Changes in Cortical 5-HT₂ Receptors Following 5-HTP and Paroxetine Administration in Rats

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Abstract

Rats were pretreated with either carbidopa (25 mg/kg 30 min previously) or phenelzine (50 mg/kg 18 h and 12 mg/kg 90 min previously) before being given 5-HTP (150 or 350 mg/kg) or paroxetine (12 mg/kg). In carbidopa-treated animals, 5-HTP (150 mg/kg) evoked many wet-dog shakes (WDS) without changing receptor numbers, but an increase in the dose of 5-HTP to 350 mg/kg paradoxically increased the number of cortical 5-HT₂ receptors. The increase was reversed by pretreating the rats with haloperidol (5 mg/kg), but this did not occur with α -methyl-para-tyrosine (250 mg/kg 16 h and 4 h before carbidopa). Apomorphine (2 mg/kg) in phenelzine-treated rats and apomorphine (10 mg/kg) in untreated rats increased 5-HT₂ receptor numbers. The reason for the failure of 5-HTP to rapidly down-regulate 5-HT₂ receptors is not known, but is thought to depend in some complex way on the proportion of 5-HTP that is decarboxylated to 5-HT outside serotonergic neurons, thus affecting the dopaminergic mechanism. In phenelzine-treated rats, paroxetine produced a 5-HT-dependent syndrome, which included WDS, and a significant reduction in cortical 5-HT₂ receptors within 3 h. WDS were unaffected by administration of propranolol (20 mg/kg) or pindolol (5 mg/kg), confirming that WDS were independent of 5-HT_{1A} receptors. The reduction of 5-HT₂ receptors was not associated with migration of the receptors into the "light-density fraction" of the cortex, isolated by density-gradient centrifugation.

Introduction

Rat cortical 5-HT₂ receptors are rapidly down-regulated by administering a 5-HT uptake inhibitor, such as fenfluramine, or 5-methoxydimethyltryptamine to animals pretreated with a monoamine oxidase (MAO) inhibitor^[1,2]. This down-regulation is temporally associated with a reduced responsiveness of brain-stem and spinal cord 5-HT₂ receptors, as evidenced from the reduced number of wet-dog

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shakes shown by the rats. In contrast, 5-HTP given to rats pretreated with MAO inhibitors, slowly and weakly down-regulates 5-HT₂ receptors despite production of many WDS, the latter being used as an *in vivo* assay of synaptic 5-HT concentration. Such findings led to the conclusion that in order for 5-HT to produce rapid receptor down-regulation, elevation of the synaptic 5-HT concentration alone may be insufficient, and that it may also be necessary to consider the involvement of dopaminergic systems, since 5-HTP can displace and release dopamine through formation of 5-HT within dopaminergic neurons^[3,4]. This hypothesis was tested in the present study.

Furthermore, we examined the process of 5-HT₂ receptor change *in vivo* following paroxetine administration in rats pretreated with phenelzine. This experimental system was chosen because of its rapid elicitation of cortical 5-HT₂ receptor down-regulation^[2]. Several workers have reported that when tissues become desensitized, receptors are removed from the cell surface and appear inside the cell. These internalized receptors are detectable in the "light-density vesicular fraction" when cell homogenates are centrifuged to equilibrium on a continuous sucrose gradient^[5,6,7]. In the present study, we measured the binding of a tritiated 5-HT₂ receptor antagonist, [³H]ketanserin, to various fractions of rat cortex isolated by density-gradient centrifugation.

Materials and Methods

Male Wistar rats (180-220 g) were used. For observation of 5-HT dependent phenomena, the rats were housed three to a cage the day before the experiment. Food and water were withdrawn 90 min before the observation period and withheld throughout the experiment. WDS occurring in individual rats were counted for 5 out of every 15 min. In some experiments, other types of 5-HT-dependent behavior were assessed. Continuous forms of behavior (hind limb abduction, tremor and Straub tail) were rated on a 0-4 scale as 0=absent, 1=perceptible, 2=weak, 3=medium, 4=maximal, as described by DICKINSON and CURZON^[8]. Intermittent forms of behavior were counted individually (WDS) or on a 0-4 scale (reciprocal forepaw treading) as 0=absent, 1=present once, 2=present several times, 3=present frequently, 4=present continuously^[8].

