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Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram

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Abstract

Rats were chronically treated with the selective serotonin re-uptake inhibitor citalopram [1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phtalancarbonitril], by means of osmotic minipumps. Using an infusion concentration of 50 mg/ml citalopram, steady-state plasma concentrations of approximately 0.3 μM citalopram were maintained for 15 days. Citalopram plasma levels dropped below pharmacologically active concentrations 48 h after removal of the minipumps. Although chronic treatment with citalopram did induce an attenuated response by extracellular levels of 5-hydroxytryptamine (5-HT) after systemic administration of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), no effect of chronic citalopram treatment was observed when 5-HT_{1B} receptor function was evaluated with a local infusion of 5-$HT_{1B/D}$ receptor agonist, sumatriptan (3-[2-dimethylamino]ethyl-*N*-methyl-1*H*-indole-5methane sulphonamide). Controversially, no augmentation of the increase of 5-HT levels was observed upon systemic administration of citalopram. It is concluded that, although chronic treatment with citalopram does induce desensitisation of 5-HT_{1A} receptors, the absence of augmented effects of citalopram on 5-HT levels indicates that other mechanisms compensate for the loss of autoreceptor control.

Keywords: Chronic treatment; 5-HT (5-hydroxytryptamine, serotonin); (Rat); Microdialysis

1. Introduction

Selective serotonin re-uptake inhibitors increase brain extracellular 5-hydroxytryptamine (5-HT) levels immediately upon administration (Fuller, 1994). The concurrent antidepressant effect in patients is, however, delayed for several weeks (Baumann, 1992).

Changes in brain pharmacology during chronic treatment of animals with selective serotonin re-uptake inhibitors has been a topic of research for many years. Most of the studies investigating adaptation of the serotonergic system upon chronic treatment of animals with selective serotonin re-uptake inhibitors have focused on the evaluation of desensitisation of 5-HT autoreceptors.

Somatodendritic 5-HT_{1A} autoreceptors decrease neuronal firing upon activation (Arborelius et al., 1994). Several authors have shown that selective serotonin re-uptake inhibitors activate these receptors indirectly by increasing extracellular levels of 5-HT in the raphe nuclei. The extent of this activation, however, is related to the dose of selective serotonin re-uptake inhibitor used (Hjorth et al., 1997; Cremers et al., 2000). Although some authors reported that the decrease of extracellular 5-HT after systemic administration of the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), wasattenuated upon chronic treatment of animals with selective serotonin re-uptake inhibitors (Invernizzi et al., 1994; Kreiss and Lucki, 1995; Le Poul et al., 1995), others have failed to observe similar effects (Hjorth and Auerbach, 1994; Bosker et al., 1995a,b).

Like the discrepancies regarding possible desensitisation of 5-HT_{1A} autoreceptors, desensitisation of 5-HT_{1B} receptors located on the serotonergic nerve terminals, is

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also somewhat controversial. Although an initial electrophysiological study provided evidence for desensitisation of 5-HT_{1B} receptors upon chronic treatment with selective serotonin re-uptake inhibitors (Chaput et al., 1986), various studies using microdialysis could not confirm this conclusion (Chaput et al., 1986; Auerbach and Hjorth, 1995; Bosker et al., 1995a,b; Moret and Briley, 1996; Davidson and Stamford, 1997).

There might be several reasons for these controversial results. First, species and animal strains may differ in their susceptibility to desensitisation (Schoups and De Potter, 1988). Secondly, as elimination of substances in animals, especially rodents, is much faster than in humans, chronic treatment regimens are characterised by high and multiple dosing of the drugs. Although a relation between the antidepressant effect and plasma levels of the selective serotonin re-uptake inhibitors is not well established, clinically effective plasma levels have been reported (Baumann, 1992). In this respect, we have recently demonstrated that selective serotonin re-uptake inhibitor-induced 5-HT_{1A} activation is critically dependent on the plasma levels of the selective serotonin re-uptake inhibitor (Cremers et al., 2000). However, in animal studies on chronic treatment with selective serotonin re-uptake inhibitors, little attention was given to the role of plasma levels. Therefore, the controversial data regarding desensitisation of autoreceptors might be explained by inadequacy of plasma levels of the selective serotonin re-uptake inhibitor during the chronic treatment.

