Naunyn-Schmiedeberg's Arch Pharmacol (1986) 332:1–7

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites

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Summary. 1. In rat brain cortex slices preincubated with [^{3}H]5-HT, the potencies of 17 5-HT receptor agonists to inhibit the electrically evoked ^{3}H overflow and the affinities of 13 antagonists (including several β-adrenoceptor blocking agents) to antagonize competitively the inhibitory effect of unlabelled 5-HT on evoked ^{3}H overflow were determined.

2. The affinities of the compounds for 5-HT_{1B} and 5-HT_2 binding sites in rat brain cortex membranes (labelled by [^{125}I]cyanopindolol = [^{125}I]-CYP in the presence of 30 µmol/l isoprenaline and [^{3}H]ketanserin, respectively), for 5-HT_{1A} binding sites in pig and rat brain cortex membranes (labelled by [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin = [^{3}H]8-OH-DPAT) and for 5-HT_{1C} binding sites in pig choroid plexus membranes (labelled by [^{3}H]mesulergine) were also determined. The affinities of the drugs for the various 5-HT recognition sites ranged over 4–5 log units (the functional experiments revealed the same range of differences between the drugs).

3. There were no significant correlations between the affinities of the drugs at 5-HT_{1C} and 5-HT_2 binding sites and their potencies or affinities, determined for the 5-HT autoreceptors. In contrast, significant correlations were found between the potencies or affinities of the drugs for the autoreceptors and their affinities at 5-HT_{1A} or 5-HT_{1B} binding sites; the best correlations were obtained with the 5-HT_{1B} binding site.

4. Some of the drugs investigated were not included in the correlation since their agonistic or antagonistic effects on the autoreceptors were weak and pEC_{30} or apparent pA_2 values could not be determined (< 5.5). Among these drugs, 8-OH-DPAT, TVX Q 7821 (2-(4-(4-(2-pyrimidin-yl)-1-piperazinyl)-butyl)-1,2-benzisothiazol-3(2H)one-1,1-dioxide) and spiperone showed a very low affinity for 5-HT_{1B} binding sites ($pK_D < 5.3$), but a high affinity for 5-HT_{1A} binding sites ($pK_D > 7.2$).

5. In conclusion, the evidence indicates that the presynaptic 5-HT autoreceptor belongs to the 5-HT_{1B} receptor subtype.

Key words: Presynaptic 5-HT autoreceptors – 5-HT release – Rat and pig brain cortex – 5-HT binding sites – 5-HT receptor subtypes

* M. Göthert was supported by a grant of the "Deutsche Forschungsgemeinschaft".
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Introduction

The first classification of 5-HT receptors proposed by Gaddum and Picarelli (1957) divided these receptors into two classes: the D receptor was located on the smooth muscle cells of the guinea pig ileum and the M receptor on the nerves of the myenteric plexus. Using radioligand binding studies, Peroutka and Snyder (1979) described 5-HT_1 and 5-HT_2 binding sites in rat brain cortex. The 5-HT_2 sites were identified as receptors and many functional correlates have since been established for these sites (Peroutka et al. 1981; Leysen et al. 1982). It is now known that the D receptor of the guinea pig ileum is identical with the 5-HT_2 receptor (Engel et al. 1985). The question of the functional correlates of the 5-HT_1 recognition site has remained a matter of debate (Peroutka et al. 1981; Middlemiss 1982; Fozard 1983), in part because of the existence of subtypes of the 5-HT_1 binding site.

5-HT_1 recognition sites were subdivided into 5-HT_{1A} and 5-HT_{1B} subtypes by means of radioligand binding studies performed with [^{3}H]5-HT (Pedigo et al. 1981; Deshmukh et al. 1982); the 5-HT_{1A} sites had high affinity for spiperone whilst the 5-HT_{1B} sites showed low affinity for this compound. Further analysis of the complex competition curves obtained for [^{3}H]5-HT binding suggested the existence of a third 5-HT_1 recognition site (Nelson et al. 1983; Engel et al. 1983). Subsequently, Palacios and collaborators by using [^{3}H]mesulergine and [^{3}H]5-HT described a further subtype, designated 5-HT_{1C} concentrated in the choroid plexus of pig and rat (Pazos et al. 1984a, b; Pazos and Palacios 1985). It is now possible to identify three distinct 5-HT_1 binding sites with specific radioligands. 5-HT_{1A} sites are labelled by [^{3}H]8-OH-DPAT in rat and pig brain membranes (Gozlan et al. 1983; Hoyer et al. 1985b). 5-HT_{1B} sites are labelled by [^{125}I]CYP (in the presence of 30 µM isoprenaline in order to exclude binding to β-adrenoceptors) (Hoyer et al. 1985a, b; Pazos et al. 1985) and 5-HT_{1C} receptors are labelled by [^{3}]mesulergine in pig choroid plexus (Pazos et al. 1984b; Hoyer et al. 1985b). Furthermore, it is established that [^{3}H]5-HT labels all three 5-HT_1 recognition sites in rat brain cortex in equal proportions (Pazos and Palacios 1985; Hoyer et al. 1985b).

