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Effects of acute and repeated administration of citalopram on extracellular levels of serotonin in rat brain

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Abstract

The effects of acute (2 days) and repeated (21 days) administration (50 mg/kg in the diet) of the selective serotonin (5-HT, 5-hydroxytryptamine) reuptake inhibitor, citalopram, on extracellular levels of 5-HT and their modulation by terminal autoreceptors in the hypothalamus of freely moving rats were compared in vivo by microdialysis. When studied without washout, extracellular levels of 5-HT were increased by both acute and repeated citalopram administration. In rats treated repeatedly, extracellular 5-HT levels were 43% (but not significantly) greater than in those treated acutely. Extracellular levels of 5-HT in control and citalopram-treated rats were similar when measured after 24 h washout. The enhancing effect of non-selective serotonergic autoreceptor antagonists, methiothepin (100 μM) or 1-(1-naphthyl)piperazine (NP) (10 μM), administered through the microdialysis probe, after 24 h washout, was similar in both control and chronically treated groups. These results suggest that repeated administration of citalopram followed by a washout of 24 h does not lead to desensitization of the terminal autoreceptor as measured in vivo in contrast to the effects we have shown previously in vitro. In rats treated chronically with citalopram without washout, methiothepin had a greater maximal effect on 5-HT outflow in comparison to rats receiving acute citalopram treatment. This finding suggests that a 5-HT autoreceptor antagonist or a combination of such a drug with a 5-HT uptake inhibitor would produce a greater increase of extracellular levels of 5-HT in hyposerotonergic states such as depression.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT uptake inhibitor; Antidepressant; Microdialysis; Hypothalamus, rat

1. Introduction

Selective serotonin (5-HT, 5-hydroxytryptamine) reuptake inhibitors have proved to be useful clinical antidepressants (Feighner and Boyer, 1991). In vitro, and in vivo, the selective 5-HT reuptake inhibitors, as their name implies, selectively inhibit the reuptake of 5-HT, and are characterized by the absence of high-affinity interactions with any known neurotransmitter receptors (Johnson, 1992). Thus, in vivo and certainly at clinical doses, these compounds can be considered to have a single acute pharmacological action. However, in spite of the rapid onset of uptake blockade in humans (in a few hours at most in platelets), the earliest signs of therapeutic improvement in depressive symptoms appear only after about 2 weeks (for a general review of selective 5-HT reuptake inhibitors, see Feighner and Boyer, 1991). The latency of the therapeutic effects has been attributed to adaptive changes brought about by long term treatment (for review, see Briley and Moret, 1993). Electrophysiological studies suggest that chronic administration of selective 5-HT reuptake inhibitors enhances the effectiveness of 5-HT neurotransmission through the desensitization of somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (Chaput et al., 1988). Administration of citalopram (50 mg/kg p.o.) to rats for 21 days followed by a washout of 24 h resulted in an increased in vitro stimulation-induced release of 5-HT from hypothalamic slices preloaded with [^{3}H]5-HT (Moret and Briley, 1990). In addition, the concentration-effect curve of the autoreceptor agonist, lysergic acid diethylamide (LSD), was significantly shifted to the right compared with control animals, indicating a desensiti-

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zation of the autoreceptor for the agonist. We suggested that repeated administration of a selective 5-HT reuptake inhibitor resulted in a decreased efficacy of the terminal autoreceptor, allowing an increased release of 5-HT (Moret and Briley, 1990). Thus desensitization of these major feedback systems after repeated selective 5-HT reuptake inhibitor administration would have the effect of allowing the synaptic level of 5-HT to rise with a consequently increased stimulation of one or more types of postsynaptic 5-HT receptor. Thus, it is only after prolonged administration that there is a breakthrough of the weakened feedback control allowing the pharmacological activity of a selective 5-HT reuptake inhibitor to be fully expressed. This may explain the latency of therapeutic action seen with these drugs in humans.

The purpose of the present study was to examine this hypothesis, particularly the rise of synaptic 5-HT, by determining the effects of acute and chronic administration with citalopram on in vivo 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) output in rat hypothalamus using microdialysis under the same conditions as those used for the in vitro release study (50 mg/kg per day p.o.) (Moret and Briley, 1990). The sensitivity of the release-controlling 5-HT_{1B} terminal autoreceptors was tested by measuring the effects of locally applied autoreceptor antagonists, methiothepin and 1-(1-naphthyl)piperazine (NP), on extracellular levels of 5-HT and 5-HIAA.

2. Materials and methods

2.1. Animals and treatments

Male Sprague-Dawley rats (OFA, Iffa Credo, France), weighing 200–210 g at the beginning of the study (290–300 g at the end), were housed singly with a 12 h light cycle at 21°C with free access to food and water. The weight of animals in the control and treated groups evolved similarly throughout the study. Four groups of animals were used, control, acute (2 days), repeated (21 days) with a 24 h washout period and repeated (21 days) without washout. Citalopram hydrobromide was incorporated into the food in proportions giving a mean consumption of 50 mg/kg per day. For repeatedly treated rats, for the first 2 weeks, 22 g of reconstituted rat chow pellets were presented to each rat per day and this amount was increased to 23 g for the last week. Both the control and the drug-treated rats consumed the totality of the food provided. Controls were fed the same quantity of a similarly prepared diet to which no drug was added. The drug-containing diet was replaced by the control diet 24 h before the microdialysis experiments in the case of repeatedly treated rats with 24 h washout. For acute and repeatedly treated animals without washout, the citalopram-containing diet was present during the microdialysis experiments. At the end of the period of drug administration, there was no apparent difference in the physical state or overt behaviour between control and drug-administered animals.

