Biochemical Pharmacology, Vol. 37, No. 23, pp. 4451–4460, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

SPECIES DEPENDENCE OF ADAPTATIONS AT THE PRE- AND POSTSYNAPTIC SEROTONERGIC RECEPTORS FOLLOWING LONG-TERM ANTIDEPRESSANT DRUG TREATMENT*

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(*Received* 20 *January* 1988; *accepted* 14 *June* 1988)

Abstract—Rats were treated daily for 3 weeks with the antidepressant amitriptyline, and adaptations following this treatment at the level of the postsynaptic 5-HT_2 receptor were studied, as well as the presynaptic serotonin re-uptake and the 5-HT autoreceptor functioning. Rabbits were treated chronically with one of the antidepressants amitriptyline, imipramine, chlorimipramine and mianserin, and the occurrence of the different pre- and postsynaptic adaptations were compared to what was observed in rat brain.

Postsynaptic 5-HT_2 receptors were down-regulated following a long-term antidepressant drug treatment in rat prefrontal cortex, but were unchanged in rabbit brain.

Two markers for presynaptic 5-HT uptake were used to evaluate differences between control and treated animals: in rat brain a decreased number of [^{3}H]imipramine binding sites was observed, however, without any change in the kinetics of the [^{3}H]5-HT accumulation. In rabbit brain, both [^{3}H]imipramine binding and [^{3}H]5-HT accumulation remained unchanged.

The function of the presynaptic serotonergic autoreceptor was affected, although differentially, in both rat and rabbit brain, following the long-term antidepressant drug treatment. In rat brain, these autoreceptors were down-regulated, whereas in rabbit brain, the results indicated that the autoreceptors were only no longer activated by endogenously released serotonin.

The authors hypothesize that the different presynaptic adaptations at the level of the 5-HT autoreceptor are responsible for the absence or presence of a postsynaptic 5-HT_2 receptor down-regulation in rat and rabbit brain following a long-term antidepressant treatment.

Because of the therapeutic time-lag before the development of clinical responses to antidepressant drugs [1], attempts have been made to find the biochemical effects that correlate with this time-lag. It has been demonstrated that chronic but not acute administration of antidepressant drugs down-regulates rat brain beta-adrenergic receptor binding [2].

In addition, chronic antidepressant drug treatment has been shown in many studies to reduce the number of postsynaptic serotonin (5-hydroxytryptamine, 5-HT‡) receptors in rat brain [3, 4]. However, it has not been possible to reproduce this postsynaptic 5-HT receptor down-regulation in other species like the cat [5] and the rabbit [6], using a similar long-term antidepressant drug therapy.

On the other hand, a loss of presynaptic α_2 receptor-coupled inhibition of norepinephrine release has been shown to occur in rat brain following long-term antidepressant drug treatment [7]. This presynaptic adaptation might be an important mediating factor in the genesis of the postsynaptic receptor desensitization, since α_2-adrenergic antagonists accelerate and enhance the down-regulation of both β-adrenergic [8, 9] and 5-HT_2 receptors [10]. Similarly, the potentation of the imipramine-induced reduction of postsynaptic 5-HT receptors by metitepin might relate to its 5-HT autoreceptor antagonistic activity [11].

Recently, a subsensitivity of the 5-HT autoreceptors in rat brain following long-term antidepressant treatment has been demonstrated both in behavioural [12] and electrophysiological studies [13, 14].

This presynaptic 5-HT autoreceptor modulates the stimulation-induced serotonin release via a negative feedback mechanism [15–17], and subsensitivity of this autoreceptor would result in enhanced synaptic concentrations of the neurotransmitter.

The aim of the present study was to investigate the effects of long-term antidepressant drug treatment on some pre- and postsynaptic aspects of serotonergic transmission in both rat and rabbit brain.

MATERIALS AND METHODS

Animal treatment. Male Wistar rats (150–250 g) and male rabbits (2–3 kg) were housed with food and water *ad lib.*, and exposed to a 12 hr light–dark cycle. Antidepressant drugs were dissolved in saline and

* This work was supported by grants from the Belgian Government (84/89-68) and the Queen Elisabeth Medical Foundation (1986).

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‡ Abbreviations used: 5-HT, serotonin; B_{max}, maximal number of binding sites.

injected at a dosage of 10 mg/kg. Control animals received saline only. The animals were killed by a blow on the neck 17 hr after the last injection and their brains were rapidly removed.

Radioreceptor binding assays. [^{3}H]ketanserin and [^{3}H]imipramine binding studies were carried out as previously described [5, 6]. Briefly, prefrontal and frontoparietal cortex for [^{3}H]ketanserin binding and total cortex for [^{3}H]imipramine binding, were dissected, frozen on dry ice and stored at −80° until assay. Then the tissue was homogenized with a Potter–Elvejhem homogenizer in 20 vol. of Tris–HCl buffer (50 mM, pH 7.8), and centrifuged (12,000 *g*, 15 min). For the [^{3}H]ketanserin binding study, the pellet was resuspended in 400 vol. of 50 mM Tris–HCl buffer, and 4 ml of this membrane suspension was incubated at 37° for 15 min with 0.2 ml [^{3}H]ketanserin (0.25–5 nM) and 0.2 ml methysergide (in a 1000-fold excess) or solvent, to determine the amount of non-specific binding. The incubation mixture contained 10 mg of tissue original wet weight, corresponding to 200–300 μg of proteins. The binding of [^{3}H]imipramine was measured at 0° after incubating the membrane suspension (in 97 vol.) with [^{3}H]imipramine (1–15 nM), and specific displacer (100 μM desipramine) in a total vol. of 1 ml for 60 min. The reaction was stopped by dilution and rapid filtration through Whatman GF/B filters under vacuum.