It has been proposed by various authors (Göthert 1982; Martin and Sanders Bush 1982a; Engel et al. 1983) that 5-HT autoreceptors are of the 5-HT_1 class, however the subtype has not been clearly established. Middlemiss (1984a) has shown that the highly selective 5-HT_{1A} agonist, 8-OH-DPAT (Middlemiss and Fozard 1983), does not activate these autoreceptors. It was further shown that pro-

pranolol and pindolol act as stereoselective antagonists at 5-HT autoreceptors (Middlemiss 1984b, 1985) and it was proposed that these autoreceptors belong to the 5-HT_{1B} subtype (Middlemiss 1984b). However, 8-OH-DPAT also shows low affinity for the 5-HT_{1C} sites, and neither propranolol nor pindolol distinguishes between 5-HT_{1A} and 5-HT_{1B} binding (Hoyer et al. 1985a, b). Furthermore function at 5-HT autoreceptors has not yet been correlated with binding data for the various 5-HT_1 receptor subtypes.

In this study we compare the pharmacology of a large series of 5-HT agonists and antagonists from different structural classes at 5-HT autoreceptors with their affinity for 5-HT_1 and 5-HT_2 receptor subtypes in radioligand binding studies. We demonstrate that 5-HT autoreceptors do not belong to the 5-HT_2, 5-HT_{1C} or 5-HT_{1A} subtypes and are best classified as 5-HT_{1B} receptors.

Materials and methods

Preparation of particulate fractions for binding studies. Male rats (Sandoz own breed, weight about 250 g) were decapitated and the brains rapidly removed and placed on ice. Pig brains were obtained from a local slaughterhouse and placed on ice immediately after death. Frontal cortices of both species and pig choroid plexi were removed and membrane homogenates were prepared as described (Hoyer et al. 1985b; Pazos et al. 1984 a, b). The membranes were stored in liquid nitrogen or kept deep frozen at $-70°$ C until used.

Radioligand binding experiments

Binding of the tritiated radioligands [^{3}H]8-OH-DPAT (5-HT_{1A}), [^{3}H]ketanserin (5-HT_2) and [^{3}H]mesulergine (5-HT_{1C}) was performed as previously described (Hoyer et al. 1985b, Pazos et al. 1984a, b).

[^{125}I]CYP binding (5-HT_{1B}) was performed in the presence of 30 µmol/l isoprenaline, in order to exclude any interference with β-adrenoceptors (Hoyer et al. 1985a, b). Briefly, 150 µl of membrane suspension (20–30 µg of protein) were added to 50 µl of [^{125}I]CYP (100–150 pmol/l) and 50 µl of drug or buffer (Tris HCl 10 mmol/l, NaCl 154 mmol/l, pargyline 10 µmol/l, pH 7.7). Membranes, drugs and the radioligand were dissolved in this buffer. The incubation was carried out in polystyrene microtiter plates (Sterilin 96 holes) in an incubator at 37° C for 90 min. The reaction was stopped by the addition of ice cold Tris HCl 50 mmol/l, pH 7.4 and bound and free ligand were separated by washing and filtering through Gelman A/E glass fiber filters on a cell harvester (Flow Laboratories). After drying under reduced suction, the filters were counted in a WW 1032 gamma counter (Kontron) at 80% counting efficiency.

Competition curves were constructed with 8 to 20 concentrations of competing drugs. As a rule all incubations were carried out in triplicate. Independent experiments were performed at least twice. Protein concentrations were determined according to the method of Bradford (1976).

