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Augmentation with a 5-HT_{1A}, but not a 5-HT_{1B} receptor antagonist critically depends on the dose of citalopram

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Abstract

Pharmacokinetic and pharmacodynamic parameters of the selective serotonin reuptake inhibitor 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phtalancarbonitril (citalopram) were determined in order to find optimal conditions for augmentation of its effect on extracellular serotonin [5-hydroxytryptamine (5-HT)] through blockade of 5-HT_{1A} and 5-HT_{1B} autoreceptors. Citalopram dose-dependently (0.3–10 μmol/kg s.c.) increased serotonin levels in ventral hippocampus of conscious rats. At plasma levels above approximately 0.15 μM, the effect of citalopram on extracellular 5-HT was augmented by both a 5-HT_{1A} [*N*-[2-[4-(2-mehoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridil)cyclohexanecarboxamide trihydrochloride (Way 100635), 1 μmol/kg s.c.] and a 5-HT_{1B} receptor antagonist (2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy]-3-(4-methylpiperazin-1-yl)phenyl]amide (GR 127935), 1 μmol/kg s.c.). However, at plasma levels of the selective serotonin reuptake inhibitor below 0.15 μM, the effects of the antagonists diverged viz. the 5-HT_{1B} receptor antagonist was still able to potentiate citalopram's effect on extracellular 5-HT, while the 5-HT_{1A} receptor antagonist was no longer effective. These results suggest that in contrast to 5-HT_{1B} autoreceptors, indirect activation of 5-HT_{1A} autoreceptors by citalopram is critically related to the dose of selective serotonin reuptake inhibitor administered. The latter may have consequences for selective serotonin reuptake inhibitor augmentation strategies with 5-HT_{1A} receptor antagonists in the therapy of depression and anxiety disorders.

Keywords: Microdialysis; 5-Hydroxytryptamine, serotonin (5-HT); Citalopram; Hippocampus, ventral; 5-Hydroxytryptamine, serotonin (5-HT) reuptake inhibitor, selective

1. Introduction

Selective serotonin reuptake inhibitors increase brain extracellular serotonin [5-hydroxytryptamine (5-HT)] concentrations in animals acutely following administration (Fuller, 1994). In contrast, the clinical effects of these substances are typically delayed for several weeks (Baumann, 1992; Fuller, 1994). This notion suggests the occurrence of adaptive changes triggered by the sustained elevated 5-HT levels. Several lines of evidence suggest that these changes involve desensitization of 5-HT autoreceptors. Attenuation of auto-inhibitory processes increases the overall serotonergic neurotransmission, and this process has been hypothesized to underlie the therapeutic effects of selective serotonin reuptake inhibitors (Blier et al., 1987).

At least three types of presynaptic 5-HT receptor regulatory mechanisms are known. Somatodendritic 5-HT_{1A} autoreceptor activation decreases 5-HT release in terminal areas via inhibition of serotonergic cell firing (Arborelius et al., 1994; Bosker et al., 1994). Several authors have reported a reduced inhibitory effect of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) on terminal 5-HT release or raphe nuclei cell firing following chronic treatment with selective serotonin reuptake inhibitors (Invernizzi et al., 1994; Kreiss and Lucki, 1995; Le Poul et al., 1995). Others, however, failed to observe any such effects (Hjorth and Auerbach, 1994; Bosker et al., 1995a,b).

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Activation of terminal 5-HT_{1B} receptors (formerly 5-$HT_{1D\beta}$) also decreases 5-HT release (Bosker et al., 1995a,b). Similarly, data concerning 5-HT_{1B} autoreceptor desensitization following chronic treatment with selective serotonin reuptake inhibitors are controversial (Chaput et al., 1986; Auerbach and Hjorth, 1995; Bosker et al., 1995a,b; Moret and Briley, 1996; Davidson and Stamford, 1997).

The third type of regulatory mechanism may be through somatodendritic 5-HT_{1D} (formerly 5-$HT_{1D\alpha}$) receptors (Starkey and Skingle, 1994; Sprouse et al., 1997). Activation of these receptors decreases 5-HT release in the cell body region, which may indirectly influence terminal 5-HT release through interplay with somatodendritic 5-HT_{1A} autoreceptors.