2.2. Surgery and microdialysis procedure

Three days before the microdialysis experiments, rats were anaesthetized (0.5–3% halothane/O_2) and placed in a stereotaxic frame. A guide cannula (CMA/12, Carnegie Medecin) was implanted unilaterally such that the subsequent insertion of a microdialysis probe terminated in the right dorsomedial hypothalamic nucleus at the following coordinates (Paxinos and Watson, 1986): 3.14 mm posterior to bregma, 0.6 mm lateral from the midline suture and 8 mm ventral from the bone. The guide cannula was fixed to the skull with stainless steel screws and dental cement. The animals were allowed a recovery period of 3 days. 16 h before the experiment, each rat was placed in a bowl-shaped cage equipped with a pivoting liquid swivel which allowed the animal's unrestricted movements. A microdialysis probe (polycarbonate membrane 2 mm long with an outer diameter of 0.5 mm and a 20000 Da molecular weight cutoff, CMA/12, Carnegie Medecin) was slowly inserted into the guide cannula. Probes were then perfused with artificial cerebrospinal fluid (ACSF) (composition in mmol/l: NaCl 125; KCl 2.5; $MgCl_2$ 1.18 and $CaCl_2$ 1.26) at 0.32 μl/min using a CMA 100 microinjection pump (Carnegie Medecin). After 16 h perfusion, the perfusate samples were collected at 30 min intervals in tubes containing 10 μl perchloric acid (0.2 N with 0.05% EDTA) and were injected immediately into a reverse-phase HPLC system with electrochemical detection for the measurement of 5-HT and 5-HIAA. After four dialysate samples had been collected for baseline measurements, tetrodotoxin (1 μM), methiothepin (100 μM) or NP (10 μM) were added, at zero time, to the perfusion fluid until the end of experiment. The composition of the mobile phase was 0.15 M NaH_2PO_4, 0.02 mM octyl sodium sulphate, 0.5 M EDTA (pH 3.8 adjusted with phosphoric acid) and 6% methanol. The mobile phase was delivered by a model 510 pump (Waters) at a flow rate of 0.2 ml/min. Analyte separation was achieved using a reversed phase column (Merck 119 mm, 2 mm internal diameter, 4 μm LiChroCart 125-2, Superspher 100RP-18). Perfusate samples were injected by a model 717 refrigerated autosampler (Waters). Detection of 5-HT and 5-HIAA was carried out with a Decade electrochemical detector (Antec, Leyden) with a glassy carbon working electrode set at +0.65 V. Data were acquired and analysed by a Millennium 2010 Chromatography Manager (Waters). At the perfusion rate

used (0.32 μl/min), the in vitro percentage recovery of 5-HT by the probes was 26.6 ± 0.9 (mean ± S.E.M., $n = 14$). The limit of detection of 5-HT was 5 fmol/sample with a signal to noise ratio of 3. After the completion of the experiment, the animals were decapitated and the brains removed and fixed in a solution of 10% formalin. After at least 2 days, 30 μm thick sections were cut for visual inspection of the probe tract.

2.3. Statistics

The mean concentration of 5-HT or 5-HIAA in the first four baseline samples before the infusion of drugs or in the last four samples was expressed as an absolute value (±S.E.M.). In some experiments, the mean of the first four basal values was taken as 100% and the subsequent samples were expressed as the percentage of the baseline value. To compare the effect of a treatment on the baseline value of 5-HT or 5-HIAA, Student's *t*-test or Wilcoxon test was used. Statistical analysis of the data on drug effects was by Student's *t*-test or Wilcoxon test after the area under the curve (AUC) was calculated or by one-way analysis of variance (ANOVA) followed by a post-hoc Dunnett's test or by two-way ANOVA of repeated measurements and with Tukey's test where appropriate.

2.4. Drugs

Citalopram hydrobromide was kindly provided by Lundbeck (Copenhagen, Denmark). Methiothepin mesylate was obtained from Research Biochemicals International and tetrodotoxin from Sigma. 1-(1-Naphthyl)piperazine (NP) hydrochloride was synthesized by the Medicinal Chemistry Division IV of Centre de Recherche Pierre Fabre.

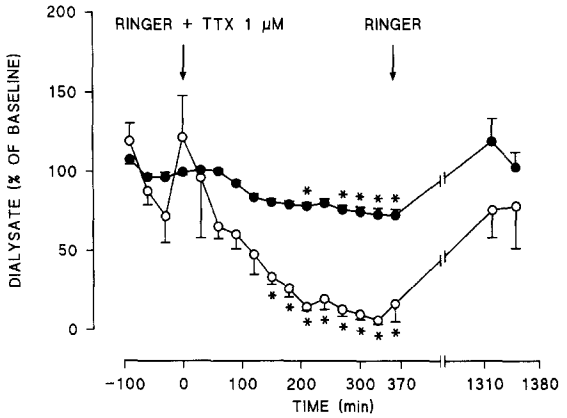

Fig. 1. Time course of microdialysis experiments studying the effect of tetrodotoxin (1 μM) on extracellular levels of 5-HT (○) or 5-HIAA (●), expressed as percentage of basal values, in the hypothalamus of the freely moving rats. Tetrodotoxin, dissolved in Ringer, was perfused from zero time (first arrow) until 360 min, and replaced by Ringer only (second arrow) until the end of experiment. Two last samples were collected 16 h after the change to Ringer only. Each point represents mean values ± S.E.M. from four rats. $^{*}P < 0.05$ (ANOVA followed by Dunnett's test).