The filters were washed three times with 5 ml of ice-cold incubation buffer, and the radioactivity on the filters was measured by liquid scintillation spectrometry (Packard, Belgium). Concentration (B_{max}) and affinity (K_d) of the receptors were determined according to Scatchard [18].

[^{3}H]5-*HT accumulation.* The [^{3}H]5-HT accumulation was determined in freshly dissected brain slices, using organ chambers as previously described [6]. Following preincubation for 20 min at 37° under a 95% O_2–5% CO_2 atmosphere, the slices were incubated with [^{3}H]5-HT for 10 min and subsequently washed during 40 min by replacing the incubation medium every 5 min. This washing procedure did not affect significantly the specifically accumulated [^{3}H]5-HT. It reduced, however, up to a minimum the amount of non-specific accumulation, as defined by the amount of radioactivity accumulated in the presence of fluoxetine (10 μM). After solubilization of the tissue slices in 0.5 ml Soluene (Packard, Belgium), the radioactivity was counted by liquid scintillation spectrometry (Packard, Belgium). V_{max} and K_m values were determined in the Lineweaver–Burk plot.

Superfusion experiments. [^{3}H]5-HT release and its modulation by the 5-HT autoreceptor was measured in freshly dissected hypothalamic slices as previously described [17]. Briefly, the slices were incubated at 37° for 2 hr with 10^{-7} M [^{3}H]5-HT in aerated Krebs–Ringer solution of the following millimolar composition: NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; glucose, 11.1, and CaNa–EDTA, 0.026. After the incubation, the slices were mounted in organ chambers to determine the [^{3}H] efflux. The slices were superfused at 1 ml/min with Krebs–Ringer solution containing chlorimipramine (10 μM) to inhibit serotonin uptake. Following a 2 hr washout period, at t = 0 min, the collection of 4 ml samples was started. The slices were stimulated twice (at t = 16 min and t = 60 min) electrically (square wave pulses, 50 mA, 7V, 2msec, 4Hz). The [^{3}H] efflux was expressed as fractional release, i.e. as a percentage of the total [^{3}H] content remaining in the tissue at the time of sampling. The [^{3}H] overflow was calculated by subtracting the basal [^{3}H] efflux immediately before stimulation, from the [^{3}H] efflux values obtained during, and two samples after, stimulation. This [^{3}H] overflow induced by the first and second stimulation were denoted S_1 and S_2 respectively, and S_2/S_1 ratios were calculated. To measure the effect of a drug on the stimulation-evoked [^{3}H]5-HT release, this drug was infused 20 min before the second stimulation, and the effect was determined by comparing this S_2/S_1 ratio with the ratio obtained in two subsequent stimulations in the absence of drug, using slices from the same animal. At the end of the superfusion, the slices were solubilized with 0.5 ml Soluene (Packard, Belgium) and radioactivity was counted in a liquid scintillation spectrometer (Packard, Belgium).

Statistical analysis. The data are expressed as means ± SEM. The number of experiments is equal to the number of rabbits used. Statistical differences were evaluated using parametrical or non-parametrical analysis, depending on the homogeneity of the variances. P- or α values smaller than 0.05 were taken as the significance level.

Drugs. [^{3}H]5-HT creatinine sulphate (sp.act. 29.2 Ci/mmol) and [^{3}H]imipramine (sp.act. 45.4 Ci/mmol) were purchased from New England Nuclear (Belgium), and [^{3}H]ketanserin (sp. act. 14.0 Ci/mmol) from Janssen Chimica (Belgium). Amitriptyline was a gift from Merck, Sharp and Dohme (Belgium), imipramine HCl, desipramine HCl and chlorimipramine from Ciba–Geigy (Belgium), and mianserin from Organon (Belgium). Fluoxetine was obtained from Eli Lilly (U.K.), methysergide bimaleate from Sandoz (Belgium), and metitepin from Hoffman-La Roche (Switzerland). Serotonin creatinine sulphate was purchased from Aldrich (Belgium).

RESULTS

Effect of long-term antidepressant drug treatment on the postsynaptic 5-HT$_2$ receptor binding in rat and rabbit brain

Saturable, monophasic, high-affinity [^{3}H]ketanserin binding was demonstrated in both rat and rabbit frontal cortex. The characteristics and the kinetic parameters indicated that the binding concerned postsynaptic 5-HT$_2$ receptors, which were similar in the two species. Amitriptyline given daily in a dose of 10 mg/kg for 3 weeks, down-regulated the [^{3}H]ketanserin binding in rat prefrontal cortex (Fig. 1). The decrease in [^{3}H]ketanserin binding represented lower specific binding, since non-specific binding, as determined by an excess methysergide, was similar in control and amitriptyline treated rats. Scatchard plots indicated a significant decrease in B_{max} value with no change in affinity (Fig. 1).