In contrast to receptor density and affinity, which can be adequately assessed by means of in-vitro binding studies (B_{max} and K_d), the desensitization of auto-inhibitory processes can only be measured in functional models, for example by using microdialysis or electrophysiological methods. In addition to using selective 5-HT_{1A} and 5-HT_{1B} receptor agonists, the selective serotonin reuptake inhibitors themselves can be used as probes for measuring desensitization processes. Eventually, the attenuation of release-restraining processes will lead to an additional increase of extracellular 5-HT. Using this latter approach, microdialysis studies have indeed reported additional increases in extracellular 5-HT following chronic treatment with selective serotonin reuptake inhibitors (Invernizzi et al., 1994; Auerbach and Hjorth, 1995), although another study failed to observe these effects (Bosker, et al. 1995a). In addition, an electrophysiological study has reported that inhibition of dorsal raphe nucleus 5-HT cell firing by 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-5-phtalancarbonitril (citalopram) was time-dependently abolished during chronic treatment (Chaput et al., 1986).

Apart from differences in animal strains (Schoups and De Potter, 1988), the discrepant findings may have a variety of causes. For instance, McQuade and Sharp (1997) have demonstrated that forebrain regions are not evenly innervated by dorsal raphe nucleus and median raphe nucleus. It can be speculated that 5-HT_{1A} autoreceptors on 5-HT cell bodies within these nuclei differ in their susceptibility to desensitization, which may explain some of the discrepancies (Kreiss and Lucki, 1994, 1997).

An obvious source of variance are differences in dose regimen of the selective serotonin reuptake inhibitors used in the studies. Since plasma half-lives of drugs in rodents are generally much shorter than in humans, multiple or very high doses are not uncommon in order to mimic human plasma levels. Pharmacologically inactive, strongly fluctuating or even toxic plasma levels, thus established, may have contributed to the varying results.

An alternative approach to test Blier's desensitization hypothesis (Blier et al., 1987) is the administration of selective serotonin reuptake inhibitors with concomitant autoreceptor blockade. Arguably, blocking the autoreceptors instantaneously mimics the desensitization process. Animal studies have demonstrated that co-administration of a 5-HT_{1A} receptor antagonist causes an additional increase of extracellular serotonin levels (Hjorth, 1993; Hjorth et al., 1996; Gobert et al., 1997), which is after all the basis of Blier's hypothesis. Consequently, co-administration of a selective serotonin reuptake inhibitor and 5-HT_{1A} and/or 5-HT_{1B} antagonists should accelerate the onset of therapeutic effect. Following this line of thought, clinical studies have reported on hastening and/or improvement of response to antidepressant treatment by co-administration of the β-adrenoceptor and 5-$HT_{1A/B}$ receptor antagonist pindolol.

Several groups have indeed claimed a more rapid onset of action, larger reductions in Hamilton depression scores in time, and sometimes beneficial effects in therapy resistant depression (Artigas et al., 1994; Perez et al., 1997; McAskill et al., 1998). On the other hand several equally well-executed studies were not able to support these findings (Berman et al., 1997; McAskill et al., 1998).

To explain this discrepancy, several explanations have been advanced such as differences in patient groups, different properties of the selective serotonin reuptake inhibitors, etc.

An alternative explanation may be found in varying selective serotonin reuptake inhibitor plasma levels between patients, which may lead to different degrees of autoreceptor activation. Arguably, this would lead to a substantial variation in antagonist effect between patients.

