacement of specific [^{3}H]5HT binding by indole-containing 5HT agonists than by piperazine-containing 5HT agonists is consistent with the markedly greater ability of indole-containing 5HT agonists to evoke brain stem-mediated myoclonus in guinea pigs. The [^{3}H]5HT binding site in guinea pig brain stem involved in the production of indoleamine-dependent myoclonus thus appears to be a 5HT-1 receptor protein which is accessible to 5HT agonists with an indole nucleus but not those with a piperazine group.

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