In the present microdialysis study, desensitisation of autoreceptors was studied after chronic treatment of rats with citalopram [1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phtalancarbonitril]. To achieve stable plasma levels, citalopram was administered by means of osmotic minipumps. Plasma concentrations of citalopram and its less potent des- and didesmethyl metabolites were followed during the 15-day period. After a wash-out period of 2 days was determined, possible changes in 5-HT receptor sensitivity were challenged by recording the effects of administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, the 5-$HT_{1B/D}$ receptor agonist, sumatriptan (3-[2-dimethylamino]ethyl-*N*-methyl-1*H*-indole-5methane sulphonamide), or citalopram itself, on extracellular 5-HT in the ventral hippocampus.

2. Materials and methods

2.1. Animals and drug administration

Male albino rats of a Wistar-derived strain (285–320 g; Harlan, Zeist, The Netherlands) were used for the experiments. The rats were housed in plastic cages (35 × 35 × 40 cm), and had free access to food and water. The experiments conformed with the Declaration of Helsinki and were approved by the animal care committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

The following drugs were used: citalopram hydrobromide [1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phtalancarbonitril], des-, didesmethyl metabolites and internal standard [1-(3-dimethylaminopropyl)-1-(4-bromophenyl)-5-phtalancarbonitril] (kindly donated by Lundbeck (Denmark), courtesy of Dr. Sanchez), (±)-8-OH-DPAT (RBI, Natick, USA), sumatriptan (3-[2-dimethylamino]ethyl-*N*-methyl-1*H*-indole-5methane sulphonamide) (Glaxo Wellcome, UK).

2.2. Surgery

Osmotic minipumps (2ML2 Alzet, USA, 5 μl/h, 2 weeks) were filled with 50 mg/ml citalopram hydrobromide under aseptic conditions. During brief isoflurane anaesthesia (2.5%, 400 ml/min N_2O, 600 ml/min O_2), minipumps were implanted subcutaneously on the left side of the back of the rat. In a subgroup, four rats were provided with an additional jugular vein cannula in order to determine plasma citalopram levels during and after 15 days of treatment.

After 15 days, the rats were anaesthetised with chloral hydrate (400 mg/kg), and the osmotic minipumps were removed. The remaining subcutaneous cavity was flushed twice with 5 ml of sterile saline. Home-made I-shaped microdialysis probes, made of polyacrylonitrile/sodium methyl sulfonate copolymer dialysis fiber (i.d. 220 μm, o.d. 0.31 μm, AN 69, Hospal, Italy) were implanted bilaterally in the ventral hippocampus. The exposed length of the membranes was 4 mm lidocaine–HCl, 10% (m/v) was used for local anaesthesia. The rats were placed in a stereotaxic frame (Kopf, USA), and probes were inserted into the ventral hippocampus (coordinates: IA: +3.7 mm; lateral: +4.8 mm; ventral: −8.0 mm from the dura mater, Paxinos and Watson, 1982) and secured with dental cement.

2.3. Pharmacokinetic and microdialysis experiments

Blood samples (0.35 ml) were drawn on days 3, 7, 12, 15, 16, and 17 after implantation of the osmotic minipumps. Samples were transferred to 1.5-ml Eppendorf vials, containing 5 μl heparin (500 IE/ml saline), mixed and immediately centrifuged for 15 min at 3000 rpm at 4°C (Chillspin, MSE, England). Plasma samples were stored at −80°C until analysis.

The rats were allowed to recover for at least 48 h after insertion of the microdialysis probes. The probes were perfused with Ringer solution containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$ at a flowrate of 1.5 μl/min (Harvard apparatus, South Natick, MA, USA). Samples were collected on-line in a 20-μl loop and injected automatically onto the column every 15 min.

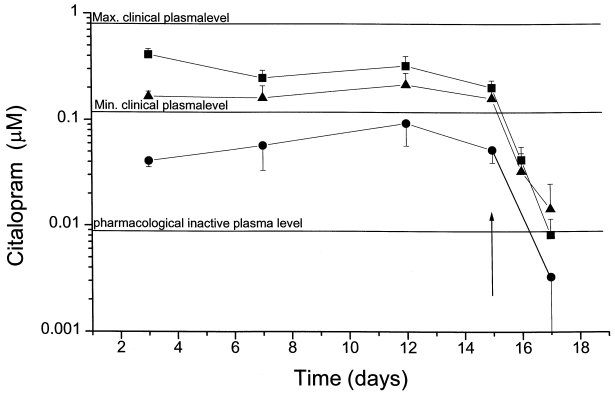

Fig. 1. Plasma levels of citalopram (■), desmethylmetabolite (●), and didesmethyl metabolite (▲) ($n = 4$) during subcutaneous infusion of 0.25 mg/h citalopram by means of osmotic minipumps. The arrow denotes removal of the minipumps.