In the present study, the contribution of 5-HT_{1A} and 5-HT_{1B} autoreceptors in restraining selective serotonin reuptake inhibitor evoked 5-HT release in rat ventral hippocampus was investigated by co-administration of selective antagonists and different doses of citalopram, currently the most selective serotonin reuptake inhibitor (Hyttel, 1994). The selective 5-HT_{1A} and 5-HT_{1B} receptor antagonists used in the study were *N*-[2-[4-(2-mehoyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridil)cyclohexanearboxamide trihydrochloride (WAY 100635) and 2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)biphenyl-4-carboxylic acid [4-methoxy]-3-(4-methylpiperazin-1-yl)phenyl]amide (GR 127935), respectively. In addition, citalopram plasma concentrations were measured to relate brain pharmacology with plasma levels of the selective serotonin reuptake inhibitor.

2. Materials and methods

2.1. Animals

Male albino rats of a Wistar-derived strain (285–320 g; Harlan, Zeist, Netherlands) were used for the experiments.

Upon surgery, rats were housed individually in plastic cages (35 × 35 × 40 cm), and had free access to food and water. Animals were kept on a 12 h light schedule (light on 7:00 am). The experiments were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

2.2. Drugs

The following drugs were used: Citalopram hydrobromide (kindly donated by Lundbeck (Denmark), courtesy of Dr. Sanchez), Way 100635, and GR 127935 (both compounds were synthesized in our laboratory, courtesy of Dr. Y. Liao and Mrs. M. Mensonides). Drugs were dissolved in saline except for GR 127935, which was dissolved in saline with a drop of acetic acid. Substances were injected subcutaneously in a volume of 1 ml/kg.

2.3. Surgery

Microdialysis experiments were performed using home made I-shaped probes, made of polyacrylonitrile/sodium methyl sulfonate copolymer dialysis fiber (i.d. 220 μm, o.d. 0.31 μm, AN 69, Hospal, Italy). The exposed length of the membranes was 4 mm.

Preceding surgery rats were anesthetized by means of an intraperitoneal injection of 400 mg/kg chloral hydrate. Lidocaine–HCl, 10% (m/v) was used for local anesthesia. 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.

Pharmacokinetic experiments were performed in a separate group of rats without microdialysis probes. Apart from this, conditions were comparable with the microdialysis animals, including anaesthesia. Blood was drawn through a canula made of silicon tubing, which was inserted into the right jugular vein for 3.8 mm, during chloralhydrate anaesthesia. The tubing was transferred subcutaneously to the scull of the rat, and a stainless steel inlet was connected to the tubing. The inlet was mounted on the skull with dental cement and surgical screws. After insertion, canulas were filled with a PVP solution (55% polyvinylpyrrolidon in 500 IE/ml heparin) in saline to prevent blood clotting.

2.4. Microdialysis experiments

Rats were allowed to recover for at least 24 h. Probes were perfused with artificial cerebrospinal fluid containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$, at a flow rate 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.

2.5. Pharmacokinetic experiments

Preceding the experiments, rats were allowed to recover from surgery for 24 h. Blood samples (0.35 ml) were drawn at 0, 15, 30, 60, 120, 240 and 360 min after injection of citalopram (3 and 10 μmol/kg s.c.). Samples were transferred to 1.5 ml eppendorf vials, containing 5 μl heparin (500 IE/ml saline), mixed and immediately transferred to a chilled centrifuge (MSE, England), and centrifuged for 15 min at 3000 rpm.

To measure citalopram plasma concentrations at the dose of 0.1 μmol/kg, it appeared necessary to introduce a concentration step. To this end animals were anesthetized by ether anesthesia 30 min after receiving the citalopram injection. Vacutainers (Becton Dickinson, England) were used in order to obtain at least 5 ml of plasma by means of cardiac puncture.

2.6. Analysis

2.6.1. Serotonin analysis

5-HT was analyzed 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: 3 μm, 100 × 2.0 mm, C18, Bester, Amstelveen, Netherlands) followed by an electrochemical detector (Antec Leyden, Leiden, 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 MeOH at a pH of 4.65. Flow-rate of the mobile phase was 0.4 ml/min. The detection limit was 1–2 fmol 5-HT per 20 μl sample (signal to noise ratio 2).