Superfusion experiments on cortex slices

Occipitoparietal cortex slices (0.3 mm thick, diameter 3 mm) of male Wistar rats weighing 200–300 g were incubated (60 min) in physiological salt solution (for composition see Engel et al. 1983) containing 0.1 µmol/l [^{3}H]5-hydroxytryptamine creatinine sulphate. Subsequently they were superfused with physiological salt solution containing paroxetine or DU 24565 (selective inhibitors of neuronal 5-HT uptake; Petersen et al. 1977; Vaastra et al. 1981; Classen et al. 1984). Two 2-min periods of electrical field stimulation (20 mA, 2 ms, 3 Hz) were applied to each slice 40 (S_1) and 90 min (S_2) after the onset of superfusion. At the end of superfusion, the slices were solubilized, and the radioactivity in the slices and in the superfusate samples was determined by liquid scintillation counting. All further details concerning superfusion, stimulation and experimental protocol were identical to those described by Engel et al. (1983).

Calculations and statistics

Untransformed data of the saturation and competition *binding* experiments with the various radioligands were analyzed by non-linear regression analysis, based on Feldman's equation (1972), using the computer modeling program SCTFIT as described in detail by DeLean et al. (1980). By *F*-Test analysis the goodness of fit between the models of one or two classes of binding sites was checked and the most appropriate model was determined (Snedecor and Cochran 1973).

Basal ^{3}H efflux and stimulation-evoked ^{3}H overflow in *superfusion* experiments were calculated according to Engel et al. (1983). Apparent pA_2 values of 5-HT receptor antagonists were determined as described by Furchgott (1972) for competitive reversible antagonism (see also Göthert et al. 1981).

Results are expressed as arithmetic means (functional studies) or geometric means (radioligand binding studies) ± SEM of n independent experiments. Linear regression lines and correlation coefficients were calculated in order to detect and quantify correlations between various drug effects.

Drugs used. [^{3}H]5-hydroxytryptamine creatinine sulphate (24.1–26.3 Ci/mmol) and [^{3}H]-ketanserin hydrochloride (64 Ci/mmol) NEN, Dreieich, FRG); [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (105–125 Ci/mmol) ([^{3}H]8-OH-DPAT; CEA, Saclay, France); 5-hydroxytryptamine creatinine sulphate (5-HT) and tryptamine hydrochloride (T; Merck, Darmstadt, FRG); 5-methoxytryptamine hydrochloride (5-OCH_3-T; Fluka, Buchs, Switzerland); 6-hydroxytryptamine (6-HT, Sigma, Munich, FRG); α-methyl-5-hydroxytryptamine creatinine sulphate (α-CH_3-5-HT; Upjohn, Kalamazoo, MI, USA); 4-hydroxytryptamine creatinine sulphate (4-HT), 5,6-dihydroxytryptamine creatine sulphate (5,6-DHT) and 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT) (Regis, Morton Grove, IL, USA); 2-methyl-5-hydroxytryptamine hydrogen maleinate (2-CH_3-5-HT), β-methyl-5-hydroxytryptamine hydrogen oxalate (β-CH_3-5-HT), ω-N-methyl-5-hydroxytryptamine oxalate (ω-N-CH_3-5-HT), N,N-dimethyl-5-hydroxytryptamine binoxalate (NN-$(CH_3)_2$-5-HT), 5-aminotryptamine hydrogenoxalate (5-NH_2-T), 5-carboxamido-tryptamine hydrogen maleinate (5-$CONH_2$-T), unlabelled 8-OH-DPAT, (±)-cyanopindolol (base), (+)-cyanopindolol (base), (−)-cyanopindolol (base), (+)-pindolol (base), (−)-pindolol (base), (±)-4[3-ter-butyl-amino-2-hydroxypropoxy]indol-2-carbonic-acid-isopropylester [(±)-21-009], (−)-[^{125}I]-iodocyanopindolol (2,175 Ci/mmol) ([^{125}I]CYP), [^{3}H]mesulergine (85 Ci/mmol) and d-LSD tartrate were synthesized by Sandoz, Basel, Switzerland; metitepin (Hoffmann-La

Table 1. Methodological data for in vitro binding assay on various 5-HT-receptors

Receptor Subtype	Ligand	Membranes	B_{max} fmol/mg protein	K_D nmol/l	Determination of non-specific binding	Reference
5-HT_{1A}	[^{3}H]8-OH-DPAT	rat brain cortex[a] pig brain cortex	127 ± 6 73 ± 5	1.9 ± 0.3 2.4 ± 0.4	10 µmol/l 5-HT	Hoyer et al. 1985b
5-HT_{1B}	[^{125}I]CYP + 30 µmol/l isoprenaline	rat brain cortex	140 ± 3	0.21 ± 0.01	10µmol/l 5-HT	Hoyer et al. 1985a
5-HT_{1C}	[^{3}H]mesulergine	pig choroid plexus	300 ± 64	1.1 ± 0.1	10 µmol/l 5-HT	Pazos et al. 1984b
5-HT_2	[^{3}H]ketanserin	rat brain cortex	175 ± 25	0.8 ± 0.2	1 µmol/l mianserin	Pazos et al. 1984a