Cortical 5-HT₂ receptors were estimated using a modification of the method of LEYSEN et al.^[9]. Rats were killed by decapitation and the cerebral cortex from each rat was homogenized for 15 s in 0.05 M Tris-HCl buffer (pH 7.7) at 4°C. The resulting homogenate was centrifuged at 50,000×g for 15 min at 4°C and the supernatant discarded. The pellet was again washed with Tris buffer, centrifuged as before and the resulting pellet resuspended in 100 vol of buffer. For experiments with haloperidol and apomorphine, the pellet was washed a third time.

The resuspended membranes were incubated with [³H]ketanserin (0.1-1.5 nM) in a final volume of 0.5 ml for 15 min at 37°C. Nonspecific binding was measured with methysergide (1 μM). Three milliliters of cold Tris buffer was added to each tube before filtration through Whatman GF/B filters. Each filter was washed twice before being placed in a scintillation vial for counting. B_{max} and K_d values were obtained from 6 point-assays by the direct linear plot method^[10]. Throughout the

text, $B_{\max}$ and K_d values are expressed as fmol/mg tissue and nM concentration, respectively.

For the experiment involving density-gradient centrifugation, each cerebral cortex was rapidly dissected and homogenized in a hypotonic solution (1 mM Tris-HCl buffer, pH 7.5) using a conical Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate (in 4 ml of buffer) was directly applied to a continuous sucrose gradient (0.5 to 1.5 M, 26 ml) formed on top of a 2-ml "cushion" of sucrose (2.0 M), and then centrifuged to equilibrium ($110,000 \times g/16$ h/ 4°C). The gradient was subsequently pumped out using a peristaltic pump and sixteen 2-ml fractions were collected and analyzed directly for 5-HT₂ receptors using the above-mentioned receptor binding method. Each fraction was incubated with 1.5 nM of [³H]ketanserin at a concentration 3–4 times that of the reported K_d ^[1,2,9].

Drugs used were paroxetine hydrochloride (Beecham Pharmaceuticals Ltd.), phenelzine and apomorphine (both hydrochloride; Sigma), carbidopa (Merck, Sharp and Dohme Ltd.), (L)-5-hydroxytryptophan (5-HTP Sigma), methysergide bimalate and ($\pm$)-pindolol hydrochloride (Sandoz Products Ltd.), the methyl ester hydrochlorides of (DL)-para-chlorophenylalanine and α -methyl-DL-para-tyrosine (α -MPT, Sigma), haloperidol (G. D. Searle) and propranolol hydrochloride (ICI Ltd.). [³H]Ketanserin was obtained from New England Nuclear. The drugs were injected into rats by the intraperitoneal route in a volume of 0.1 ml/100 g body weight.

Results

1. 5-HTP after carbidopa

The frequency of WDS during a 6-h period after administration of 5-HTP (150 mg/kg) to carbidopa-treated rats (25 mg/kg, 30 min previously) is shown in Fig. 1. At the end of this period, the number of cortical 5-HT₂ receptors was found to be unchanged when compared with those in a control group of carbidopa-treated rats given saline instead of 5-HTP; $b_{\max} = 12.1 \pm 0.6$ fmol/mg brain ($K_d = 0.36 \pm 0.02$ nM, $n = 6$) compared with $B_{\max} = 11.5 \pm 0.5$ ($K_d = 0.36 \pm 0.02$ nM, $n = 6$) in the control group. This extends the period of observation without any receptor change from the 3 h previously reported^[2] to 6 h.