2.6.2. Citalopram analysis

Citalopram was measured according to Øyehaug et al. (1982), with minor modifications. Briefly, to 150 μl plasma samples, 75 μl of the internal standard LU 10–202 (2 μM) and 30 μl of 1 N NaOH were added. 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; 50 μl samples were injected onto the column. Extraction recovery of citalopram, metabolites and internal standard were approximately 95%. Citalopram plasma levels were corrected for recovery of the internal standard.

With the experiments in which 5 ml plasma was obtained via the vacutainer, a similar protocol was used, but the plasma sample was now extracted three times with 10 ml of ether. After extraction, the combined ether layers were evaporated into 150 μl of 0.1 N HCl, thereby concentrating the samples 33.3 times. Under these conditions, relative recovery of the extraction for citalopram, metabolites and internal standard was lower and amounted to approximately 70%. Citalopram 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, 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 30 μl/l triethylamine, at a pH value of 3.0. Flow rate in this system was 0.75 ml/min. The detection limits for ''on column injections'' of citalopram, desmethyl-citalopram, and didesmethyl-citalopram were 8, 7 and 5 nM, respectively (signal to noise = 2).

2.7. Data presentation and statistics

Four consecutive microdialysis samples with less then 20% variation were taken as control and set at 100%. Data are presented as percentages of control level (mean ± 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 Dunnett's test. Level of significance level was set at $p < 0.05$. Dose response curves and ED_{50}s were calculated using origin software.

Pharmacokinetic data were fitted using Multifit (copyright Dr. J.H. Proost, Dept. of pharmacokinetics and drug delivery, University of Groningen, Netherlands).

3. Results

3.1. Pharmacokinetic experiments

Plasma levels were determined of 3 doses of citalopram (0.1, 3 and 10 μmol/kg). Subcutaneous administration of 10 μmol/kg citalopram rapidly established plasma levels of about 0.5 μM. During the time span of the pharmacological experiment (240 min.), plasma levels gradually decreases to about 0.15 μM. Following administration of 3 μmol/kg citalopram, plasma levels of about 0.13 μM were found to occur within 15 min. After 240 min, plasma levels decreased to approximately 0.01 μM (Fig. 1). Desmethyl and didesmethyl metabolites of citalopram could not be detected in the plasma after administration of either dose.

The three doses of citalopram showed a linear relation with the respective plasma concentrations (Fig. 2). From the plasma time–concentration profile after 10 μmol/kg s.c. citalopram, several pharmacokinetic parameters were

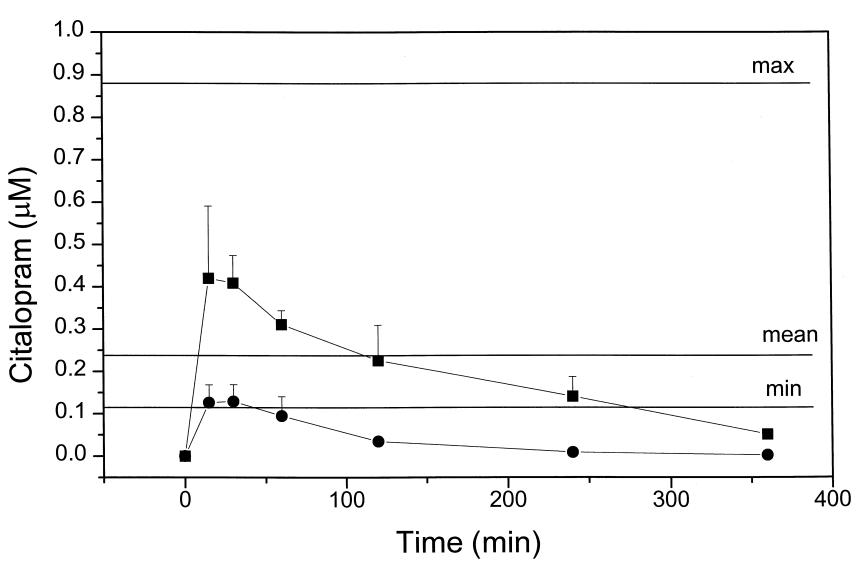

Fig. 1. Plasmaconcentrations in time following subcutaneous injection of 3.0 ($n = 4$, ●) and 10 μmol/kg ($n = 4$, ■) citalopram. Min, mean, and max denote the respective minimal, mean, and maximal borders of the human clinically effective therapeutic window.