[a] 5-HT_{1A} binding was determined using pig brain cortex membranes. 5-HT_{1A} receptors from rat and pig brain cortex are identical (Hoyer et al. 1985b)

Roche, Basel Switzerland); quipazine (Miles, Elkhart, IN, USA); metergoline (Farmitalia, Milan, Italy); DU 24565 (6-nitro,2-(1-piperazinyl)quinoline; Philips-Duphar, Weesp, The Netherlands); (±)-propranolol hydrochloride (ICI, Plankstadt, FRG); 6-chloro-2-(1-piperazinyl)pyrazine hydrochloride (MK 212; Merck, Sharpe and Dohme, Munich, FRG or synthesized by Sandoz, Basel, Switzerland); unlabelled ketanserin and spiroperidol (spiperone) (Janssen, Beerse, Belgium); TVX Q 7821 (2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl-1,2-benzoisothiazol-3(2H)one-1,1-dioxide hydrochloride) (Troponwerke, Köln, FRG); cyproheptadine hydrochloride (Merck, Sharpe and Dohme, Munich, FRG); mianserin hydrochloride (Organon, Oss, Netherlands); isoprenaline hydrochloride (Sigma, Taufkirchen, FRG), paroxetine hydrochloride (Ferrosan, Copenhagen, Denmark).

Results

Binding experiments

Table 1 summarizes the values of maximal capacities (B_{max}), the apparent dissociation constants (K_D) and the conditions by which non-specific binding in the different assay systems was determined. The competition curves with [^{3}H]8-OH-DPAT as well as those with [^{125}I]CYP in the presence of isoprenaline were monophasic for 5-HT receptors agonists and antagonists. The same held true for [^{3}H]mesulergine binding in pig choroid plexus. The 5-HT_2 binding sites were labelled by [^{3}H]ketanserin in rat brain cortex. Some 5-HT receptor agonists, especially 5-HT itself, showed flat or biphasic competition curves with [^{3}H]ketanserin. Since the reason for the biphasic shape of the competition curves obtained with 5-HT receptor agonists is unknown only the pK_D of the main component was taken into account and indicated in Table 2.

Functional experiments

Unlabelled 5-HT inhibited the electrically evoked ^{3}H overflow in a concentration-dependent manner, a 60% decrease representing the maximum effect. Qualitatively similar results were obtained with the other indolethylamines investigated (Göthert and Schlicker 1983; Engel et al. 1983) and with LSD; the negative logarithms of the concentrations producing the half maximum effect (pEC_{30} values) are listed in Table 2 (in the concentration range investigated the compounds mentioned so far did not affect the basal ^{3}H-efflux). The evoked ^{3}H overflow was not affected by TVX Q 7821 (up to 3.2 µmol/l), but was inhibited by unlabelled 8-OH-DPAT 3.2 and 10 µmol/l (by 18 and 38%, respectively). Since, from 1 µmol/l onward, 8-OH-DPAT and TVX Q 7821 enhanced basal ^{3}H efflux, the effects of both drugs on the evoked ^{3}H overflow in this concentration range must be regarded with caution.

Metitepin, metergoline and quipazine caused a parallel shift to the right of the concentration-response curve of unlabelled 5-HT for its inhibitory effect on evoked ^{3}H overflow (Engel et al., 1983). The same held true for a series of β-adrenoceptor antagonists including (±)-cyanopindolol and its enantiomers (Schlicker et al., 1985), (±)-propranolol, (±)-21009 and (−)-pindolol as well as for the piperazine derivative MK 212 (data not shown).

From the shifts at the level of the EC_{30} value of 5-HT, apparent pA_2 values of the antagonists were calculated (Table 2). (+)-Pindolol, spiroperidol and cyproheptadine, each up to 3.2 µmol/l, did not affect the concentration-response curve of 5-HT. The inhibitory effects of other indolethylamines and of LSD were also antagonized by metitepin (Langer and Moret 1982; Göthert and Schlicker 1983). By contrast, metitepin or (±)-cyanopindolol, each 1 µmol/l, did not affect the inhibitory effect of 8-OH-DPAT 10 µmol/l (data not shown).