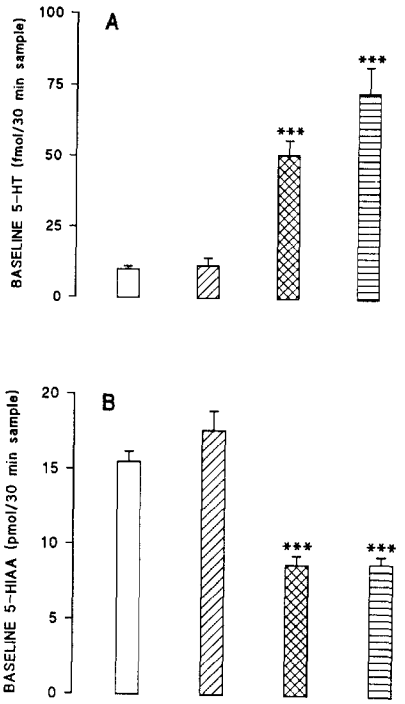

Fig. 2. Effect of citalopram administration on basal (mean of the first four samples) extracellular levels of 5-HT (A) or 5-HIAA (B) expressed as fmol/30 min sample or pmol/30 min sample, respectively, in the hypothalamus of the freely moving rats. Each column represents mean values ± S.E.M. Open, striped, cross-hatched and horizontal filled columns represent control animals ($n = 28$), animals repeatedly treated with 24 h washout ($n = 11$), acute animals ($n = 10$) and animals repeatedly treated without washout ($n = 11$), respectively. $^{***}P < 0.001$ when compared to respective controls or repeatedly treated rats with 24 h washout (Student's *t*-test or Wilcoxon test).

3. Results

Infusion of tetrodotoxin (1 μM) reduced the basal extracellular outflow of 5-HT to negligible levels in comparison to predrug baseline values. A subsequent perfusion with Ringer solution only, from 360 min until the end of experiment, restored the normal basal levels when measured 16 h later (Fig. 1). A decrease of 5-HIAA levels was also observed and, although the extent of this effect was less than for 5-HT (Fig. 1), the effect was also reversible. The baseline levels of 5-HT and 5-HIAA in the animals which received the drug-free diet (whether they were controls for the acute drug treatment, the repeated drug treatment with 24 h washout, or the repeated treatment without washout), did not differ significantly. These basal values were,

therefore, pooled and the global value used for comparisons with each of the different groups of treated animals.

Baseline 5-HT levels in repeatedly treated rats with 24 h washout were not different from the controls, however those of acute and repeatedly treated animals without washout (i.e. with the drug still present) were increased in comparison with controls and with those treated repeatedly with 24 h washout (Fig. 2A). In repeatedly treated rats without washout the increase was 43% greater than in acutely treated animals, however this effect did not reach statistical significance ($P = 0.0844$) (Fig. 2A). There was no change in the baseline levels of 5-HIAA between controls and rats treated repeatedly with 24 h washout. The levels of 5-HIAA in acute and chronic without washout groups were reduced compared to controls, but there was no difference between acute and chronic groups (Fig. 2B).

The effect of methiothepin or NP, added after the first four basal samples during the microdialysis experiment, on the extracellular levels of 5-HT are represented in Figs. 3–5. After 24 h washout, in animals treated repeatedly with citalopram, methiothepin, added to the perfusion medium at 100 μM from zero time until the end of the experiment, increased the extracellular levels of 5-HT as in control rats (Fig. 3A). Although the methiothepin-induced 5-HT outflow tended to be slightly lower in the chronic citalopram group, the areas under the curve were not significantly different between the two groups. Similar findings were obtained with 10 μM NP (Fig. 3B). Methiothepin had no effect on the outflow of 5-HIAA in control or repeatedly treated rats, and NP decreased this measure slightly but significantly from the basal value in both groups (data not shown). The decrease was similar in both groups.

In acutely and repeatedly treated rats without washout (Fig. 4A and Fig. 5A, respectively), the enhancing effect of methiothepin (100 μM) was similar in treated and control animals. The areas under the curve,