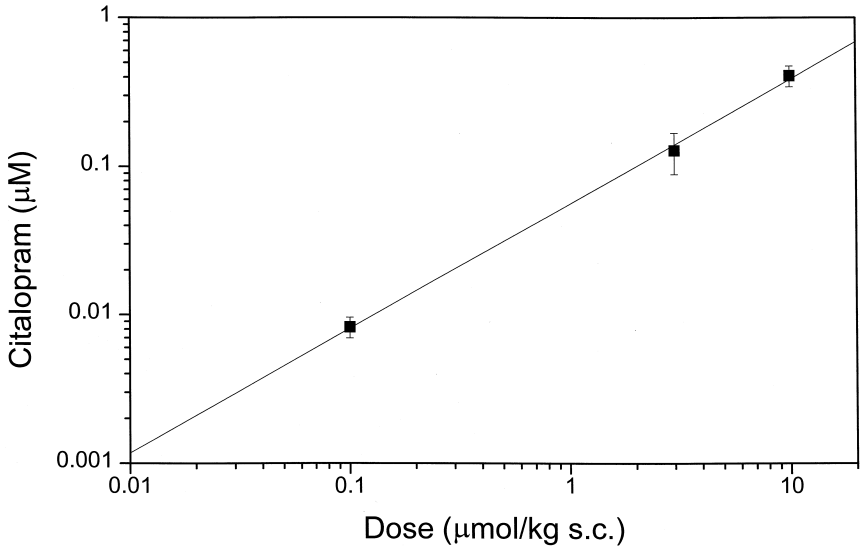

Fig. 2. Linear relationship between the administered dose of citalopram and the plasma concentration measured at $t = 30$ min ($n = 4$) ($r = 1.00$).

calculated. $T_{1/2}$ was 110 ± 13 min, volume of distribution 20 ± 4 l/kg, and clearance 0.13 ± 0.02 l/kg/min.

3.2. Pharmacodynamic experiments

3.2.1. Basal extracellular 5-HT levels

Basal levels of 5-HT in ventral hippocampus were 7.8 ± 0.7 fmol/15 min fraction (mean ± S.E.M., $n = 72$). No significant differences were observed between the different treatment groups.

3.2.2. Effect of subcutaneous administration of citalopram on extracellular 5-HT levels

Subcutaneous administration of 0.1, 0.3, 1.0, 3.0 and 10.0 μmol/kg citalopram dose-dependently increased extracellular 5-HT in ventral hippocampus (Fig. 3).

A dose of 0.1 μmol/kg citalopram had no significant effect on extracellular 5-HT in ventral hippocampus ($F(1,116) = 0.729$, $P > 0.05$). The next dose induced a short lasting increase of extracellular 5-HT to about 225% of control values ($F(1,107) = 2.66$, $P < 0.05$). Post-hoc analysis revealed that the effect was significant at $t = 60$