Correlations between binding affinities and functional effects

The pEC_{30}, apparent pA_2 and pK_D-values of the 5-HT receptor agonists or antagonists for 5-HT autoreceptor-mediated effects and for the affinities at the various 5-HT binding sites ranged over 4 – 5 log units (Fig. 1). No significant correlation was found between the affinities of the drugs (expressed as pK_D values) at 5-HT_{1C} or 5-HT_2 binding sites and the pEC_{30} values of the agonists for the inhibition of evoked ^{3}H-overflow (Figs. 1C and D) or the apparent pA_2 values of antagonists for the antagonism of the inhibition produced by unlabelled 5-HT (Figs. 1G and H; see Table 3 for statistical parameters). However, there were significant correlations between pEC_{30} or apparent pA_2-values and the affinities of the drugs at 5-HT_{1B} or 5-HT_{1A} binding sites (Figs. 1A, B, E, F). The correlation coefficients (r) for the

Table 2. pEC_{30} and apparent pA_2 values of 5-HT receptor agonists and antagonists at 5-HT-autoreceptors and pK_D values of these drugs at $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1C}$ and $5\text{-}HT_2$-binding sites

Compounds[a]		pEC_{30} (agonists)[b] or apparent pA_2 (antag.)[c]: 5-HT autoreceptors	pK_D[d]: 5-HT binding sites			
			$5\text{-}HT_{1B}$	$5\text{-}HT_{1A}$	$5\text{-}HT_{1C}$	$5\text{-}HT_2$
Agonists						
1 5-HT		6.76	7.63 ± 0.12 (10)	8.51 ± 0.08 (6)	7.48 ± 0.07 (3)	5.53 ± 0.37 (5)
2 $5\text{-}OCH_3$-T		6.45	6.40 ± 0.23 (3)	8.04 ± 0.15 (4)	7.36 ± 0.31 (4)	5.59 ± 0.32 (4)
3 $2\text{-}CH_3$-5-HT		4.21	4.44 ± 0.16 (3)	5.60 ± 0.04 (3)	5.84 ± 0.19 (2)	4.99 ± 0.37 (3)
4 (±)α-CH_3-5-HT		4.62	6.00 ± 0.25 (4)	7.07 ± 0.17 (5)	7.23 ± 0.21 (4)	6.90 ± 0.18 (4)
5 (±)β-CH_3-5-HT		6.46	5.51 ± 0.20 (3)	7.05 ± 0.08 (3)	6.31 ± 0.16 (2)	5.60 ± 0.32 (2)
6 $N,N(CH_3)_2$-5-HT		5.38	6.04 ± 0.30 (4)	7.60 ± 0.28 (4)	7.15 ± 0.09 (4)	6.42 ± 0.12 (2)
7 $5\text{-}CONH_2$-T		7.65	8.29 ± 0.12 (3)	9.67 ± 0.08 (3)	6.23 ± 0.15 (3)	4.83 ± 0.03 (2)
8 4-HT		6.21	5.98 ± 0.22 (4)	7.02 ± 0.04 (2)	7.39 ± 0.30 (3)	6.14 ± 0.15 (2)
9 5,6-DHT		5.47	5.21 ± 0.13 (3)	6.02 ± 0.08 (4)	6.15 ± 0.05 (2)	5.01 ± 0.04 (2)
10 5,7-DHT		4.27	3.66 ± 0.34 (3)	4.90 ± 0.04 (3)	4.29 ± 0.16 (3)	< 4 (3)
11 $5\text{-}NH_2$-T		5.96	6.17 ± 0.13 (4)	6.15 ± 0.17 (4)	7.15 ± 0.17 (2)	5.49 ± 0.24 (2)
12 6-HT		5.91	5.23 ± 0.31 (4)	5.80 ± 0.04 (2)	5.26 ± 0.13 (3)	4.94 ± 0.29 (2)
13 ω-N-CH_3-5-HT		7.16	7.35 ± 0.27 (2)	8.31 ± 0.14 (3)	6.55 ± 0.21 (2)	6.74 ± 0.31 (3)
14 T		5.31	4.99 ± 0.34 (3)	6.77 ± 0.05 (2)	7.33 ± 0.02 (2)	–
15 8-OH-DPAT		< 5.5	4.22 ± 0.10 (4)	8.73 ± 0.11 (7)	5.24 ± 0.19 (4)	5.04 ± 0.09 (4)
16 LSD		5.50	6.82 ± 0.15 (3)	8.59 ± 0.06 (6)	7.93 ± 0.06 (5)	8.62 ± 0.06 (6)
17 TVXQ 7821		< 5.5	3.87 ± 0.27 (3)	7.73 ± 0.10 (3)	4.53 ± 0.27 (3)	5.07 ± 0.11 (3)
Antagonists (in brackets: autoreceptor studies, concentrations investigated, µmol/l)						
18 metitepin	(1.0)	6.99	7.28 ± 0.03 (5)	7.10 ± 0.13 (5)	7.56 ± 0.07 (4)	8.76 ± 0.09 (7)
19 quipazine	(10.0)	5.29	6.51 ± 0.14 (6)	5.49 ± 0.05 (7)	6.73 ± 0.09 (3)	6.20 ± 0.03 (5)
20 metergoline	(1.0)	6.29	7.39 ± 0.09 (8)	8.10 ± 0.10 (5)	9.19 ± 0.10 (3)	9.03 ± 0.14 (6)
21 MK 212	(320)	4.04	5.03 ± 0.13 (4)	5.32 ± 0.03 (5)	6.16 ± 0.15 (2)	4.76 ± 0.14 (5)
22 (±)cyanopindolol	(0.032)	8.29	8.28 ± 0.11 (6)	8.27 ± 0.12 (4)	4.44 ± 0.5 (3)	4.53 ± 0.13 (4)
23 (+)cyanopindolol	(1.0)	6.83	7.25 ± 0.13 (4)	7.27 ± 0.16 (3)	–	4.60 ± 0.13 (3)
24 (−)cyanopindolol	(0.032)	8.30	8.74 ± 0.05 (3)	8.64 ± 0.11 (3)	–	4.58 ± 0.04 (3)
25 (+)pindolol	(3.2)	< 5.5	5.29 ± 0.08 (4)	5.88 ± 0.05 (5)	4.18 ± 0.3 (3)	4.42 ± 0.08 (7)
26 (−)pindolol	(1.0)	6.57	7.19 ± 0.03 (10)	7.66 ± 0.04 (6)	4.31 ± 0.07 (5)	4.53 ± 0.20 (3)
27 (±)propranolol	(0.32)	6.76	7.07 ± 0.10 (4)	6.48 ± 0.06 (3)	–	6.12 ± 0.22 (4)
28 (±)21-009	(0.075)	7.49	8.53 ± 0.04 (7)	7.76 ± 0.14 (4)	5.05 ± 0.05 (3)	4.58 ± 0.21 (4)
29 spiperone	(3.2)	< 5.5	5.27 ± 0.06 (3)	7.18 ± 0.26 (4)	5.94 ± 0.02 (3)	8.76 ± 0.03 (3)
30 cyproheptadine	(3.2)	< 5.5	5.32 ± 0.22 (3)	6.45 ± 0.07 (3)	7.86 ± 0.32 (4)	8.46 ± 0.14 (8)