2.4. Analysis

2.4.1. Serotonin

5-HT was analysed using high-pressure liquid chromatography (HPLC) with electrochemical detection. The HPLC pump (Shimadzu LC-10 AD liquid chromatograph) was connected to a reversed phase column (Phenomenex hypersil 3: C18, 3 μm, 100 × 2.0 mm, C18, Bester, Amstelveen, The Netherlands) followed by an electrochemical detector (Antec Leyden, Leiden, The Netherlands), working at a potential setting of 500 mV vs. Ag/AgCl reference. The mobile phase consisted of 5 g/l di-ammoniumsulfate, 500 mg/l ethylene diamino tetra acetic acid (EDTA), 50 mg/l heptane sulphonic acid, 30 μl/l of triethylamine, and 4.5% v/v methanol, at a pH of 4.65. The flow-rate of the mobile phase was 0.4 ml/min. The detection limit was 0.2 fmol 5-HT per 20-μl sample (signal-to-noise ratio = 2).

2.4.2. Citalopram

Citalopram was measured according to Oyehaug et al. (1982), with minor modifications. Briefly, 75 μl of the internal standard LU 10-202 (2 μM) and 30 μl of 1 N NaOH were added to 150-μl plasma samples. Samples were extracted twice by mechanically shaking for 3 min with 3 ml of diethyl ether. The ether layers were then transferred to 10-ml evaporating tubes, and 150 μl of 0.1 N HCl was added. The ether was evaporated in a water-bath at 40°C under a stream of nitrogen. The HCl layer was washed once with 0.5 ml ether. Samples, 50 μl, were injected onto the column. Extraction recovery of citalopram, metabolites and internal standard was approximately 95%. Plasma levels were corrected for recovery of the internal standard.

An HPLC/auto-injector (1084B Liquid Chromatograph, Hewlett-Packard) was used, in combination with a fluorescence detector (470 Scanning Fluorescence detector, Waters, England) operating at an absorption wavelength of 240 nm, an emission wavelength of 296 nm, and a slit-width of 12 nm. Separation was performed using a Supelcosil HPLC column (5 μm, C18, 250 × 46 mm, Supelco, The Netherlands), at ambient temperature. The mobile phase consisted of 46% v/v acetonitrile, 54% v/v potassium dihydrogen phosphate buffer (4.3 g/l), and 1 mM tetramethylammonium, at pH 3.0. The flowrate in this system was 0.75 ml/min. The detection limits for ''on column injections'' of citalopram, desmethyl-citalopram and didesmethyl-citalopram were 5, 5 and 3 nM, respectively (signal-to-noise ratio = 2).

2.5. Data presentation and statistics

Four consecutive microdialysis samples with less than 20% variation were taken as control and set at 100%. The data are presented as percentages of the control level (means ± S.E.M.). Statistical analysis was performed using Sigmastat for Windows (Jandel). Treatment effects were compared using two-way analysis of variance (ANOVA) for repeated measurements, followed by the Mann–Whitney Rank Sum Test. Significance was set at $P < 0.05$.

3. Results

3.1. Citalopram plasma levels

Infusion rate calculations, based on acute subcutaneous administration of citalopram ($Cl_{calculated} = 0.142$ l/min), predicted that plasma levels of 500 nM would be obtained when the osmotic minipumps were filled with 100 mg/ml. Empirical evaluation showed, however, that these plasma levels were around 1 μM (data not shown).