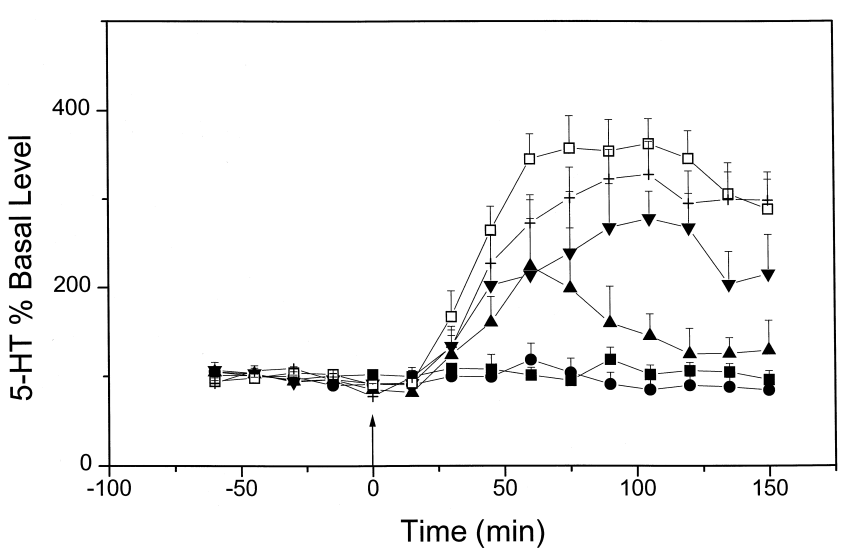

Fig. 3. Effect of subcutaneous administration of citalopram on extracellular levels of 5-HT in ventral hippocampus of conscious and freely moving rats. Citalopram or vehicle was administered at $t = 0$ min: Saline ($n = 7$; ■), Citalopram 0.1 μmol/kg ($n = 5$; ●), Citalopram 0.3 μmol/kg ($n = 4$; ▲), Citalopram 1.0 μmol/kg ($n = 5$; ▼), Citalopram 3.0 μmol/kg ($n = 5$; □), Citalopram 10 μmol/kg ($n = 6$; +).

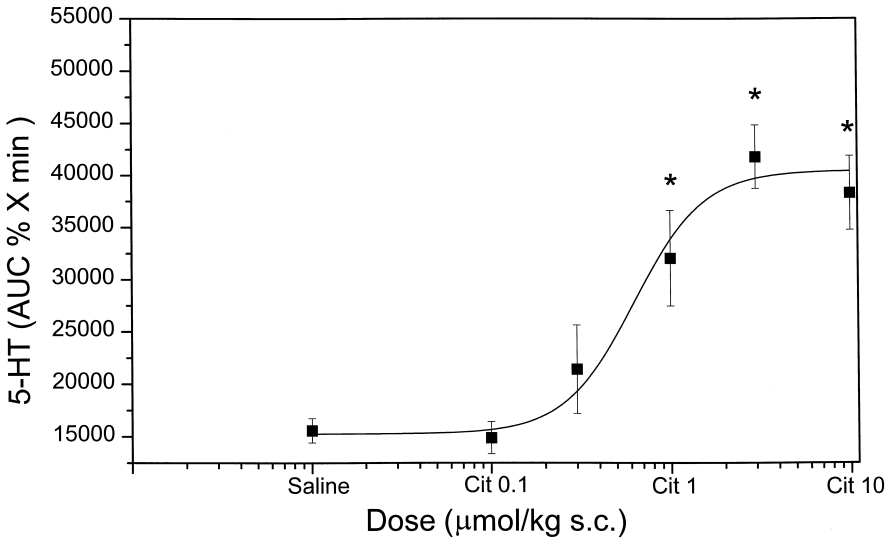

Fig. 4. Dose–response curve of citalopram induced 5-HT increases in ventral hippocampus release. Data were calculated as AUC from $t = 0$ to $t = 150$ min. Dose 1.0, 3.0 and 10 μmol/kg elicit an statistical difference in AUC vs. Saline AUC ($P < 0.05$). The calculated ED_{50} of this effect was 0.62 μmol/kg s.c.

min. Increasing the dose to 1 μmol/kg elevated 5-HT levels significantly to 275% for the remainder of the experiment ($F(1,121) = 5.26$, $P < 0.05$). Post-hoc analysis revealed significant increases from $t = 90–120$ min. A dose of 3 μmol/kg elicited an increase in extracellular 5-HT of 350% ($F(1,121) = 22.3$, $P < 0.05$). Post-hoc analysis revealed significant differences from $t = 30$ to $t = 150$ min. A dose of 10 μmol/kg increased 5-HT to a comparable extent ($F(1,131) = 18.8$, $P < 0.05$). Post-hoc analysis revealed significant effects from $t = 45$ to $t = 150$. From the dose–response curve, using the area under the curve (AUC) from $t = 0$ to $t = 150$ min, the half-maximal dose was estimated to be 0.62 μmol/kg (Fig. 4).