In rabbit prefrontal plus frontoparietal cortex, a 3-week treatment of the rabbits, either once daily

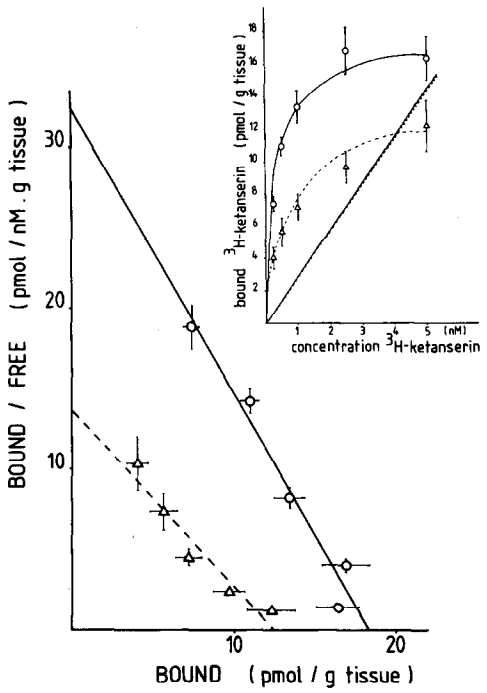

Fig. 1. Effect of long-term amitriptyline treatment on the 5-HT_2 receptor binding in rat brain. Rats were treated i.p. with saline (○——○) or amitriptyline (10 mg/kg) (△- - -△) daily for 21 days. [^{3}H]Ketanserin binding was assayed as described in Materials and Methods. For each experiment, the prefrontal cortices from two rats were pooled, and measurements were performed in duplicate. Inset: saturation curve for specific [^{3}H]ketanserin binding in saline treated and amitriptyline treated rats. The straight lines represent non-specific binding as determined by a 1000-fold excess of methysergide. Values are means ± SEM of five experiments. Below: Scatchard plots. K_d values are 0.64 ± 0.08 nM and 1.05 ± 0.29 nM respectively, not significantly different. B_{max} values for saline treated rats amounted to 20.2 ± 1.9 pmol/g tissue; for amitriptyline treated rats to 13.1 ± 1.5 pmol/g tissue, significantly different from the control rats (Student's t-test for unpaired observations, $P < 0.05$).

with chlorimipramine (10 mg/kg) or twice daily with amitriptyline or imipramine (10 mg/kg) did not change [^{3}H]ketanserin binding. No change in B_{max} or K_d could be detected following either of the treatments of the rabbits (Table 1).

Effect of long-term antidepressant drug treatment on the [^{3}H]imipramine binding and the [^{3}H]5-HT accumulation in rat and rabbit brain

Saturable, monophasic, high-affinity [^{3}H]imipramine binding was demonstrated on cortical membranes from both rat and rabbit brain. In rat brain, a daily administration of amitriptyline (10 mg/kg) for 21 days caused a significant decrease in the number of these binding sites (Table 2). In rabbit brain, the number of [^{3}H]imipramine binding sites remained unchanged, even after a twice daily injection of amitriptyline for 3 weeks (Table 2). On the other hand, both in rat and rabbit brain, the chronic treatment decreases the affinity of the binding site, as revealed by an enhanced K_d value (Table 2).

The [^{3}H]5-HT accumulation was studied using freshly dissected slices. With concentrations of [^{3}H]5-HT ranging between 20 and 500 nM, a monophasic, saturable accumulation of [^{3}H]5-HT could be demonstrated. Non-specific accumulation was linear. A similar saturable, high-affinity, specific accumulation of [^{3}H]5-HT was observed for rat and rabbit brain. The K_m and V_{max} values obtained by Lineweaver–Burk analysis for both rat and rabbit brain, are given in Table 2. Daily injections of amitriptyline (10 mg/kg) for 21 days tended to reduce the K_m and V_{max} value from rat and rabbit brain but the differences were never significant (Table 2).

Effect of long-term amitriptyline treatment on the [^{3}H]5-HT release and its modulation in rat brain

Following preincubation with [^{3}H]5-HT and a subsequent washout period, the [^{3}H] content of the hypothalamic slices amounted to 0.39 ± 0.04 pmol/mg of tissue (N = 12) for control rats, and to 0.42 ± 0.04 pmol/mg tissue (N = 8) for hypothalamic slices obtained from rats which were treated with

Table 1. 5-HT_2 receptor binding in frontal cortex of rabbits following long-term antidepressant treatment

Rabbit treatment	B_{max} (pmol/g tissue)	K_d (nM)
1 × 10 mg/kg (21 days)		
Saline	11.5 ± 1.4 (6)	0.92 ± 0.11 (6)
Chlorimipramine	10.7 ± 0.9 (6)	0.95 ± 0.07 (6)
2 × 10 mg/kg (21 days)		
Saline	15.8 ± 1.9 (5)	0.78 ± 0.08 (5)
Amitriptyline	15.0 ± 1.5 (5)	0.91 ± 0.14 (5)
Imipramine	17.0 ± 2.2 (5)	0.83 ± 0.11 (5)

Rabbits were treated for 3 weeks with 10 mg/kg i.p., either once daily with chlorimipramine, or twice daily with amitriptyline or imipramine. Control rabbits received saline only. [^{3}H] Ketanserin binding was assayed as described in Materials and Methods. Values are means ± SEM, with the number of experiments denoted in parentheses, and each performed in triplicate. Differences between the parameters for control and treated rabbits were evaluated using Student's t-test for unpaired observations; no significant differences were revealed.

Table 2. [^{3}H]Imipramine binding and [^{3}H] accumulation in brains of rats and rabbits following long-term antidepressant treatment

Treatment	[^{3}H]Imipramine binding		[^{3}H]5-HT accumulation	
	B_{max} (pmol/g tissue)	K_d (nM)	V_{max} (pmol/g tissue)	K_m (nM)
Rat				
Saline	14.5 ± 1.6	6.0 ± 0.8	481 ± 52	115 ± 12
Amitriptyline	8.2 ± 1.7*	9.8 ± 0.7**	360 ± 45	99 ± 11
Rabbit				
Saline	18.1 ± 1.6	7.9 ± 1.1	380 ± 64	300 ± 63
Amitriptyline	19.7 ± 1.5	12.8 ± 0.7**	333 ± 65	199 ± 34

Rats were treated once daily, and rabbits twice daily, with the antidepressant amitriptyline (10 mg/kg) for 21 days. Control animals received saline only. [^{3}H]Imipramine binding and [^{3}H]5-HT accumulation were assayed as described in Materials and Methods. Values are the means ± SEM of five experiments, each performed in triplicate. Differences between the kinetic parameters for control and treated animals were evaluated using Student's t-test for unpaired observations (* $P < 0.05$; ** $P < 0.01$).

amitriptyline once daily for 21 days. At the end of the superfusion, this [^{3}H] content was reduced to 0.26 ± 0.01 pmol/mg tissue (N = 19) and 0.26 ± 0.03 pmol/mg tissue (N = 8) for control and amitriptyline treated rats respectively.