Fig. 1 shows that treatment of rats with osmotic minipumps (50 mg/ml citalopram), resulted in stable citalopram plasma levels of around 0.3 μM (0.30 + 0.03

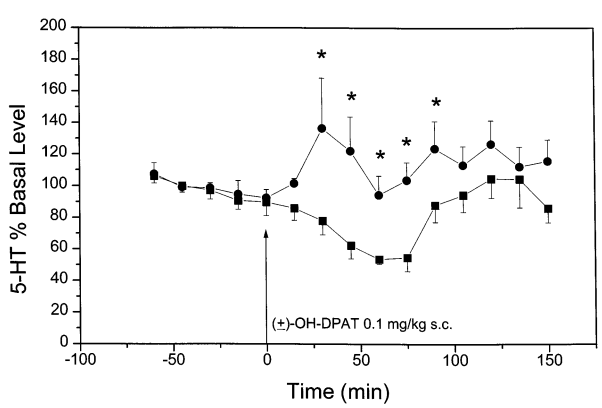

Fig. 2. Effect of chronic treatment of rats for 15 days with citalopram on 5-HT_{1A} receptor agonist (±)-8-HO-DPAT 0.1 mg/kg-induced decrease in 5-HT levels. Citalopram-treated ($n = 5$; ●); saline-treated ($n = 8$; ■). * $P < 0.05$.

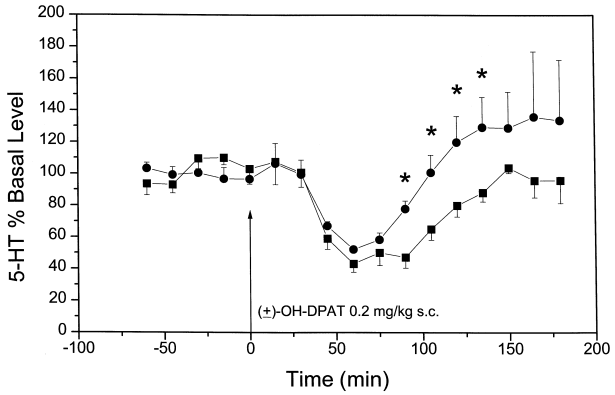

Fig. 3. Effect of chronic treatment of rats for 15 days with citalopram on 5-HT_{1A} receptor agonist (±)-8-HODPAT 0.2-mg/kg induced decrease in 5-HT levels. Citalopram-treated ($n = 6$; ●); saline-treated ($n = 6$; ■). * $P < 0.05$.

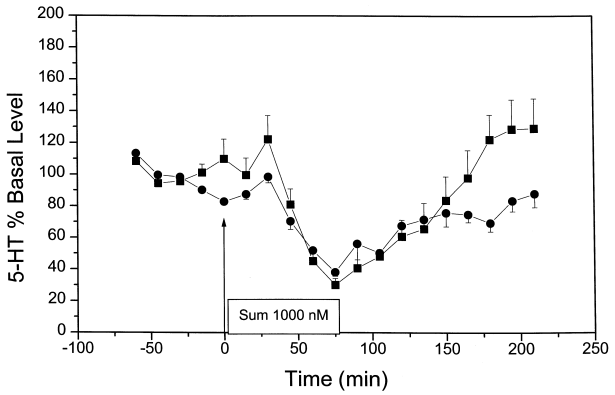

Fig. 5. Effect of chronic treatment of rats for 15 days with citalopram on the decrease in 5-HT levels produced by local infusion of 1000 nM of 5-$HT_{1B/D}$ receptor agonist, sumatriptan. Citalopram-treated ($n = 8$; ●); saline-treated ($n = 8$; ■).

μM, Fig. 1). Desmethyl- and di-desmethyl metabolites were found to be around 0.065 and 0.2 μM, respectively).

Upon removal of the minipumps, a gradual decline in plasma levels of citalopram and metabolites was observed. After 48 h, the plasma levels of citalopram had dropped to below 9 nM.

3.2. Basal 5-HT levels

The basal levels of 5-HT in dialysates did not differ between citalopram- and saline-treated rats 48 h after removal of the minipumps: saline-treated rats 6.66 + 0.82 fmol/sample ($n = 26$); citalopram-treated rats 7.26 + 0.56 fmol/sample ($n = 27$); $P = 0.219$, n.s.).

3.3. 8-OH-DPAT challenge

The effect of 8-OH-DPAT (0.1 mg/kg s.c.), that clearly decreased 5-HT levels in ventral hippocampus, was completely abolished after chronic treatment of the animals with citalopram ($F(1,108) = 2.65$, $P < 0.05$). Post hoc analysis revealed significant differences from $t = 30$ to $t = 90$ min (Fig. 2).