3.2.3. Effect of co-administration of the 5-HT$_{1A}$ receptor antagonist WAY 100635 and citalopram on extracellular 5-HT levels

A low dose of citalopram (1 μmol/kg) increased 5-HT levels, but co-administration of the 5-HT$_{1A}$ receptor antagonist did not potentiate this effect ($F(1,117) = 1.129$, $P > 0.05$) (Fig. 5). Co-administration of an intermediate dose of citalopram (3 μmol/kg) and the 5-HT$_{1A}$ receptor antag-

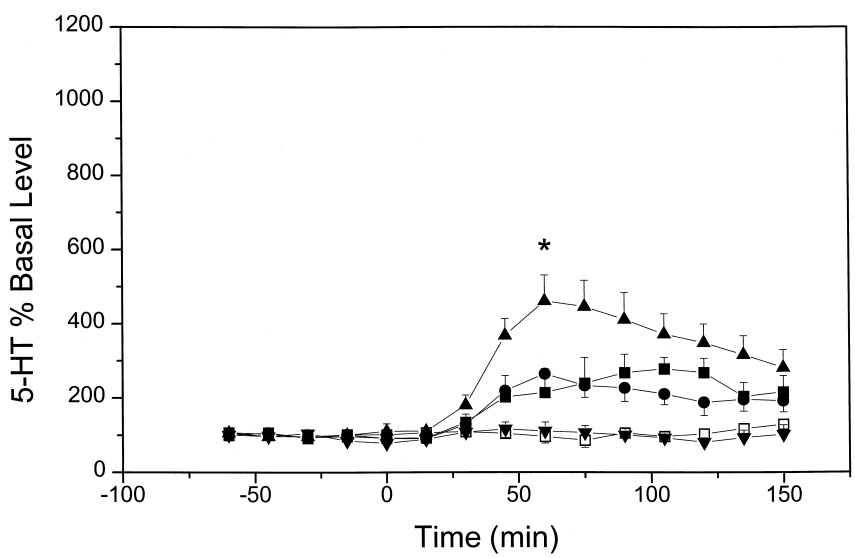

Fig. 5. Effects of citalopram 1.0 μmol/kg and WAY 100635 1.0 μmol/kg or GR 127935 1.0 μmol/kg, alone, or co-administered, on extracellular 5-HT levels in ventral hippocampus. Citalopram 1.0 μmol/kg (■, $n = 5$), WAY 100635 (□, $n = 3$), citalopram 1.0 μmol/kg and WAY 100635 1.0 μmol/kg (●, $n = 6$), GR 127935 (▼, $n = 4$), citalopram 1.0 μmol/kg and GR 127935 1.0 μmol/kg (▲, $n = 5$) ($^{*}P < 0.05$).