A larger dose of 5-HTP (350 mg/kg) given to carbidopa-treated rats produced a more severe syndrome characterized by persistent tremor and reciprocal forepaw treading; WDS were also evoked (Fig. 1). Unexpectedly, there was a small but significant (10%; $P < 0.02$) increase in receptor number at 3 h, whereas at 6 h there was no change in receptor number and by 24 h, a significant decrease was apparent (Fig. 2). The increase in receptor number observed at 3 h was reversed by haloperidol (5 mg/kg); smaller doses of this drug were ineffective (Fig. 3). In a control group given haloperidol (5 mg/kg), the carbidopa receptor number was not altered 3 h after administration of saline (instead of 5-HTP) when compared with another control group that received only carbidopa and saline ($B_{\max} = 11.2 \pm 0.6$, $K_d = 0.41 \pm 0.03$, $n = 8$ compared with $B_{\max} = 11.1 \pm 0.4$, $K_d = 0.43 \pm 0.05$, $n = 8$ in the control group). Total WDS observed in the 5-min observation periods over 3 h

were 105.5 ± 8.7 ($n = 15$) in carbidopa-treated rats receiving 5-HTP (350 mg/kg), compared with 104.0 ± 11.0 ($n = 6$), 51.7 ± 20.4 ($n = 6$) and 45.1 ± 11.0 ($n = 8$) in rats also receiving haloperidol at 0.1, 1 and 5 mg/kg respectively.

However, in rats that were pretreated with α -MPT (250 mg/kg 16 h and 4 h before carbidopa), 5-HTP (350 mg/kg) still provoked an increase in receptor number (Fig. 3); $B_{\max} = 14.6 \pm 0.5$ compared with a $B_{\max}$ of 13.5 ± 0.6 in rats given saline instead of 5-HTP ($K_d = 0.43 \pm 0.03$ and 0.45 ± 0.01 respectively, $n = 9$ in each group).

2. Paroxetine after phenelzine

Propranolol (20 mg/kg) given 45 min before paroxetine to rats pretreated with 2 injections of phenelzine (50 mg/kg 18 h and 12 mg/kg 90 min previously) did

Fig. 1 Mean numbers of wet-dog shakes (WDS) occurring in rats pretreated with carbidopa (25 mg/kg 30 min previously) and given either 5-HTP (150 mg/kg) (—●—) or 5-HTP (350 mg/kg) (—○—) at zero time. Each value is the mean for 6 rats, the bars indicating S. E. M.

ANOVA, * $p < 0.05$ ** $p < 0.02$

Fig. 2 Time course of changes in $B_{\max}$ of [^3H]ketanserin binding to cortical membranes prepared from rats pretreated with carbidopa (25 mg/kg) 30 min before injection of 5-HTP (350 mg/kg). The numbers of rats are given in the columns. The results are expressed as percentages of the $B_{\max}$ obtained in simultaneously run rats pretreated with carbidopa and given saline instead of 5-HTP. The mean $B_{\max}$ for the control rats was 14.0 ± 0.6 fmol/mg tissue ($K_d = 0.33 \pm 0.02$, $n = 23$).

Fig. 3 Effects of haloperidol (0.1, 1.0 and 5.0 mg/kg) and α -MPT(250 mg) on 5-HT₂ receptor number 3 h after administration of 5-HTP (350 mg/kg) to carbidopa(25 mg/kg 30 min previously)-treated rats. The numbers of rats are given in the columns. The results are expressed as percentages of the $B_{\max}$ obtained in simultaneously run carbidopa-treated rats not given either haloperidol or α -MPT and given saline instead of 5-HTP.

not affect the total number of WDS observed in twelve 5-min observation periods, but it significantly ($P < 0.05$, Mann-Whitney U-test) reduced the occurrence of other phenomena (Table 1). Pindolol (5 mg/kg) showed a similar selectivity (data not shown). In rats given propranolol before paroxetine, the 5-HT₂ receptor number at 3h was 10.4 ± 0.5 ($K_d = 0.45 \pm 0.02$, $n = 8$) compared with 12.0 ± 0.4 ($K_d = 0.46 \pm 0.02$, $n = 8$) in rats given saline instead of paroxetine, a reduction of 13% ($P < 0.02$).

