European Journal of Pharmacology, 163 (1989) 145–149
Elsevier

EJP 20345

Short communication

Rapid desensitization and down-regulation of 5-HT_2 receptors by DOM treatment

Josée E. Leysen *, Paul F.M. Janssen and Carlos J.E. Niemegeers

Department of Biochemical Pharmacology and Janssen Research Council, Janssen Research Foundation, B-2340 Beerse, Belgium

Received 15 December 1988, accepted 31 January 1989

The regulation of the 5-HT_2 receptor-mediated head twitch response and of 5-HT_2 receptor binding in the frontal cortex was studied in rats treated repeatedly with the 5-HT_2 agonist 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (2.5 mg/kg, s.c.). Four injections in 24 h produced a near maximal reduction in the behaviour (−70%) and in the B_{max} for [^{3}H]ketanserin binding (−41%). The K_D values tended to increase slightly. 5-HT_2 receptors reappeared, with half-lives of 5.5 to 3 days. In view of the reported anomalous 5-HT_2 receptor regulation by antagonists and the regular regulation by agonists, we propose a refinement in the receptor regulation theory.

5-HT_2 receptor down-regulation; 5-HT_2 receptor agonists; DOM (1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane)

1. Introduction

5-Hydroxytryptamine$_2$ (5-HT_2) receptors in rat brain have been reported to be down-regulated by treatment with various antidepressants (Peroutka and Snyder, 1980) and with 5-HT antagonists (see Conn and Sanders-Bush, 1987; Leysen et al., 1986). Several observations point to an anomalous regulation of these receptors: (i) the down-regulation by antidepressants, thought to be a consequence of enhanced serotonergic transmission, still occurs in the absence of endogenous serotonin, (ii) depletion or destruction of serotonergic neurones does not affect receptor numbers, (iii) treatment with various serotonin antagonists produces desensitization and down-regulation instead of an expected supersensitivity and up-regulation. The effects of agonist treatment have been less investigated and findings are difficult to interpret because of the mixed antagonistic properties of the drugs (ergot derivatives), the lack of selectivity for the 5-HT_2 receptor (phenylpiperazines) or a too low affinity of drugs (mescaline) (for review see Conn and Sanders-Bush, 1987).

Recently, we found that the 5-HT_2 receptor-mediated phosphatidyl inositol response in cultured calf aorta smooth muscle cells became rapidly desensitized by treatment with 5-HT and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) (Pauwels et al., submitted). The latter drug has been described as a relatively selective 5-HT_2 agonist and is one of the most potent agonists known for these receptors (Shannon et al., 1984; Hoyer, 1988). In order to explore further 5-HT_2 receptor regulation in vivo, we investigated the 5-HT_2 receptor-mediated behavioural response and the binding properties of the 5-HT_2 receptor in rats treated repeatedly with DOM.

2. Materials and methods

Male Wistar rats (200 g) were subcutaneously (s.c.) treated with 2.5 mg/kg of DOM. An equal

* To whom all correspondence should be addressed: Department of Biochem. Pharmacol., Janssen Research Foundation, B-2340 Beerse, Belgium.

number of control rats received saline. Injections were given every 8 h, starting at 8:00 a.m. Immediately after each injection, the head twitch response was scored for 90-180 min.

In a first type of experiments, groups of rats were treated with DOM 2-, 4- and 10-times. Twenty-four hours after the last injection, the animals were killed and various brain areas were immediately dissected, frozen in liquid nitrogen and stored at −80 °C. Radioligand binding assays for the following receptors were performed in twice washed total membrane fractions: 5-HT_2: [^{3}H]ketanserin in the frontal cortex; 5-HT_{1A}: [^{3}H]N,N-dipropyl-8-hydroxy-2-aminotetralin (8-OHDPAT) in the hippocampus; 5-HT_{1B}: [^{3}H]5-HT in the presence of spiroxatrine in the hippocampus; α_2-adrenergic: [^{3}H]clonidine in the cortex; β_1-adrenergic: [^{3}H]dihydroalprenolol in the presence of ICI 118-551 in the cortex; dopamine D_2: [^{3}H]spiperone in the presence of R 43 448 in the striatum. The experimental conditions and materials were as described in Leysen et al. (1986; in press). Saturation binding curves, with at least 8 concentrations of radioligand, were made in duplicate with tissue pooled from 3 rats. Tissues from the treated and control rats were always assayed in parallel. The K_D and B_{max} values were derived by Scatchard analysis as described by Leysen et al. (1986). There were 15 treated and 15 control rats in a group, yielding 5 separate tissue pools of treated and controls per brain area. A treatment was given to 2 or 4 groups of rats.

In a second type of experiment, groups of rats were treated 4 times with DOM or saline and the animals were killed after 24, 48, 72 and 144 h. 5-HT_2 receptors were assayed in the frontal cortex.