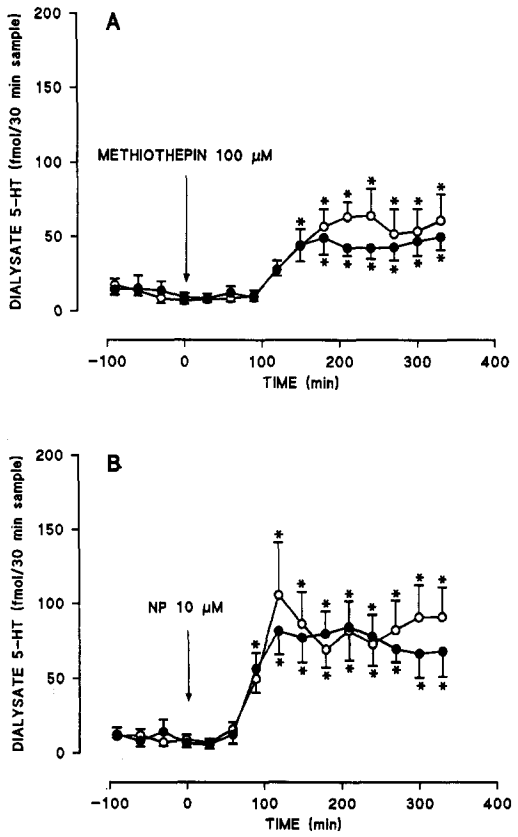

Fig. 3. Time course of microdialysis experiments studying the effect of methiothepin (100 μM) (A) or NP (10 μM) (B) on extracellular levels of 5-HT, expressed as fmol/30 min sample, in the hypothalamus of freely moving rats. Methiothepin or NP, dissolved in Ringer, was perfused from zero time (arrow) until the end of the experiment. ○ Controls, ● repeatedly (21 days) treated animals with 24 h washout. Each point represents mean values ± S.E.M. of five to six rats. $P < 0.05$ in comparison to respective basal values (ANOVA followed by Dunnett's test).

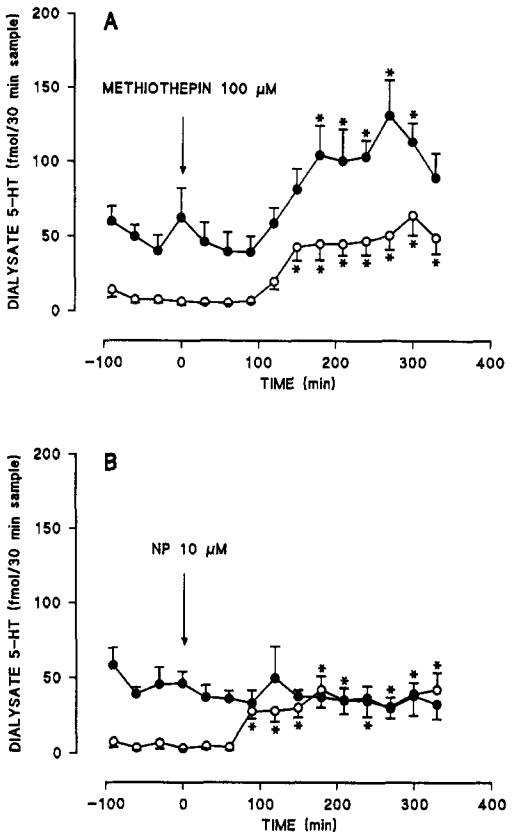

Fig. 4. Time course of microdialysis experiments studying the effect of methiothepin (100 μM) (A) or NP (10 μM) (B) on extracellular levels of 5-HT, expressed as fmol/30 min sample, in the hypothalamus of freely moving rats. Methiothepin or NP, dissolved in Ringer, was perfused from zero time (arrow) until the end of the experiment. ○ Controls, ● acutely (2 days) treated animals without washout. Each point represents mean values ± S.E.M. of four to six rats. $P < 0.05$ in comparison to respective basal values (ANOVA followed by Dunnett's test).

representing the effect of methiothepin, were not significantly different between the two groups of control and treated rats. Since the basal level of extracellular 5-HT was increased by citalopram, however, the maximal levels achieved were significantly higher in the citalopram-treated animals (Fig. 6). In contrast, NP (10 μM) increased the extracellular levels of 5-HT in control animals but was devoid of effect in the treated groups (Fig. 4B and Fig. 5B). 5-HIAA levels remained unchanged in the presence of methiothepin or NP in control and acutely treated rats but were decreased slightly but significantly with both compounds in chronically treated animals, and NP also decreased these levels of 5-HIAA in corresponding controls (data not shown).

Fig. 6 presents, in absolute values, the extracellular levels of 5-HT after administration through the probe of methiothepin or NP, as measured in the last four samples which were chosen as a best estimation of the maximal effect. In acutely and repeatedly treated

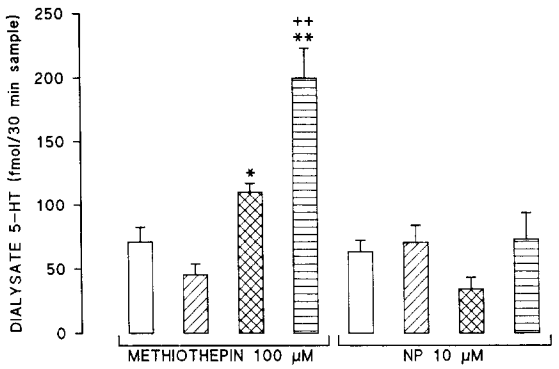

Fig. 6. Effect of methiothepin (100 μM) or NP (10 μM) on extracellular levels of 5-HT measured in the last four samples and expressed as fmol/30 min sample in the hypothalamus of the freely moving rats. Each column represents mean values ± S.E.M. of four to six animals. Open, striped, cross-hatched and horizontal filled columns represent control animals, animals repeatedly treated with 24 h washout, acute animals and animals repeatedly treated without washout, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$ when compared to the respective controls (Student's t-test or Wilcoxon test). $^{++}P < 0.01$ when compared to acutely treated animals (Wilcoxon test).