Figure 4 shows the data obtained from analysis of the sucrose gradients prepared from cortices taken 3 h after injection of either saline or paroxetine (12 mg/kg) into rats pretreated with phenelzine (50 mg/kg 18 h and 12 mg/kg 90 min previously). It can be seen that in both samples, most of the specific binding occurred at a sucrose molarity of approximately 9.5 M. Of the total specific binding, 60–70% was present in the five fractions around each peak. The most dramatic effect was a significant decrease in the specific binding of [^3H]ketanserin in the paroxetine-treated group, but there was clearly no increase of specific binding in the "light-density" fraction.

Table 1 Effects of propranolol on paroxetine-induced 5-HT-dependent behaviors

group	WDS	tremor	HLA	RFPT	Straub tail
	(mean $\pm$ SEM)	(median)			
control	74.2 $\pm$ 20.8	9	27	15	5
propranolol	66.2 $\pm$ 15.1	1*	2*	4*	1*

Propranolol (20 mg/kg) was given 45 min before paroxetine (12 mg/kg) to rats pretreated with 2 injections of phenelzine (50 mg/kg 18h and 12 mg/kg 90 min previously), $n=9$. In the control group, rats were given saline instead of propranolol, $n=9$. Types of 5-HT-dependent behaviors were measured for 3h by the procedures described in Materials and Methods (with the exception of WDS maximum rated score of 48 from twelve 5-min observation periods).

WDS: wet-dog shakes

HLA: hind limb abduction

REPT: reciprocal forepaw treadng

* $p<0.05$ (Mann-Wihtney U-test)

3. Other drug treatments

A single injection of para-chlorophenylalanine (300 mg/kg) 3 days previously had no effect on ($P>0.05$) 5-HT₂ receptors; 11.1 ± 0.48 ($K_d=0.25 \pm 0.01$, $n=12$) compared with 10.5 ± 0.51 ($K_d=0.29 \pm 0.02$, $n=12$) in the control group.

Apomorphine (2 mg/kg) significantly elevated 5-HT₂ receptors within 3 h when given to phenelzine-treated rats, B_{max} increasing from 9.2 ± 0.7 to 11.1 ± 0.5 ($n=9$ in each group) without affecting K_d (0.35 ± 0.02 and 0.36 ± 0.03 respectively). A similar change in 5-HT₂ receptor number was also obtained 3 h after a single injection of apomorphine (10 mg/kg), whereas a lower dose of apomorphine (2

Fig. 4 Specific binding of [³H]ketanserin to cerebral cortices homogenized and centrifuged to equilibrium in sucrose gradients. Cortices were dissected 3 h after injection of either saline (—○—, $n=6$) or paroxetine (—●—, $n=5$) in to rats pretreated with phenelzine (50 mg/kg 18 h and 12 mg/kg 90 min previously).

Fig. 5 Effects of apomorphine on 5-HT₂ receptor number in either phenelzine- or non-phenelzine-treated rats. The numbers of rats are given in the columns. The results are expressed as percentages of the B_{max} obtained in simultaneously run control rats given saline instead of apomorphine.

mg/kg) had no effect when given to untreated rats (Fig. 5).