After subcutaneous administration of a higher dose of 8-OH-DPAT (0.2 mg/kg), 5-HT levels decreased to about 40% of the controls. Chronic pretreatment with citalopram did not prevent the maximum effect, but attenuated the effect on 5-HT levels during the rebound phase ($F(1,83) = 2.64$, $P < 0.05$). The effects were significantly different from $t = 90$ to $t = 135$ (Fig. 3).

3.4. Sumatriptan challenge

Local infusion of 500 nM of sumatriptan in the ventral hippocampus for 60 min decreased the levels of 5-HT to about 50% of the controls. No significant differences were observed between citalopram- and saline-treated animals ($F(1,128) = 0.389$, n.s.) (Fig. 4).

Local infusion of 1000 nM of sumatriptan for 60 min decreased the levels of 5-HT to about 30% of the controls. Again, no significant differences were observed between citalopram- and saline-treated animals ($F(1,175) = 1.549$, n.s.) (Fig. 5).

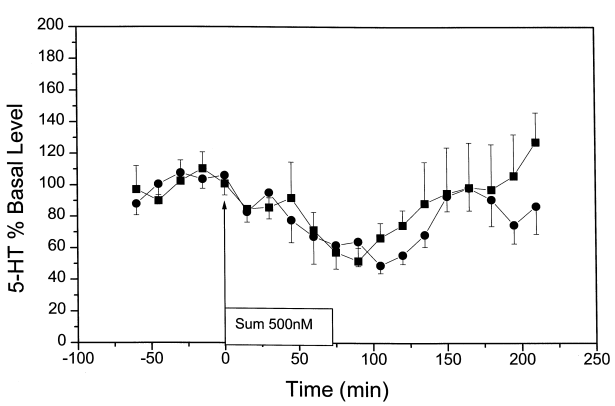

Fig. 4. Effect of chronic treatment of rats for 15 days with citalopram on the decrease in 5-HT levels produced by local infusion of 500 nM of 5-$HT_{1B/D}$ receptor agonist, sumatriptan. Citalopram-treated ($n = 4$; ●); saline-treated ($n = 8$; ■).

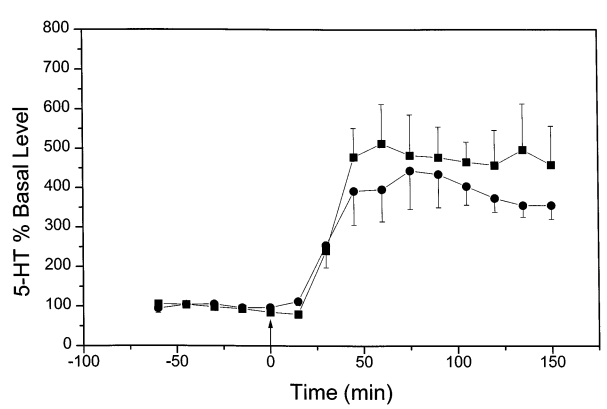

Fig. 6. Effect of chronic treatment of rats for 15 days with citalopram on citalopram induced increase in 5-HT levels. Citalopram-treated ($n = 4$; ●); saline-treated ($n = 5$; ■).

3.5. Citalopram challenge

No differences were found between treated and untreated animals after systemic administration of 10 μmol/kg citalopram ($F(1,98) = 0.959$, n.s.). The maximum increase in both groups was about 450%. No differences in time–effect were observed (Fig. 6).

4. Discussion

Desensitisation of 5-HT autoreceptors upon chronic treatment of animals with selective serotonin re-uptake inhibitors has been a topic of interest for several years. Although various studies have found evidence for the occurrence of these processes, others have consistently failed to show any of these effects. As pharmacokinetic measurements are not common in pharmacological research, dose regimens used were based on estimates rather than on pharmacokinetic data. The present study showed that, if a properly pharmacokinetically validated chronic treatment regimen is used, 5-HT_{1A} autoreceptor desensitisation can be observed.