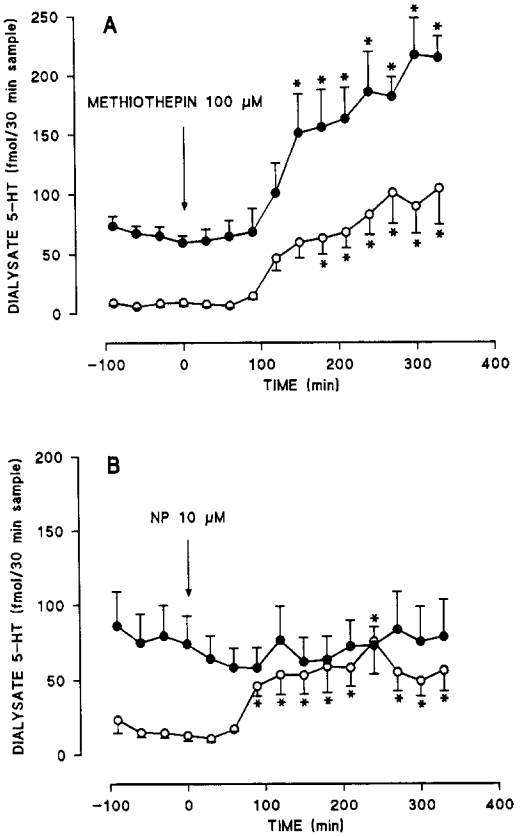

Fig. 5. Time course of microdialysis experiments studying the effect of methiothepin (100 μM) (A) or NP (10 μM) (B) on extracellular levels of 5-HT, expressed as fmol/30 min sample, in the hypothalamus of freely moving rats. Methiothepin or NP, dissolved in Ringer, was perfused from zero time (arrow) until the end of the experiment. ○ Controls, ● repeatedly (21 days) treated animals without washout. Each point represents mean values ± S.E.M. of four to six rats. $P < 0.05$ in comparison to respective basal values (ANOVA followed by Dunnett's test).

(without washout) rats, methiothepin increased the amount of 5-HT in comparison with controls or repeatedly treated rats with 24 h washout (Fig. 6). In addition the effect of methiothepin was significantly greater in repeatedly treated rats in comparison to acutely treated animals (Fig. 6). An analysis of variance of repeated measures showed a statistically significant difference ($P < 0.05$) for the last four samples in the effect of methiothepin between acutely and repeatedly treated animals (curves in Fig. 4A and Fig. 5A). In the case of NP, the output of 5-HT was similar in the four groups of animals (Fig. 6). The extracellular levels of 5-HIAA were decreased by methiothepin or NP in acutely and repeatedly treated animals without washout, and were increased by NP in the group of repeatedly treated rats with 24 h washout (data not shown).

4. Discussion

The purpose of the present study was to replicate previous in vitro findings using an in vivo method. We have already shown that chronic treatment with the selective 5-HT reuptake inhibitor, citalopram, at 50 mg/kg for 21 days with 24 h washout induces a down-regulation of the serotonergic terminal autoreceptor, allowing an increase of 5-HT neurotransmission (Moret and Briley, 1990). This conclusion was based on the fact that this repeated administration with citalopram in vivo enhanced the amount of [^{3}H]5-HT released by electrical stimulation and reduced the inhibitory effect of the agonist, LSD, in hypothalamic slices in vitro.

The present study used the same dose of citalopram (50 mg/kg), the same route of administration (in the diet, with the same preparation) and the same brain region (the dorsomedial hypothalamic nucleus which is included in the hypothalamic slice preparation used in the in vitro release experiments). Rats were freely moving from the beginning of the repeated administration with citalopram until the end of the measurement of extracellular levels of 5-HT and its metabolite, 5-HIAA. The levels of 5-HT measured by microdialysis are very low and close to the limit of detection. For this reason, the use of an autoreceptor agonist to determine the sensitivity of the terminal autoreceptor (i.e. by demonstrating a decrease in the extracellular levels of 5-HT) was not possible. This situation could have been overcome by the addition of a selective 5-HT reuptake inhibitor to the perfusion medium to boost basal outflow measures as is often reported (see for example Bosker et al., 1995). In view of our interest in studying the effect of a systemic treatment with a selective 5-HT reuptake inhibitor, infusion of a selective 5-HT reuptake inhibitor locally would have led to uninterpretable results. For these reasons we chose to use 5-HT autoreceptor antagonists, methiothepin and NP to test autoreceptor sensitivity.

In the hypothalamus of freely moving rats, the dialysate content of 5-HT was reduced below the level of detection following the blockade of voltage-dependent Na^+ channels by tetrodotoxin, indicating that under our experimental conditions extracellular 5-HT is of neural origin and results from neurotransmitter release. Similar results were obtained by Matos et al. (1990) in the lateral hypothalamus of freely moving rats, and by Lawrence and Marsden (1992) and Moret and Briley (1995) in conscious guinea-pig frontal cortex and substantia nigra pars reticulata, respectively. Interestingly, the levels of 5-HIAA were far less sensitive to the presence of tetrodotoxin suggesting that much of the extracellular 5-HIAA is not due to neurotransmitter release but probably to non-specific leakage.

The lack of change in the basal extracellular levels of 5-HT following a repeated treatment with citalopram with 24 h washout in comparison to control animals suggests that citalopram does not down-regulate the receptor responsible for the regulation of 5-HT release. This finding is in contrast to the results we obtained previously in vitro (Moret and Briley, 1990).