Discussion

The present results extend earlier findings that in rats pretreated with an MAO inhibitor, 5-HT synthesized from exogenous 5-HTP does not affect 5-HT₂ receptors in the same way as endogenous 5-HT released spontaneously from nerve terminals (in the presence of a 5-HT uptake inhibitor) or by a releasing agent^[1,2]. After fenfluramine or a 5-HT uptake inhibitor, endogenous 5-HT rapidly produced down-regulation of the receptors, whereas after 5-HTP, the amount of down-regulation was small and it occurred only slowly, i.e., after 6 h in phenelzine-treated rats^[2] and after 24 h in carbidopa-treated animals. An increased dose of 5-HTP in carbidopa-treated rats had a paradoxical effect of increasing receptor number in the short term, although by 24 h there was a small but significant decrease in receptor number. On the other hand, although endogenous 5-HT was decreased by para-chlorophenylalanine (300 mg/kg 3 days previously), its administration did not affect the 5-HT₂ receptor number. The reasons why rapid down-regulation was not obtained by 5-HTP are not known. 5-HTP is decarboxylated to 5-HT by any cell containing aromatic amino acid decarboxylase and it can, for example, displace and release dopamine through formation of 5-HT within dopaminergic neurons^[3,4]. The finding of decreased WDS after a larger dose of 5-HTP was attributed to the inverted U-shaped dose-response curve of 5-HTP in carbidopa-treated rats, originally described by BEDARD and PYCOCK^[11], with the

maximum number of WDS being evoked by 5-HTP at a dose of 150 mg/kg. Although dopamine receptor stimulation inhibits 5-HTP-induced WDS^[11] and quipazine-induced head twitches^[12], the reduction in frequency of WDS after a larger dose of 5-HTP could not be explained by dopamine release, because the frequency of WDS was further reduced by haloperidol (1 and 5 mg/kg). Nevertheless, the up-regulation of 5-HT₂ receptors was gradually reversed by pretreating the rats with increasing doses of haloperidol, suggesting modulation of 5-HT₂ receptors by a dopaminergic mechanism. The likelihood of this reversal being due to residual haloperidol interfering with the receptor assay was minimized by extra washing of the homogenate; furthermore, control experiments in carbidopa-treated rats with and without haloperidol showed that the drug did not affect either $B_{\max}$ or K_d . The K_i for haloperidol-induced inhibition of [³H]ketanserin binding is 22 nM^[9], which compares with an estimated brain concentration of 12 μ M after haloperidol (5mg/kg) administration, assuming an equal distribution throughout the body.

One finding to support a dopaminergic influence on 5-HT₂ receptor number was the increase in receptor number after apomorphine administration. However, after depletion of catecholamines with α -MPT, 5-HTP still produced an increase in receptor number. We have no explanation for this paradox. The reasons why 5-HTP did not produce effects similar to those of other agents affecting the serotonergic system (5-HT uptake inhibitors, releasing agents or 5-HT-mimetics) are not known, but the 5-HTP-elicited receptor increase must depend in some complex way on the proportion of 5-HTP that is decarboxylated to 5-HT outside serotonergic neurons, thus affecting the dopaminergic mechanism.

WDS are mediated exclusively via 5-HT₂ receptors when evoked by a serotonergic mechanism, whereas other aspects of the 5-HT-dependent syndrome involve both 5-HT_{1A} and 5-HT₂ receptors^[13,14]. Pindolol and propranolol, acting as 5-HT_{1A} receptor antagonists^[15], antagonized tremor, hind-limb abduction, reciprocal forepaw treading and Straub tail, while sparing WDS. Propranolol did appear to reduce the down-regulation of 5-HT₂ receptors 3 h after administration of paroxetine to phenelzine-treated rats. It is not known whether this was due either to β -adrenoceptor or 5-HT_{1A} receptor blockade affecting 5-HT₂ receptor regulation or to possible inhibitory effects of such a blockade on 5-HT synthesis^[16].

In addition, we examined the process of 5-HT₂ receptor change *in vivo* following paroxetine administration in rats pretreated with phenelzine (50 mg/kg 18 h and 12 mg/kg 90 min previously). This experimental system was chosen, since it produces a situation where receptors are exposed to an increased level of agonist, thus inducing rapid cortical 5-HT₂ receptor down-regulation^[1,2]. We demonstrated that this drug schedule led to a remarkable decrease in the number of cortical 5-HT₂ receptors without causing the receptors to migrate into the "light-density fraction" isolated by continuous sucrose density-gradient centrifugation. This result suggests that the *in vivo* down-regulation of 5-HT₂ receptors in the brain does not involve receptor internalization.

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