[a] For explanation of abreviations see Methods (Drugs used)

[b] Derived from concentration-response curves for the inhibitory effects of 5-HT receptor agonists on the electrically evoked ^3H overflow from rat brain cortex slices preincubated with [^3H]5-HT. Neuronal 5-HT uptake was blocked throughout superfusion by paroxetine 3.2 µmol/l (indolethylamines, LSD) or DU 24565 1 µmol/l (8-OH-DPAT, TVX Q 7821). The pEC_{30} values of indolethylamines were taken from Göthert and Schlicker (1983) and Engel et al. (1983)

[c] Apparent pA_2 values of the compounds for their antagonism against unlabelled 5-HT were determined as described in the text. Neuronal 5-HT uptake was blocked throughout superfusion by DU 24565 1 µmol/l. The apparent pA_2 values of metitepin, quipazine, metergoline and cyproheptadine were taken from Engel et al. (1983). Absolute values of ^3H efflux in the control experiments (5-HT and 5-HT receptor antagonists absent): basal efflux determined immediately before S_2, 0.15 ± 0.03 *n*Ci/5 min (corresponding to a fractional rate of ^3H efflux of 0.0052 ± 0.0002/min; related to tissue tritium); ^3H overflow evoked by S_2, 0.17 ± 0.04 *n*Ci (corresponding to 2.80 ± 0.30% of tissue tritium)

[d] Geometric means ± SEM of (*n*) independent experiments. For $n = 2$ the maximal deviations from the mean value are indicated

correlations with $5\text{-}HT_{1B}$ binding sites (Figs. 1A and E, Table 3) were higher than those for the correlations with $5\text{-}HT_{1A}$ binding sites (Figs. 1B and F, Table 3). In the binding studies, the best discrimination between $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ binding sites could be obtained with unlabelled 8-OH-DPAT and TVX Q 7821, which selectively displayed a high affinity at the $5\text{-}HT_{1A}$ binding sites (pK_D $5\text{-}HT_{1A}$ = 8.73 and 7.73 respectively; Table 2). Our findings that the electrically evoked ^3H overflow was only marginally inhibited by 8-OH-DPAT (3.2 µmol/l) and not affected at all by TVX Q 7821 were not compatible with similarities between the sites mediating the functional effects and the $5\text{-}HT_{1A}$ binding sites, but rather with similarities between the functional sites and the $5\text{-}HT_{1B}$ binding sites (pK_D of 4.22 and 3.87, respectively).