Following the washout period, a constant spontaneous [^{3}H] outflow was measured from the rat hypothalamic slices which in the period t = 12–16 min amounted to 2.58 ± 0.13 (N = 14) and 2.56 ± 0.12% (N = 8) percent of total tissue tritium for saline and amitriptyline treated rats respectively.

Thus, long-term amitriptyline treatment did not influence the [^{3}H] content of the slices following the incubation, nor the basal [^{3}H] efflux during superfusion (Student's t-test for unpaired observations).

Electrical stimulation (4Hz, 50 mA) of the brain slices caused a [^{3}H] overflow, significantly above the basal [^{3}H] efflux in these slices. This [^{3}H] overflow, denoted S_1 and expressed as fractional release, amounted to 0.85 ± 0.12% (N = 10) of total tissue tritium for hypothalamic slices from control rats. Treatment of the rats with amitriptyline resulted in a significant (Student's t-test for unpaired observations, $P < 0.05$) increase of this electrically evoked [^{3}H] overflow, to 1.24 ± 0.13% (N = 8) of total tissue tritium.

After the first stimulation, the [^{3}H] outflow decreased in all the tissue preparations to the pre-stimulation basal [^{3}H] efflux value. Following a second electrical stimulation, a new [^{3}H] overflow flow (S_2) could be induced in all the brain slices. The S_2/S_1 ratio (0.83 ± 0.09 for control rats) remained unchanged, independent of the foregoing treatment of the rats.

In controls rats, the S_2/S_1 ratio was modulated by the infusion of serotonin or metitepin in the superfusion medium 20 min before the second stimulation. The stimulation-induced [^{3}H] release was significantly (Student's t-test for paired observations, $P < 0.02$) decreased by the infusion of serotonin (10^{-6} M), whereas metitepin (10^{-5} M) significantly ($P < 0.001$) augmented the [^{3}H]5-HT release (Fig. 2).

Amitriptyline treatment of rats for 3 weeks completely abolished the modulating effect of both serotonin and metitepin on the stimulation-induced [^{3}H]5-HT release (Fig. 2).

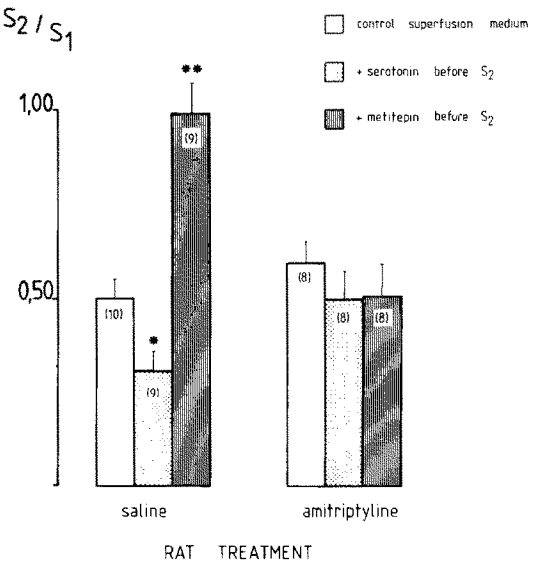

Fig. 2. Effect of serotonin and metitepin on the stimulation evoked [^{3}H]5-HT release from superfused rat hypothalamic slices, and abolishment of this modulating effect following long-term amitriptyline treatment. Rats were treated with saline or with amitriptyline (1 × 10 mg/kg) i.p. for 21 days. After dissection, the hypothalamic slices were incubated with 0.1 μM [^{3}H]5-HT and subsequently superfused. [^{3}H] Overflow was induced twice by electrical stimulation at t = 16 (S_1) and t = 60 (S_2). Serotonin (10^{-6} M) or metitepin (10^{-5} M) were infused in the control superfusion medium 20 min before the second stimulation. S_2/S_1 is the ratio of [^{3}H] overflow evoked by the second stimulation to the [^{3}H] overflow induced by the first stimulation. The results are the means ± SEM for the number of rats denoted between brackets. * Significantly different from the value obtained for hypothalamic slices (of the same rats) which were continuously superfused with control superfusion medium; Student's t-test for paired observations (* $P < 0.02$, ** $P < 0.001$).

Effect of long-term antidepressant drug treatment on the [^{3}H]5-HT release and its modulation in rabbit brain

Rabbits were treated for 21 days, either once daily with the antidepressant chlorimipramine, or twice daily with one of the antidepressants amitriptyline, imipramine or mianserin.

The [^{3}H] content of the hypothalamic slices as a result of the incubation of the brain slices with [^{3}H]5-HT (0.1 μM), was determined immediately after the washout period, and at the end of the superfusion experiment. An overview of these data for control (saline treated) and drug treated rabbits is represented in Table 3. None of the treatments caused a significant change in the amount of [^{3}H] accumulated in the slices (analysis of variance).