Recent work has shown that selective serotonin re-uptake inhibitor induced activation of 5-HT autoreceptors is critically related to the dose and concurrent plasma- and brain levels of the selective serotonin re-uptake inhibitor as well as the autoreceptor antagonist (Hjorth et al., 1997). In a previous study, we have shown that activation of 5-HT_{1A} autoreceptors is only observed on acute administration of citalopram when plasma levels of citalopram are above 150 nM. Selective serotonin re-uptake inhibitor-induced activation of 5-HT_{1B} receptors was already observed at much lower plasma levels of the selective serotonin re-uptake inhibitor (Cremers et al., 2000). As elimination of exogenous substances is, in general, much faster in rodents than in humans, chronic treatment of animals with selective serotonin re-uptake inhibitors is characterised by daily multiple high dosing. Consequently, high plasma levels followed by a rapid decline to low and inactive concentrations might explain why several authors have failed to observe any desensitisation of 5-HT autoreceptors. As plasma levels of the selective serotonin re-uptake inhibitor are critically important and fluctuations in plasma levels are inherent to systemic administration, the present study made use of osmotic minipumps in an attempt to achieve stable plasma levels, which were shown to produce autoreceptor activation.

From pharmacokinetic data obtained after acute subcutaneous administration of citalopram, we calculated which infusion concentration was needed to establish citalopram plasma levels known to activate both autoreceptors. From the time–concentration profile of acutely administrated 10 μmol/kg citalopram in rats who received surgery under isoflurane anaesthesia, a clearance (Cl) of 0.142 l/min was calculated (data not shown). Calculating the infusion rate (R_o) with the equation $R_o = C_{ss} \times \mathrm{Cl}$, we first evaluated minipumps filled with 100 mg/ml saline (aiming at a steady state (C_{ss}) of 500 nM in 300-g rats). The plasma levels, however, were found to be around 1 μM, which was above clinically effective concentrations. Lowering the infusion concentration to 50 mg/ml was found to produce stable plasma levels for 2 weeks, ensuring chronic activation of autoreceptors. In addition, these levels were also within the limits of clinically effective plasma levels of citalopram in humans (0.12–0.84 μM, Baumann, 1992). Desmethyl and didesmethyl metabolites of citalopram were quantified during chronic treatment. The drug-to-metabolites ratios observed in rats were somewhat different from those observed in humans. Whereas, in rats, the ratio between parent, desmethyl and didesmethyl was about 10:1:5, respectively, the corresponding ratios in humans are about 10:5:1, respectively. However, as the affinity of the metabolites for the re-uptake site is at least 10 times lower than that of the parent compound, their effects, if any, will not be predominant (Hyttel, 1994).

Upon removal of the minipumps the $T_{1/2}$ value for elimination was dramatically increased (2 days vs. 2 h, Cremers et al., 2000). Apparently, the pharmacokinetic parameters of citalopram change after chronic treatment because of redistribution in the body. This dramatic decrease in elimination has consequences for chronic experiments, as pharmacological challenges on termination of the treatment should be postponed until selective serotonin re-uptake inhibitor concentrations are below pharmacologically active levels. In a previous study (Cremers et al., 2000), we determined the threshold of the pharmacologically active plasma level to be around 9 nM. Forty eight hours after removal of the minipumps, the plasma levels of citalopram were indeed below this border. Although the didesmethyl metabolite did not reach this limit within 48 h, due to lower affinity for the 5-HT re-uptake side (Hyttel, 1994), it is assumed to be without interfering effects. It is of interest to note that the basal 5-HT levels 48 h after removal of the minipumps did not different between saline- and citalopram-treated rats, which also indicates the absence of interfering amounts of residual citalopram.

Systemic administration of the putative 5-HT_{1A} receptor agonist, 8-OH-DPAT, had marked dose-dependent effects after chronic treatment with citalopram. Whereas the effect of a 0.1-mg/kg dose was completely abolished after chronic treatment, administration of 0.2 mg/kg produced near similar maximal responses, with the citalopram-treated rats showing a faster recovery of 5-HT levels. Apparently, chronic treatment of animals with citalopram induces a shift in the response curve of 5-HT_{1A} receptor agonists, indicative of somatodendritic 5-HT_{1A} receptor desensitisation. Although as mentioned above, not all authors have observed this desensitisation upon chronic treatment. Several authors also found attenuated effects of 8-OH-DPAT after chronic treatment with selective serotonin re-uptake inhibitors (Invernizzi et al., 1994; Kreiss and Lucki, 1995;