However, the lack of effect of chronic administration with citalopram with 24 h washout on the basal levels of 5-HT is consistent with the results of Sleight et al. (1989) after chronic amitriptyline (10 mg/kg p.o. for 21 days, with 24 h washout), Hjorth and Auerbach (1994) after citalopram (5 mg/kg s.c., b.i.d. for 2 weeks, with 24 h washout), Invernizzi et al. (1994) after citalopram (10 mg/kg i.p. twice daily for 14 days with 24 h washout) and Bosker et al. (1995) after chronic fluvoxamine (6.7 mg/kg for 21 days, s.c. osmotic minipumps with 3 days washout), in the rat frontal cortex or dorsal hippocampus. Extracellular levels of 5-HIAA which were not modified in the treated animals under these conditions are in concordance with the absence of effect on 5-HT levels, since they change in parallel.

In order to keep the same route of administration as for the repeated treatment with 24 h washout, the acute administration of citalopram was carried out with a drug-containing diet given for 2 days. Under these conditions the extracellular levels of 5-HT were increased 5-fold, which was similar to the 7-fold increase obtained after a single subcutaneous injection of citalopram (20 mg/kg) in naive rats (Moret, unpublished data). Most studies have found, after an acute administration with selective 5-HT reuptake inhibitors, an increase (greater or lesser depending on regions examined and the dose of selective 5-HT reuptake inhibitor utilized) of extracellular levels of 5-HT in terminal regions with the cortex being the most resistant to increase. This has been discussed extensively by Fuller (1993). The decrease of 5-HIAA is explained by the blockade of the 5-HT reuptake mechanism preventing the intracellular metabolism of released and retaken up 5-HT.

A similar pattern in the levels of 5-HT and 5-HIAA was observed after chronic administration of citalopram without washout, with a tendency for extracellular levels of 5-HT to be higher in comparison to acute treatment conditions. The 7-fold increase of 5-HT in the present study is comparable to the 6-fold increase obtained by Bel and Artigas (1993) in the frontal cortex after a chronic treatment with fluvoxamine for 2 weeks (1 mg/kg per day s.c. osmotic minipumps in place during microdialysis experiments).

Methiothepin is a potent, non-selective serotonergic receptor antagonist. In rat and guinea-pig hypothalamic slices it antagonizes the inhibitory effect of an autoreceptor agonist on the electrically evoked release of [^{3}H]5-HT. In contrast to other antagonists it is also able to enhance release when applied by itself, which has led to the suggestion that it may possess inverse agonist properties at the terminal 5-HT autoreceptor (Moret and Briley, 1993). Negative efficacy or inverse agonism of methiothepin has also been shown at the $5\text{-HT}_{1D\alpha}$ receptor subtype (Thomas et al., 1995). In in vitro release experiments on guinea-pig substantia nigra or hypothalamic slices, NP behaves as a terminal serotonergic autoreceptor antagonist without any action at the 5-HT uptake site (Moret and Briley, 1995). In microdialysis experiments performed on substantia nigra of freely moving guinea-pigs, methiothepin (Moret and Briley, 1993) and NP (Moret and Briley, 1995), when applied locally via the microdialysis probe, increased the extracellular levels of 5-HT. This effect,

which was tetrodotoxin-sensitive, suggests that 5-HT autoreceptors are tonically activated and exert a potent inhibitory tone on the release of 5-HT and that the enhancing effect operates through a neuronal mechanism. Similar findings have been obtained in the guinea-pig hypothalamus (Moret, unpublished results). In the present study similar results were found in the rat. Fig. 3 shows the effects of methiothepin (100 μM) and NP (10 μM) added through the microdialysis probe in rats treated repeatedly with 24 h washout. The lack of difference between control and chronically treated animals concords with the results of Sleight et al. (1989) after 21 days of treatment with amitriptyline (10 mg/kg) with 24 h washout. The ability of the 5-HT autoreceptor agonist, 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole succinate, RU 24969 (10 mg/kg i.p.) to reduce extracellular levels of 5-HT was not modified by chronic amitriptyline. Similarly, Bosker et al. (1995) have shown that RU 24969 administered at a concentration of 100 nM through the microdialysis probe decreased the extracellular levels of 5-HT to the same extent in saline- and fluvoxamine-treated rats (6.7 mg/kg for 21 days with 3 days washout). Therefore chronic treatment with citalopram followed by 24 h washout, when no drug is left in the rat brain (Hyttel et al., 1984), did not cause any down-regulation of the terminal 5-HT autoreceptor with the consequence of no change in the basal extracellular levels of 5-HT. This is in contrast to a study by Chaput et al. (1986) who found, using in vivo electrophysiological methods, a desensitization of the terminal 5-HT autoreceptor after a chronic administration with citalopram (20 mg/kg per day for 14 days with 10–12 h washout). It is possible that in this case the duration of this washout was not enough to exclude completely the presence of the drug at the time of the experiment. In the present study a rapid restoration of receptor sensitivity cannot be fully excluded as suggested by Bosker et al. (1995). In Bosker's study washout was for 3 days, whereas in the present study a 24 h washout was used. If adaptive changes occurred, it is unlikely that these effects would be lost after only 24 h washout.