An unchanged spontaneous [^{3}H] efflux was measured from hypothalamic slices from saline treated rabbits or rabbits which were treated chronically with one of the antidepressants. For brain slices obtained from control rabbits, this basal [^{3}H] efflux amounted to 1.83 ± 0.05% (N = 24) of total tissue tritium at t = 12 min, corresponding to an outflow rate of 14 ± 1 fmol/mg tissue per 4 min. Table 3 gives an overview of the basal [^{3}H] efflux for control and treated rabbits. Treatment of the rabbits with one of the antidepressants did not cause any significant change in the spontaneous [^{3}H] efflux compared with saline treated rabbits (analysis of variance). Beside these values, Table 3 also includes the data from experiments in which the brain slices were superfused in the presence of metitepin (10^{-5} M) from the beginning of the superfusion.

Electrical stimulation (4Hz, 50 mA) of the slices at t = 16 min, induced a [^{3}H]overflow in all the tissue preparations. For control rabbits, this overflow value amounted to 3.21 ± 0.21% (N = 23) of total tissue tritium, in control superfusion medium. An overview of the [^{3}H] overflow values obtained for control and treated rabbits, and for superfusion circumstances in control medium or in the presence of metitepin from the beginning of the superfusion, is represented in Table 3. Only after a long-term treatment with mianserin, a significantly lower [^{3}H] overflow was observed in control superfusion medium, compared with the value obtained for saline treated rabbits (Mann–Whitney U-test; $P < 0.05$). The other antidepressant treatments did not cause any change in the electrically induced [^{3}H] overflow (analysis of variance between saline treated, chlorimipramine treated, imipramine treated and amitriptyline treated rabbits).

Treatment of the rabbits with one of the antidepressants also did not affect the [^{3}H] overflow values obtained when the slices were superfused in medium containing metitepin from the start of the experiment (Kruskal–Wallis analysis).

After the first electrical stimulus, the [^{3}H] efflux from all the tissues decreased again to the pre-stimulation value, and a second stimulation at t = 60 min evoked a second [^{3}H] overflow (S_2), slightly smaller than at the first stimulation period. For hypothalamic slices obtained from control rabbits, the S_2/S_1 ratio amounted to 0.80 ± 0.03 (N = 21). Infusion of serotonin before the second stimulation decreased the

Table 3. Effect of long-term drug treatment on the accumulation in, and the release of tritium from, rabbit hypothalamic slices loaded with [^{3}H]5-HT

Rabbit treatment	[^{3}H] Content (pmol/mg tissue)		Basal [^{3}H] efflux (%)		[^{3}H] Overflow (%)	
	At t = 0 min	At t = 80 min	Control superfusion medium	Superfusion in the presence of metitepin (10^{-5} M)[a]	Control superfusion medium	Superfusion in the presence of metitepin (10^{-5} M)[a]
Saline	0.81 ± 0.04 (24)	0.47 ± 0.03 (24)	1.83 ± 0.05 (24)	1.82 ± 0.14 (7)	3.21 ± 0.21 (23)	4.17 ± 0.65 (7)
Chlorimipramine	0.75 ± 0.03 (10)	0.44 ± 0.03 (10)	1.93 ± 0.04 (10)	—	2.69 ± 0.31 (10)	—
Imipramine	0.81 ± 0.04 (8)	0.45 ± 0.04 (8)	1.81 ± 0.08 (8)	2.13 ± 0.06 (6)*	3.77 ± 0.25 (8)	3.76 ± 0.22 (6)
Amitriptyline	0.79 ± 0.05 (8)	0.46 ± 0.04 (8)	1.81 ± 0.10 (8)	1.99 ± 0.11 (6)	3.90 ± 0.43 (8)	3.73 ± 0.44 (6)
Mianserin	0.80 ± 0.07 (8)	0.44 ± 0.05 (8)	2.05 ± 0.08 (8)	—	2.93 ± 0.15 (8)	—

Rabbits were treated i.p. for 21 days, either once daily with chlorimipramine (10 mg/kg), or twice daily with one of the antidepressants amitriptyline, imipramine or mianserin (10 mg/kg). Control rabbits received saline only. After dissection, the hypothalamic slices were incubated with [^{3}H]5-HT (0.1 μM) and subsequently superfused. The [^{3}H] content was determined immediately after the washout period (at t = 0 min) and at the end of the superfusion. The basal [^{3}H] efflux was determined in the interval t = 12–16 min. Electrical stimulation (4Hz, 50 mA) was induced at t = 16 min. [^{3}H] Efflux and electrically stimulated [^{3}H] overflow are expressed as per cent of the total [^{3}H] content present in the tissue at that moment. The electrically stimulated [^{3}H] overflow is calculated by subtracting the pre-stimulation basal [^{3}H] efflux value from the [^{3}H] efflux values obtained during, and two samples after, stimulation. The results are the means ± SEM, for the number of rabbits denoted in parentheses.