This assumption is further supported by two 5-HT receptor antagonists, spiroperidol and cyproheptadine, which discriminate also quite clearly between the $5\text{-}HT_{1B}$ and the $5\text{-}HT_{1A}$ site. Both spiperone and cyproheptadine were inactive at the autoreceptor (up to a concentration of 3.2 µmol/l) and had low affinities for $5\text{-}HT_{1B}$ sites (pK_D values: 5.27 and 5.32, respectively), whereas their affinities for $5\text{-}HT_{1A}$ sites were higher by about 1−2 orders of magnitude (7.18 and 6.45, respectively).

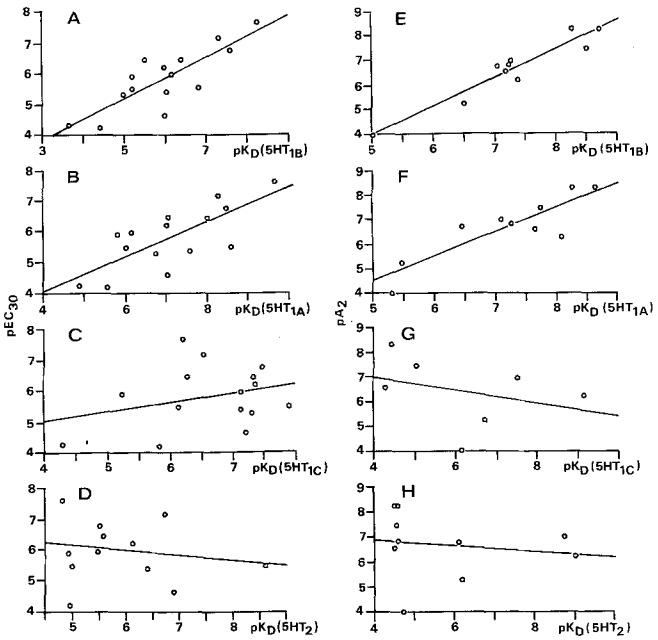

Fig. 1A–H. Correlation of pEC_{30} or apparent pA_2 values from superfusion experiments with pK_D values from binding studies. The affinity values and the potencies for the calculation of the linear regression are taken from Table 2. The correlation lines were calculated with pEC_{30} values for 5-HT receptor agonists (*left side*: **A, B, C, D**) and with apparent pA_2 values for 5-HT receptor antagonists (*right side*: **E, F, G, H**) and with pK_D values derived from specific binding experiments. The correlation coefficients and the statistical significance are given in Table 3

Table 3. Parameter estimation ($y = mx + b$) from linear regression analysis in Fig. 1

Parameter	5-HT_{1B}	5-HT_{1A}	5-HT_{1C}	5-HT_2
5-HT receptor agonists (pEC_{30} versus pK_D)				
m	0.67	0.56	0.28	−0.16
b	1.81	1.82	3.93	6.92
r	0.82	0.74	0.28	0.18
p	0.001	0.0018	0.32	0.57
5-HT receptor antagonists (apparent pA_2 versus pK_D)				
m	1.15	0.98	−0.26	−0.11
b	−1.73	−0.41	8.03	7.33
r	0.95	0.86	0.33	0.15
p	0.001	0.0013	0.48	0.67

m = slope; b = intercept on the *y*-axis; *r* = correlation coefficient; *p* = significance level

Discussion

The aim of this study was to characterize the pharmacology of 5-HT autoreceptors of the rat brain cortex and to determine to which 5-HT receptor subtype autoreceptors belong. This was done by combining functional and radioligand binding studies.