Le Poul et al., 1995). Extrapolating the data on citalopram pharmacokinetics to the chronic treatment regimens used in these latter experiments, shows that researchers using higher and frequent doses were more likely to find these effects than when lower doses were used (Chaput et al., 1986; Moret and Briley, 1990, 1996; Invernizzi et al., 1994). Although in the present study, the effect of 8-OH-DPAT was indeed abolished, or at least attenuated, it cannot be neglected that this attenuation could have been due to decreased availability of the agonist. Differences in elimination due to induction of kinetics, however, should not be very relevant, as citalopram is described to be without major interaction with other drugs (Sproule et al., 1997). The possibility of blunted absorption due to tissue necrosis produced by the osmotic minipump was eliminated by injection of the agonist on the side contralateral to the area were the osmotic minipump was positioned. Although there are no reasons to suspect lower bioavailability of the agonist in citalopram- vs. saline-treated rats, local application in the raphe, or pharmacokinetic experiments should elucidate this point.

Local administration of two concentrations of the 5-$HT_{1B/D}$ receptor agonist, sumatriptan, decreased 5-HT levels equally in citalopram- and saline-treated rats. Chronic treatment of rats with plasma levels, which were shown to activate 5-HT_{1B} receptors apparently does not desensitise these autoreceptors (Cremers et al., 2000). Chaput et al. (1986), using an indirect electrophysiological approach, found evidence for desensitisation of this receptor, but no study using the microdialysis method has ever shown desensitisation of this receptor (Auerbach and Hjorth, 1995; Bosker et al., 1995a,b; Moret and Briley, 1996; Davidson and Stamford, 1997). As 5-HT_{1B} receptors have been shown to be activated by low doses of selective serotonin re-uptake inhibitor (Rollema et al., 1996; Cremers et al., 2000), faster desensitisation than of 5-HT_{1A} receptors would be expected. Rapid resensitisation of the 5-HT_{1B} receptor is a possible explanation for the observed absence of effects. As the current protocol was based on challenge of the system after elimination of the selective serotonin re-uptake inhibitor, such effects cannot be excluded.

Although terminal 5-HT_{1B} receptors apparently were not desensitised in our setup, the 5-HT_{1A} receptors were. This would mean that, upon challenge with a dose of selective serotonin re-uptake inhibitor, which activates 5-HT_{1A} as well as 5-HT_{1B} receptors, selective serotonin re-uptake inhibitor-evoked increases in 5-HT levels would be augmented in citalopram-treated animals. However, in our experiments, this effect was not observed. There might be several reasons for this observation. First, since citalopram treatment induced a shift in 5-HT_{1A} receptor agonist-induced response, rather than abolished the effect, any remaining 5-HT_{1A} autoreceptor function might have sufficed to preserve proper autoreceptor function. Treatment of animals for longer periods should elucidate whether this would lead to enhancement of the shift in receptor function, with consecutive augmentation of selective serotonin re-uptake inhibitor-induced effects. Second, as desensitisation of the serotonin transporter has been described after chronic administration of selective serotonin re-uptake inhibitor, this effect might counteract the desensitisation of autoreceptors (Blier and Bouchard, 1994). Chronic treatment with selective serotonin re-uptake inhibitor would then be a ''resetting of the system'', rather than an enhancement of serotonergic neurotransmission. Based on this possibility, chronic treatment of animals with selective serotonin re-uptake inhibitors followed by physiological activation of the serotonergic system should be tried in order to unravel the function of desensitisation of the 5-HT_{1A} autoreceptor.

In conclusion, the present study demonstrated that rational chronic treatment is only possible if it is accompanied by proper pharmacokinetic validation. Several discrepancies in the literature regarding effects after chronic treatment with drugs might be strongly related to failure to produce relevant and stable plasma levels of drugs in animals. In addition, if stable and relevant plasma levels are ensured, pharmacological changes would be easier to extrapolate to a clinical setting. Although, in the present study, 5-HT_{1A} desensitisation was observed, no augmented 5-HT levels were observed after a single selective serotonin re-uptake inhibitor challenge. If this augmentation were not relevant for the antidepressive action, but instead, the desensitisation of 5-HT_{1A} receptors, which control the firing rate of the serotonergic system, co-administration of a 5-HT_{1A} receptor antagonist with selective serotonin re-uptake inhibitors would be more useful than co-administration of a 5-HT_{1B} receptor antagonist.

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