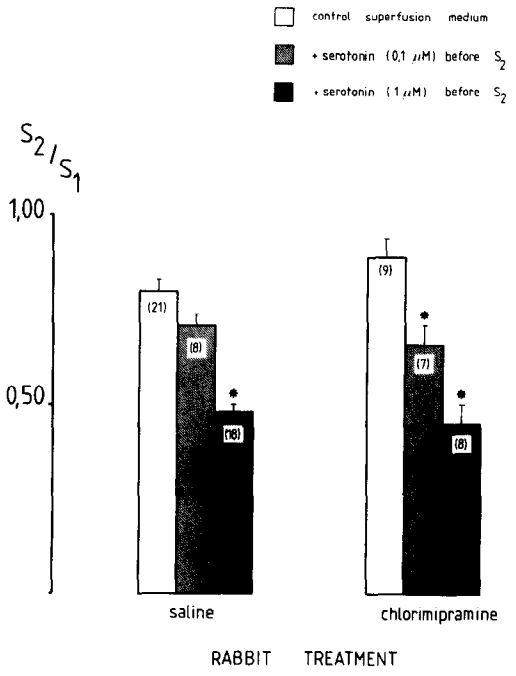

Fig. 3. Effect of serotonin on the stimulation evoked [^{3}H]5-HT release from superfused rabbit hypothalamic slices in saline and chlorimipramine treated rabbits (1 × 10 mg/kg i.p. for 21 days). After dissection, the hypothalamic slices were incubated with 0.1 μM [^{3}H]5-HT and subsequently superfused. [^{3}H] Overflow was induced twice by electrical stimulation at $t = 16$ min (S_1) and $t = 60$ min (S_2). Serotonin (10^{-7} or 10^{-6} M) was infused in the control superfusion medium 20 min before the second stimulation. S_2/S_1 is the ratio of [^{3}H] overflow evoked by the second stimulation to the overflow induced by the first stimulation. The results are the means ± SEM for the number of rabbits denoted between brackets. * Significantly different from the value obtained for hypothalamic slices (of the same rabbits) which were continuously superfused with control superfusion medium; Student's t-test for paired observations ($P < 0.01$).

stimulation-induced [^{3}H] overflow on a concentration-dependent manner (Fig. 3). The addition of metitepin (10^{-5} M) to the superfusion medium, on the contrary, augmented the [^{3}H] overflow induced by the second stimulation (Fig. 4).

In hypothalamic slices from rabbits chronically treated with one of the antidepressants, serotonin still caused a decrease in the electrically evoked [^{3}H]5-HT release (Fig. 3 and Table 4). However, metitepin completely lost its enhancing effect in the chronically treated rabbits (Fig. 4).

For the slices that were superfused throughout the duration of the experiments with medium containing metitepin (10^{-5} M), serotonin (10^{-6} M) no longer

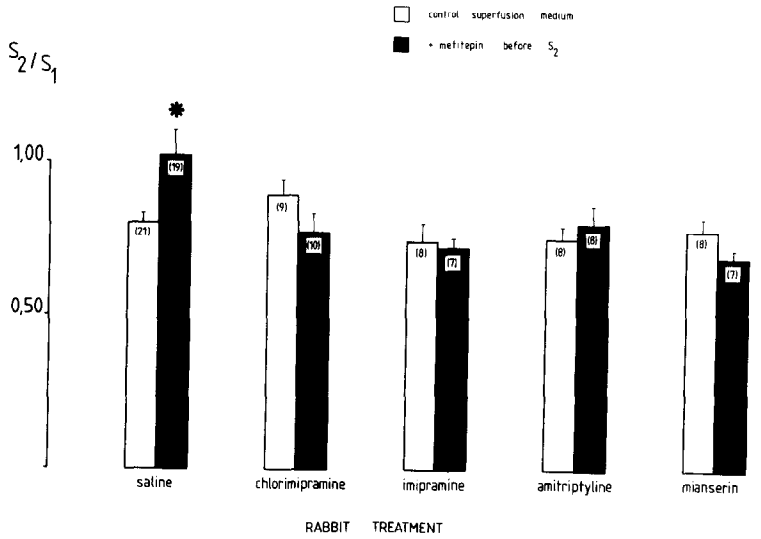

Fig. 4. Effect of metitepin on the stimulation evoked [^{3}H]5-HT release from superfused rabbit hypothalamic slices, and abolishment of this modulating effect following long-term antidepressant drug treatment. Rabbits were treated with saline, chlorimipramine (1 × 10 mg/kg), amitriptyline, imipramine or mianserin (2 × 10 mg/kg) i.p. for 21 days. After dissection, the hypothalamic slices were incubated with 0.1 μM [^{3}H]5-HT and subsequently superfused, [^{3}H] Overflow was induced twice by electrical stimulation at $t = 16$ min (S_1) and $t = 60$ min (S_2). Metitepin (10^{-5} M) was infused in the control superfusion medium 20 min before the second stimulation. S_2/S_1 is the ratio of [^{3}H] overflow evoked by the second stimulation to this [^{3}H] overflow induced by the first stimulation. The results are the means ± SEM for the number of rabbits denoted between brackets. * Significantly different from the value obtained for hypothalamic slices (of the same rabbits) which were continuously superfused with control superfusion medium. The Wilcoxon test was used to evaluate the differences within the saline treated group and within the imipramine treated group. Student's t-test for paired observations was used within the chlorimipramine, the amitriptyline and the mianserin treated group.

Table 4. Effect of long-term antidepressant drug treatment on the modulation of the electrically induced [^{3}H]5-HT release by serotonin in rabbit hypothalamic slices

(A) Slices superfused with control superfusion medium		
	S_2/S_1	
Treatment	Control[c]	+ Serotonin (10^{-6} M)
Saline	0.80 ± 0.03 (21)	0.48 ± 0.02 (18)[a*]
Imipramine	0.74 ± 0.06 (8)	0.47 ± 0.02 (8)[b*]
Amitriptyline	0.75 ± 0.04 (8)	0.53 ± 0.05 (8)[a*]
Mianserin	0.78 ± 0.04 (8)	0.46 ± 0.06 (8)[a*]
(B) Slices superfused in the continuous presence of metitepin (10^{-5} M)		
Treatment	Control[c]	+ Serotonin (10^{-6} M)
Saline	0.68 ± 0.05 (7)	0.77 ± 0.09 (7)[a]
Imipramine	0.63 ± 0.03 (6)	0.59 ± 0.03 (5)[a]
Amitriptyline	0.71 ± 0.05 (6)	0.70 ± 0.04 (6)[a]