In order to understand the difference between our in vitro results (Moret and Briley, 1990) and the present in vivo findings, it is important to bear in mind the various factors implicated in in vivo 'release' as measured by microdialysis. Indeed the present experiments measure extracellular levels of 5-HT and not synaptic release as in in vitro experiments. Another difference is that microdialysis studies measure endogenous 5-HT whereas the release of exogenous preloaded 5-HT is measured in slice experiments. The extracellular levels of 5-HT are the result of a number of factors. The release of 5-HT is dependent on the terminal autoreceptors and the firing rate of the raphe nucleus neurones which is under the control of 5-HT_{1A} receptors (Chaput et al., 1986; Sprouse and Aghajanian, 1987; Hjorth and Magnusson, 1988; Jolas et al., 1993). The absence of this factor in the slice preparation may be another important difference between these studies. The synthesis of 5-HT is dependent on the rate-limiting enzyme, tryptophan hydroxylase, which is influenced by neuronal firing and possibly by terminal autoreceptors (Hamon et al., 1988; Hjorth et al., 1995). The removal of 5-HT depends on the activity of the specific 5-HT uptake carrier and, to a lesser extent, on extracellular catabolic enzymes such as monoamine oxidase. Thus it may not be possible to distinguish a change in the sensitivity of the terminal autoreceptor by studying the levels of 5-HT if there are concomitent changes in other parts of the system.

Methiothepin enhances release after both acute and repeated citalopram treatment without washout. The maximal effect of methiothepin, estimated by the mean of the last four sample values, is increased in acute and repeatedly treated animals without washout in comparison with control or repeatedly treated animals with 24 h washout. This result is to be expected if the effect of methiothepin is to inhibit a negative feedback mechanism. The greater the amount of extracellular 5-HT (caused here by the presence of citalopram acting at the 5-HT uptake site), the greater the effect of methiothepin. A similar effect has been shown in vitro with methiothepin and citalopram in rat hypothalamic slices (Galzin et al., 1985).

More interestingly, after 21 days of citalopram treatment without washout, methiothepin produces a greater effect than in acutely treated rats. This could be due to an accumulation of citalopram and thus a greater inhibition of the uptake of 5-HT. Alternatively it could suggest that changes in other parts of the system, such as a desensitisation of the 5-HT_{1A} somatodendritic receptor governing the firing of the raphe neurones, result in a greater potential release of 5-HT which is held in check by the terminal autoreceptor.

The effect of methiothepin given alone reaches a level similar to that induced by citalopram (Fig. 4A and Fig. 5A). Thus a 5-HT autoreceptor antagonist acting specifically at the terminal would increase immediately the synaptic levels of 5-HT, and combination with a 5-HT uptake inhibitor would be expected to reinforce this effect.

In contrast to methiothepin, NP loses its effect in acute and repeatedly treated animals without washout, in other words when the uptake blocker is still present. It could be argued that the enhancing effect of NP is a fenfluramine-like action and the presence of a 5-HT uptake inhibitor would suppress it, as has previously been shown with milnacipran or imipramine with the serotonergic displacer, 4-methyl-α-ethyl-*m*-tyramine (H75/12) (Moret et al., 1985). We have shown however that the enhancing effect of NP, also found in guinea-

pig brain, is tetrodotoxin-sensitive, like methiothepin (Moret and Briley, 1995), whereas fenfluramine or amphetamine have been shown to release monoamines by tetrodotoxin-insensitive processes (Carboni and Di Chiara, 1989; Matos et al., 1990). The effect of NP could also be explained by a blockade of the uptake of 5-HT which could be prevented by the previous action of citalopram. However in slices of guinea-pig brain we have shown that at 10 μM NP does not inhibit 5-HT uptake (Moret and Briley, 1995), as already mentioned above. NP has been shown to be a partial (ant)agonist in vitro (Moret and Briley, 1995), however the presence of high concentrations of the terminal autoreceptor agonist, 5-HT, should accentuate the antagonist properties of the compound. Thus at present, it is difficult to find an explanation for the loss of effect of NP in the presence of citalopram.

Thus, in summary, the present study shows no evidence for the desensitisation of the terminal 5-HT autoreceptor at the level of the hypothalamus after 21 days administration of citalopram with 24 h washout. The effect of the non-selective antagonist, methiothepin, is to increase extracellular levels of 5-HT over and above those in the presence of citalopram. This effect is significantly greater after 21 days of treatment with citalopram as compared to acute administration. This is interpreted to indicate that the effect of autoreceptor blockade is not lost after citalopram treatment but is indeed enhanced. Thus the administration of a reuptake inhibitor or an autoreceptor antagonist are both capable of increasing extracellular 5-HT levels. Furthermore the two effects are additive or possibly synergistic. This suggests that this combination of a reuptake inhibitor and an autoreceptor antagonist may be potentially useful in the treatment of depressive disorders resistant to therapy by a single drug.

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References

Bel, N. and F. Artigas, 1993, Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei, Synapse 15, 243.

Bosker, F.J., K.E. Van Esseveldt, A.A. Klompmakers and H.G.M. Westenberg, 1995, Chronic treatment with fluvoxamine by osmotic minipumps fails to induce persistent functional changes in central 5-HT_{1A} and 5-HT_{1B} receptors, as measured by in vivo microdialysis in dorsal hippocampus of conscious rats, Psychopharmacology 117, 358.

Briley, M. and C. Moret, 1993, Neurobiological mechanisms involved in antidepressant therapies, Clin. Neuropharmacol. 16, 387.