DOM was synthesized for local use in the Organic Synthesis Department, Janssen Research Foundation, Beerse, Belgium.

3. Results

The in vitro binding affinity of DOM to various neurotransmitter receptors was found to be (K_i values in nM): 5-HT_2, 154; 5-HT_{1A}, 6240; 5-HT_{1B}, 1770; α_1-adrenergic, 6200; α_2-adrenergic, 1640; histamine-H_1, 43000; dopamine-D_2 and β_1-adrenergic, > 10000.

In preliminary experiments it was observed that 2.5 mg/kg DOM produced an intensive head twitch response (average of 40 twitches per 30 min initially), starting within 2 min following the injection and lasting for about 3 h. The effect was antagonized by a low dose of the 5-HT_2 antagonist, ritanserin (ED_{50} 0.08 mg/kg).

The effect of a repeated treatment with DOM (2.5 mg/kg s.c.; 2, 4 and 10 times) on the head twitch response and on the binding parameters of [^{3}H]ketanserin for 5-HT_2 receptors in the frontal cortex are shown in table 1A,B. Two injections already produced a significant reduction by 27% in the behavioural response and a significant decrease by 24% in the B_{max} value for [^{3}H]ketanserin binding. After 4 and 10 injections, the head twitches were reduced by 70 and 80% and the B_{max} values by 41 and 46%, respectively. The mean K_D values showed a slight but significant increase after 4 and 10 injections of DOM. When the binding data were analyzed per treated group, consisting of five separate tissue pools, the B_{max} value was consistently reduced in all the groups. However, the increase in the K_D value was only significant in 2 out of the 4 groups for the 4 times treated and in one of the two groups for the 10 times treated animals. The binding parameters of 5-HT_{1A}, 5-HT_{1B}, α_2- and β_1-adrenergic and of dopamine-D_2 receptors in rat brain areas remained unchanged after 10 DOM injections (see table 1C).

In the second type of experiment, we studied the time course over which the binding parameters of [^{3}H]ketanserin for 5-HT_2 receptors returned to control values in rats that had received 4 injections of 2.5 mg/kg DOM. The data are shown in table 2. As observed in the first series of experiments, the B_{max} value was significantly decreased by 48% 24 h after the last injection. A similar decrease was still seen after 48 h. The effect decreased after 72 h and after 144 h the reduction was only 16%. In this series of experiments, the K_D values were unchanged. Calculation of the rate of receptor reappearance according to Mauger et al. (1982) revealed a biphasic curve. The estimated rate constants were 0.0053 h^{-1} between 1 and 3,

TABLE 1

Behavioural response (A), [^{3}H]ketanserin binding in frontal cortex (B) and various receptor binding assays in brain tissue following repeated treatment of rats with DOM (2.5 mg/kg s.c. per 8 h). Mean values ± S.D. (number of determinations).

(A) Number of treatments	Head twitches number in 1 h	Δ%
1	75.4 ± 33.4 [c] (96)	
2	55.1 ± 42.2 [a] (84)	−27
4	23.0 ± 26.9 [a] (72)	−70
10	15.1 ± 16.2 [a] (24)	−80

(B) Number of treatments	Matched controls		Treated			
	K_D nM	B_{max} fmol/mg tissue	K_D nM	Δ%	B_{max} fmol/mg tissue	Δ%
2	0.47 ± 0.13	25.73 ± 3.17 (10)	0.58 ± 0.16	+23	19.62 ± 3.45 [a] (10)	−24
4	0.49 ± 0.09	27.16 ± 2.02 (20)	0.61 ± 0.13 [b]	+24	16.12 ± 3.21 [a] (20)	−41
10	0.47 ± 0.06	25.74 ± 3.89 (10)	0.77 ± 0.16 [a]	+63	13.85 ± 4.49 [a] (10)	−46

(C) Receptor [d]	Controls		Treated	
	K_D nM	B_{max} fmol/mg tissue	K_D nM	B_{max} fmol/mg tissue
5-HT_{1A}	1.17 ± 0.04	30.34 ± 1.02	1.22 ± 0.03	31.68 ± 0.80
5-HT_{1B}	1.91 ± 0.12	6.84 ± 0.30	2.14 ± 0.18	7.02 ± 0.37
α_2-A	1.36 ± 0.05	10.40 ± 0.33	1.30 ± 0.08	10.38 ± 0.24
β_1-A	1.38 ± 0.13	5.30 ± 0.47	1.79 ± 0.39	6.08 ± 0.92
D_2	0.041 ± 0.002	31.12 ± 1.25	0.042 ± 0.002	31.83 ± 0.87

Significant difference from controls according to Student's t-test (two tailed): [a] $P < 0.001$, [b] $P < 0.005$, no indication: not significant; [c] control value for the behavioural response; [d] measurements after 10 injections of DOM, mean values of 5 determinations. The rats were killed 24 h after the last injection.

and 0.0097 h^{-1} between 3 and 6 days after drug withdrawal, corresponding to half-lives of 5.5 and 3 days.