Rabbits were treated with saline, imipramine, amitriptyline or mianserin (2 × 10 mg/kg i.p.) for 21 days. After dissection, the hypothalamic slices were incubated with 0.1 μM [^{3}H]5-HT and subsequently superfused. [^{3}H] Overflow was induced twice by electrical stimulation at t = 16 min (S_1) and T = 60 min (S_2). Serotonin (10^{-6} M) was infused in the superfusion medium 20 min before the second stimulation. S_2/S_1 is the ratio of [^{3}H] overflow evoked by the second stimulation to this overflow induced by the first stimulation. The values are the means ± SEM for the number of rabbits denoted in parentheses.

[a] Student's t-test for paired observations, to evaluate the effect of the infusion of serotonin within one treatment group; * significant difference ($P < 0.01$).

[b] Wilcoxon test, ibid.

[c] Analysis of variance, to evaluate the effect of the animal treatment on the control S_2/S_1 ratio; (A): no difference ($F = 1.525$, df = 4.49, $\alpha = 0.210$); (B): no difference ($F = 0.68$, df = 2.16, $\alpha = 0.521$).

reduced the stimulation-evoked [^{3}H]5-HT release, when infused before the second stimulation. This was the same for saline treated rabbits and for the rabbits chronically treated with one of the antidepressants (Table 4).

DISCUSSION

The present study investigated species differences in the down-regulation of brain 5-HT$_2$ receptors following long-term antidepressant drug treatment. Chronic administration of the tricyclic antidepressant amitriptyline significantly reduced the density (B_{max}) of 5-HT$_2$ receptors in rat brain, as has been reported by several other investigators [3, 4]. However, rabbit brain 5-HT$_2$ receptors seem to be unaffected by long-term antidepressant drug treatment, as has been demonstrated in a former study using a daily 2-week treatment with one of the antidepressants imipramine, desipramine, amitriptyline, zimelidine or maprotiline [6], and by this study using a more intensive (until twice daily) and longer treatment of the rabbits with either chlorimipramine, imipramine or amitriptyline. In a previous study [6], the [^{3}H]ketanserin labelled sites in rabbit brain were characterized and found to be very similar to the postsynaptic 5-HT$_2$ receptors in rat brain. Also the pharmacokinetics and the immediate inhibitory effects of the antidepressant drugs on the serotonin re-uptake are very similar in the brains of the two species [6]. This is important since in the first instance, the re-uptake inhibition was said to play a major role in the genesis of the postsynaptic receptor down-regulation [3]. Another hypothesis concerning antidepressants suggested that their mechanism of action would relate to their binding capacity at the postsynaptic 5-HT$_2$ receptor [19, 20]. However, we did not find support for this theory since no down-regulation of the 5-HT$_2$ receptor could be demonstrated following long-term treatment with antidepressant drugs which have shown to possess potent 5-HT$_2$ receptor blocking activity in the rabbit brain both *in vitro* [6] and *in vivo* [21]. In order to clarify this problem, we focused our attention on the presynaptic adaptations following a long-term antidepressant treatment in both rat and rabbit brain, since these presynaptic changes could be determining factors in the subsequent evolving postsynaptic adaptations.

One of these presynaptic aspects concerns the serotonin re-uptake mechanism, and the theory that this mechanism is being modulated by the imipramine recognition site [22]. It has been suggested that tricyclic antidepressants which bind to this regulatory site, would down-regulate this site on chronic administration [23]. As a result, the 5-HT re-uptake would be facilitated, finally leading to an increased firing rate of the serotonergic axons to compensate

the rapid adaptation of the 5-HT uptake mechanism. An enhanced release of a co-transmitter would then bring about the postsynaptic 5-HT_2 down-regulation [24]. A decreased B_{max} of the [^{3}H]imipramine binding site and an increased V_{max} for the [^{3}H]5-HT uptake, observed by Barbaccia *et al.* [23] in rat brain following long-term imipramine or desipramine treatment, agrees with this hypothesis.

In our study, we also demonstrated a reduced number of [^{3}H]imipramine binding sites in rat brain following long-term amitriptyline treatment, whereas this number remained unchanged in rabbit brain.

However, this phenomenon could not be responsible for the difference in postsynaptic changes, since also in rat brain, the desensitized [^{3}H]imipramine site was not accompanied by a change in the re-uptake mechanism, which was a condition for the hypothesis proposed by Costa and Barbaccia.

Going back to the original hypothesis concerning the mechanism of the down-regulation of rat brain 5-HT_2 receptors, Peroutka and Snyder [3] suggested that increased synaptic 5-HT levels as a consequence of the chronic re-uptake inhibition by tricyclic antidepressants, would be responsible for the postsynaptic adaptation.

Increased synaptic levels of serotonin could also be obtained by an increased release of serotonin as a result of the 5-HT autoreceptor down-regulation. This presynaptic 5-HT autoreceptor is known to play an important role in modulating the stimulation induced release of serotonin via a negative feedback system, a mechanism counteracted by such antagonists as metitepin, for example. This autoregulatory system is observed both in rat [15, 16, 25] and rabbit brain [17].