In rat brain cortex membranes, [^{125}I]CYP (in the presence of 30 μmol/l isoprenaline) (Hoyer et al. 1985a, b; Pazos et al. 1985) and [^{3}H]ketanserin (Leysen et al. 1982; Pazos et al. 1984a, b) label 5-HT_{1B} and 5-HT_2 binding sites, respectively. In pig cortex and choroid plexus membranes, [^{3}H]-8-OH-DPAT (Hoyer et al. 1985b) and [^{3}H]mesulergine (Pazos et al. 1984b) are suitable to identify 5-HT_{1A} and 5-HT_{1C} binding sites respectively. Furthermore, it has been shown that all three subtypes of 5-HT_1 binding sites occur in the rat brain cortex (Hoyer et al. 1985b).

Under the conditions of the present investigation, the electrically evoked ^{3}H overflow from the rat brain cortex slices reflects quasi-physiological serotonin release (Göthert and Weinheimer 1979; Classen et al. 1984), the latter is under the modulatory control of inhibitory presynaptic autoreceptors (for reviews, see Héry and Ternaux 1981; Starke 1981; Göthert 1982, 1985).

Tryptamine and its 5-hydroxy, 5-methyl, 5-methoxy, 5-amino, and 5-carboxamido derivatives as well as LSD, act as agonists at the presynaptic 5-HT autoreceptors (Göthert 1982, Göthert and Schlicker 1983, Engel et al. 1983). 8-OH-DPAT 1 μmol/l did not affect the evoked serotonin release in the present study an observation which confirms that of Middlemiss (1984a). Hamon et al. (1984), however, found a decrease in evoked 5-HT release by 34% (basal tritium efflux not affected), which could be antagonized by metergoline. In our study, 8-OH-DPAT inhibited the evoked serotonin release only at concentrations exceeding 1 μmol/l, at which basal ^{3}H efflux was markedly enhanced. The inhibitory effect of 8-OH-DPAT 10 μmol/l was not attenuated by metitepin or (±)-cyanopindolol, excluding that 5-HT autoreceptors are involved. TVXQ 7821, a putative anxiolytic, which displays affinity for 5-HT_{1A} binding sites in the nanomolar range (Dompert et al. 1985), did not evoked ^{3}H overflow at 3.2 μmol/l.

The 5-HT receptor antagonists, metitepin, quipazine and metergoline have been shown to produce parallel shifts to the right of the concentration-response curve of unlabelled 5-HT for its inhibitory effect on 5-HT release from the rat brain cortex and hypothalamus (Göthert 1980, Schlicker and Göthert 1981, Martin and Sanders-Bush 1982b, Middlemiss 1984a). Data suggesting the possiblility of antagonism by MK 212 at the 5-HT autoreceptor was first described by Baumann and Waldmeier (1981), and the parallel shift by MK 212 of the concentration-response curve of 5-HT, found in the present study, supports this suggestion.

Propranolol and pindolol were characterized as stereoselective antagonists at the 5-HT autoreceptors of the rat brain cortex by Middlemiss (1984b, 1985) and Richards (1985); in addition, the β-adrenoceptor antagonists (±)-cyanopindolol and its enantiomers (present study, Schlicker et al. 1985) and (±)-21009 (present study) block the autoreceptors.

There were no significant correlations between the affinities of the drugs at 5-HT_{1C} and 5-HT_2 binding sites and their potencies or affinities determined for the 5-HT autoreceptor-mediated effect, thus excluding the possibility that these binding sites and receptors have properties in common. In contrast, significant correlations were found between the potencies or affinities of the drugs determined for the 5-HT autoreceptors and their affinities at 5-HT_{1A} and 5-HT_{1B} binding sites. However, this is not surprising since the pharmacological profile of 5-HT_{1A} and 5-HT_{1B} recognition sites for tryptamine derivatives is rather similar. Nevertheless the correlations were best with 5-HT_{1B} sites, both when agonists and when antagonists were investigated. An even more clear cut distinction between both recognition sites becomes evident when selective agonists which are not tryptamine

derivatives, (like 8-OH-DPAT and TVX Q 7821) and 5-HT antagonists (like spiroperidol and cyproheptadine) are taken into account. These drugs had no measurable affinity to the autoreceptors and very low affinity to the 5-HT_{1B} sites, but displayed moderate to high affinity to the 5-HT_{1A} sites with pK_D values greater than 6.4.

In conclusion the results of the present investigation indicate that the presynaptic 5-HT autoreceptors of the rat brain cortex belong to the 5-HT_{1B} receptor subtype.

Acknowledgements. The technical assistance of Miss Breitwieser, Mr. Bauert and Mr. Girod is gratefully acknowledged. We thank the respective companies for generous gifts of drugs.

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Received May 28/Accepted September 19, 1985