Carboni, E. and G. Di Chiara, 1989, Serotonin release estimated by transcortical dialysis in freely-moving rats, Neuroscience 32, 637.

Chaput, Y., C. De Montigny and P. Blier, 1986, Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain, Naunyn-Schmied. Arch. Pharmacol. 333, 342.

Chaput, Y., P. Blier and C. De Montigny, 1988, Acute and long-term effects of antidepressant serotonin (5-HT) reuptake blockers on the efficacy of 5-HT neurotransmission: electrophysiological studies in the rat central nervous system, Adv. Biol. Psychiat. 17, 1.

Feighner, J.P. and W.F. Boyer, 1991, Selective Serotonin Reuptake Inhibitors (Wiley, Chichester).

Fuller, R.W., 1993, Do reuptake inhibitors increase extracellular 5-HT in all terminal regions? Microdialysis studies with 5-HT reuptake inhibitors, Trends Pharmacol. Sci 14, 397.

Galzin, A.-M., C. Moret, B. Verzier and S.Z. Langer, 1985, Interaction between tricyclic and nontricyclic 5-hydroxytryptamine uptake inhibitors and the presynaptic 5-hydroxytryptamine inhibitory autoreceptors in the rat hypothalamus, J. Pharmacol. Exp. Ther. 235, 200.

Hamon, M., C.-M. Fattaccini, J. Adrien, M.-C. Gallissot, P. Martin and H. Gozlan, 1988, Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine$_{1A}$ agonists with potential anxiolytic properties, J. Pharmacol. Exp. Ther. 246, 745.

Hjorth, S. and S.B. Auerbach, 1994, Lack of 5-HT_{1A} autoreceptor desensitization following chronic citalopram treatment, as determined by in vivo microdialysis, Neuropharmacology 33, 331.

Hjorth, S. and T. Magnusson, 1988, The 5-HT_{1A} agonist 8-OH-DPAT preferentially activates cell body 5-HT autoreceptors in rat brain in vivo, Naunyn-Schmied. Arch. Pharmacol. 338, 463.

Hjorth, S., C.S. Suchowski and M.P. Galloway, 1995, Evidence for 5-HT autoreceptor-mediated, nerve impulse-independent, control of 5-HT synthesis in the rat brain, Synapse 19, 170.

Hyttel, J., K. Fredricson Overo and J. Arnt, 1984, Biochemical effects and drug levels in rats after long-term treatment with the specific 5-HT uptake inhibitor, citalopram, Psychopharmacology 83, 20.

Invernizzi, R., M. Bramante and R. Samanin, 1994, Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: role of presynaptic 5-HT_{1A} receptors, Eur. J. Pharmacol. 260, 243.

Johnson, A.M., 1992, Paroxetine – a pharmacological review, Int. Clin. Psychopharmacol. 6, 15.

Jolas, T., S. Haj-Dahmane, L. Lanfumey, C.-M. Fattaccini, E.J. Kidd, J. Adrien, H. Gozlan, B. Guardiola-Lemaitre and M. Hamon, 1993, (−)Tertatolol is a potent antagonist at pre- and postsynaptic serotonin 5-HT_{1A} receptors in the rat brain, Naunyn-Schmied. Arch. Pharmacol. 347, 453.

Lawrence, A.J. and C.A. Marsden, 1992, Terminal autoreceptor control of 5-hydroxytryptamine release as measured by in vivo microdialysis in the conscious guinea-pig, J. Neurochem. 58, 142.

Matos, F.F., H. Rollema and A.I. Basbaum, 1990, Characterization of monoamine release in the lateral hypothalamus of awake, freely moving rats using in vivo microdialysis, Brain Res. 528, 39.

Moret, C. and M. Briley, 1990, Serotonin autoreceptor subsensitivity and antidepressant activity, Eur. J. Pharmacol. 180, 351.

Moret, C. and M. Briley, 1993, The unique effect of methiothepin on the terminal serotonin autoreceptor in the rat hypothalamus could be an example of inverse agonism, J. Psychopharmacol. 7, 331.

Moret, C. and M. Briley, 1995, In vitro and in vivo activity of 1-(1-naphthyl)piperazine at terminal 5-HT autoreceptors in guinea-pig brain, Naunyn-Schmied. Arch. Pharmacol. 351, 377.

Moret, C., M. Charvéron, J.P.M. Finberg, J.P. Couzinier and M. Briley, 1985, Biochemical profile of midalcipran (F 2207), 1-phenyl-1-diethyl-aminocarbonyl-2-aminomethyl-cyclopropane (Z)

hydrochloride, a potential fourth generation antidepressant drug, Neuropharmacology 24, 1211.

Paxinos, G. and G. Watson, 1986, The Rat Brain in Stereotaxic Coordinates (Academic Press, Sydney).

Sleight, A.J., R.J. Smith, C.A. Marsden and M.G. Palfreyman, 1989, The effects of chronic treatment with amitriptyline and MDL 72394 on the control of 5-HT release in vivo, Neuropharmacology 28, 477.

Sprouse, J.S. and G.K. Aghajanian, 1987, Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists, Synapse 1, 3.

Thomas, D.R., S.A. Faruq and A.M. Brown, 1995, Characterisation of [^{35}S]GTPγS binding to CHO cell membranes expressing human 5-$HT_{1D\alpha}$ receptors: evidence for negative efficacy of 5-HT receptor antagonists, Br. J. Pharmacol. 114, 153P.