Hypothalamic slices were incubated with a low [^{3}H]5-HT concentration, to insure selective uptake into serotonergic neurons [15, 26]. The amount of [^{3}H] accumulated in the slices following the incubation with [^{3}H]5-HT was unchanged by a long-term treatment of rats or rabbits with one of the antidepressants imipramine, chlorimipramine, amitriptyline or mianserin. This is in line with the higher mentioned experiments which demonstrated that an antidepressant drug treatment of rats or rabbits did not cause any change in the kinetics of the presynaptic uptake mechanism for serotonin.

When subsequently superfused, a basal [^{3}H] efflux was observed, which remained unchanged following an antidepressant drug treatment of rats or rabbits. It should, however, be mentioned that the trend towards a slightly enhanced basal [^{3}H] efflux from brain slices of mianserin treated rabbits, probably relates to the observation that mianserin, in contrast to imipramine, induces an *in vitro* release of [^{3}H]5-HT from synaptosomes [27].

Electrical stimulation caused a [^{3}H] overflow, which consists mainly of unchanged [^{3}H]5-HT [see ref. 28], and which probably reflects exocytotic action potential-evoked release of [^{3}H] serotonin from serotonergic axons [15, 17].

Apart from the mianserin treated rabbits, where a significantly lower value for this stimulation-induced [^{3}H] overflow was noted, no differences were observed between the release of [^{3}H]5-HT in hypothalamic slices from saline treated rabbits, or from rabbits treated with any other antidepressant drug. In rat brain, on the contrary, a remarkably enhanced release of [^{3}H]5-HT at stimulation was observed in hypothalamic slices following a long-term treatment of the rats with amitriptyline.

We subsequently investigated whether an antidepressant treatment changed the modulating effect of serotonergic drugs on the stimulation-induced release of serotonin.

In control rats, exogenous serotonin decreased the stimulation-induced [^{3}H]5-HT overflow, whereas metitepin added to the superfusion medium caused an increase in the stimulation-evoked [^{3}H]5-HT overflow. In rats which had been treated with amitriptyline for 3 weeks, both serotonin and metitepin had completely lost their effect on the release of [^{3}H]5-HT. This observation suggests an uncoupling of the presynaptic 5-HT autoreceptor from its modulating effect following an antidepressant drug therapy in rats. The enhanced stimulation-evoked release of [^{3}H]5-HT measured in the brain slices from these treated rats is consistent with the idea of subsensitive or uncoupled 5-HT autoreceptors.

Down-regulation of the 5-HT autoreceptor following a long-term antidepressant drug treatment of rats and mice, was also demonstrated by other investigators. Studying the same functional parameter, namely modification of the stimulation-evoked [^{3}H]5-HT release, Maura and Raiteri [29] demonstrated sub- or supersensitive 5-HT autoreceptors in superfused rat brain synaptosomes following long-term stimulation or blockade. Also electrophysiological studies in rat brain [13, 14], and behavioural studies with mice [12], demonstrated attenuated presynaptic 5-HT autoreceptor-mediated response by long-term antidepressant drug treatment and electroconvulsive shocks.

Thus, our findings as well as the results of other recent studies clearly indicate a down-regulation of the 5-HT autoreceptor in rat brain following a long-term antidepressant drug treatment.

We also studied the presynaptic autoreceptor in rabbit brain. In control rabbits, exogenously added serotonin concentration-dependently inhibited the stimulation-induced [^{3}H]5-HT release, in a very similar way as observed in rat brain. The serotonergic antagonist metitepin, when present from the start of the superfusion, blocked the effect of infused unlabelled serotonin. Moreover, it increased the [^{3}H]5-HT release when added on its own to the superfusion medium. The enhancing effect of a 5-HT autoreceptor antagonist on the stimulation-evoked release of [^{3}H]5-HT is shared by other antagonists such as cyanopindolol, for example (data not shown).

Following long-term antidepressant drug treatment, exogenous serotonin was still effective in decreasing the stimulation-evoked [^{3}H]5-HT release, in the same concentration-dependent manner as observed in saline treated rabbits.

Metitepin, on the other hand, no longer enhanced the release of [^{3}H]5-HT evoked by the second stimulation when added to the superfusion medium 20 min before this stimulus.

However, when agonist and antagonist were present during the stimulation period, the reducing

effect of serotonin on the [^{3}H]5-HT release was still antagonized by the presence of metitepin. Thus, the loss of the enhancing effect of metitepin on its own, cannot be explained as a down-regulation of the 5-HT autoreceptor, nor as a selective decrease in the affinity for the antagonist. The observation that added unlabelled serotonin still inhibits the [^{3}H]5-HT release, and that metitepin still antagonizes this effect, indicates that the autoreceptor remained drug-sensitive and that this receptor-mediated negative feedback mechanism can still be activated. The only explanation for the observation that metitepin had become inactive on its own, is that less serotonin was present in the synaptic cleft, and that its concentration was too low to activate the autoreceptor. Indeed, the enhancing effect of the antagonist on the stimulus-evoked release of serotonin is due to a blockade of the autoinhibition by the released neurotransmitter. For the same reason, antagonists were inactive in the synaptosome model [30], after depletion by reserpine [31], or after inhibition of the 5-HT synthesis [32].

In conclusion, rat and rabbit brain adapt differentially on chronic administration of antidepressant drugs. Postsynaptic 5-HT$_2$ receptors become down-regulated in rat brain, whereas they remain unchanged in rabbit brain. Our results indicate that desensitization of the presynaptic 5-HT autoreceptor, concomitant with an enhanced release at nerve stimulation, possibly plays an important role in the genesis of the postsynaptic 5-HT$_2$ receptor down-regulation. However, more research is certainly necessary to elucidate the mechanism of action of antidepressant drugs.

Acknowledgements—We wish to thank L. Van den Eynde for typing the manuscript, and Y. Onsea for technial assistance.

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