4. Discussion

Our in vitro binding data show, in agreement with previous findings (Shannon et al., 1984), that DOM binds relatively selectively to the 5-HT_2 receptor. The head twitch response, which is elicited in rats by DOM, and the antagonism of the effect by a low dose of ritanserin confirmed that the drug acts as an agonist. We showed that treatment of rats with a pharmacologically active dose of DOM, which is active for about 3 h, rapidly caused desensitization of the behavioural response, in parallel with a substantial decrease in the number of 5-HT_2 receptors in the frontal cortex. Both parameters were already significantly reduced after 2 injections, and 4 injections given over a 24 h period produced a near maximal reduction (−70% in the behavioural response, −41% in the B_{max} value; see table 1A,B). In binding studies, a reduction by nearly half of the total number of receptor sites is considered to be a very profound effect. We could demonstrate that these changes were reproducible (see Results). In contrast, changes in the K_D value of less than a factor of 2 are usually not considered to be very important. Moreover, a slight change in the K_D value for a labelled antagonist does not provide information on possible changes in the affinity for the agonist. The changes now observed in the binding parameters in the frontal cortex of the treated animals are not likely to be due to persistence of

TABLE 2

Time curve for the recovery of [^{3}H]ketanserin binding in the frontal cortex after repeated treatment of rats with DOM (4 injections of 2.5 mg/kg s.c. per 8 h). Mean values ± S.D. (5).

Time of withdrawal	K_D nM	B_{max} fmol/mg tissue	Δ%
Control	0.51 ± 0.07	28.31 ± 2.03	
24 h	0.48 ± 0.09	14.68 ± 2.48 [a]	−48
48 h	0.40 ± 0.02	16.32 ± 2.23 [a]	−42
72 h	0.47 ± 0.09	18.98 ± 1.92 [a]	−33
144 h	0.51 ± 0.09	23.68 ± 2.48 [a]	−16

Significant difference from controls according to Student's t-test (two tailed); [a] $P < 0.001$, no indication: not significant.

the drug in the tissue. This was verified in experiments where frontal cortical homogenates were incubated for 10 min at 37 °C with 10^{-7} and 10^{-6} M DOM., followed by the usual centrifugation and washing procedure for the membrane preparation. In these tissues, the K_D and B_{max} values for [^{3}H]ketanserin binding were similar to control values, indicating that even high concentrations of DOM are removed during membrane preparation (data not shown). It can be concluded that DOM treatment effectively reduces the total number of 5-HT_2 receptor sites, and this probably underlies the behavioural desensitisation. Indeed, receptor regulation is commonly thought to involve changes in receptor numbers, starting with internalisation of the receptors, followed by receptor degradation (Sibley et al., 1987). Since external as well as internalized receptors are measured in membrane preparations, it can be inferred that 5-HT_2 receptors are rapidly metabolized. The ratio of receptor resynthesis over degradation was apparently lower in the first days after cessation of the agonist treatment. The receptor half-life of 3 days, derived after 3 to 6 days drug withdrawal, corresponded to the time of receptor reappearance, which has been measured earlier following treatment with the 5-HT_2 antagonist, setoperone (Leysen et al., 1986).

DOM did not affect other serotonergic, adrenergic or dopaminergic receptor subtypes (table 1C): the drug showed a 10-40 times lower or no affinity for these receptors. It is possible that these receptors were not sufficiently occupied at the concentration used. Heterologous regulation of these receptors by 5-HT_2 receptor stimulation appeared to be absent.

Although 5-HT_2 receptors show an anomalous response to receptor denervation or blockade (see introduction), a normal response to agonist treatment was found. To explain this phenomenon we propose a refinement in the interpretation of the receptor regulation theory. It can be assumed that, in general, receptors have the ability to be up- and down-regulated. However, it could be that a receptor exists in a state of either supersensitivity or subsensitivity under normal conditions in vivo. A receptor that receives a tonic stimulation under physiological conditions, such as the dopamine receptor, would exist in a desensitized state. Acute blockade of such a receptor would produce profound behavioural effects and chronic blockade would rapidly give rise to receptor supersensitivity. In contrast, receptors that receive little impetus under normal conditions, such as, maybe, 5-HT_2 receptors, would normally exist in a fully supersensitive state. Acute blockade of such a receptor would not disrupt the normal situation and would cause no observable effect. This is the case when a 5-HT_2 antagonist is administered in the absence of an exogenous agonist. Such receptors cannot develop further supersensitivity, but they are very sensitive to agonists and rapidly develop desensitization. An indication that a large population of serotonergic neurones are scarcely active under normal conditions has been obtained from electrophysiological studies (Yen and Blum, 1984; Trulson and Jacobs, 1979). Hence, receptor regulation remains a general phenomenon, but it may preferentially occur in one or another direction, depending on the state of the receptor under normal conditions.

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