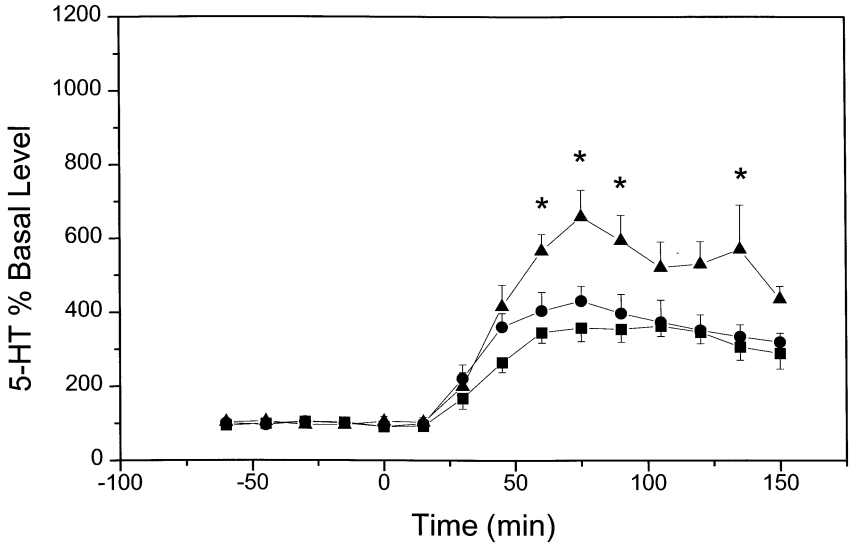

Fig. 6. Effects of citalopram 3.0 μmol/kg and WAY 100635 1.0 μmol/kg or GR 127935 1.0 μmol/kg, on extracellular 5-HT levels in ventral hippocampus. Citalopram 3.0 μmol/kg (■, $n = 5$), citalopram 3.0 μmol/kg and Way 100635 1.0 μmol/kg (●, $n = 5$), citalopram 3.0 μmol/kg and GR 127935 1.0 μmol/kg (▲, $n = 5$) ($^{*}P < 0.05$).

onist showed a tendency towards an additional increase. However, this effect was not statistically significant ($F(1,108) = 0.648$, $P > 0.05$) (Fig. 6). At a dose of 10 μmol/kg citalopram, the increase in extracellular 5-HT was significantly augmented by the 5-HT_{1A} receptor antagonist ($F(1,118) = 2.71$, $P < 0.05$) (Fig. 7).

From the AUC (0–150 min), it can be seen that augmentation by WAY 100635 occurs at doses of the selective serotonin reuptake inhibitor exceeding 3 μmol/kg (Fig. 8).

3.2.4. Effect of co-administration of the 5-HT_{1B} receptor antagonist GR 127935 and citalopram on extracellular 5-HT levels

Co-administration of the selective 5-HT_{1B} receptor antagonist GR 127935 clearly potentiated the citalopram induced increase in extracellular 5-HT. The effect was already significant at a dose of 1 μmol/kg of citalopram ($F(1,79) = 3.09$, $P < 0.05$) (Fig. 5). Augmentation was observed to become progressively stronger when the dose of citalopram was increased (citalopram 3 μmol/kg s.c.

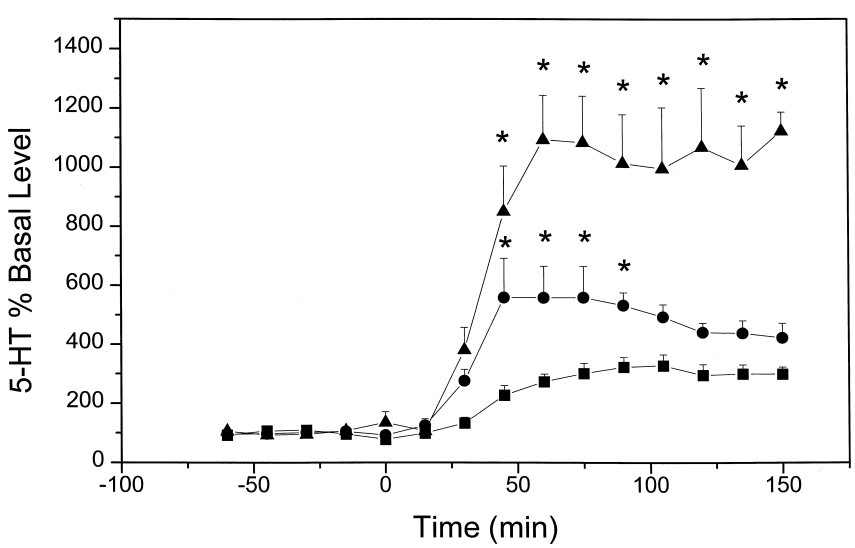

Fig. 7. Effects of citalopram 10.0 μmol/kg and WAY 100635 1.0 μmol/kg or GR 127935 1.0 μmol/kg, on extracellular 5-HT levels in ventral hippocampus. Citalopram 10.0 μmol/kg (■, $n = 6$), citalopram 10.0 μmol/kg and WAY 100635 1.0 μmol/kg (●, $n = 5$), citalopram 10.0 μmol/kg and GR 127935 1.0 μmol/kg (▲, $n = 5$) ($^{*}P < 0.05$).

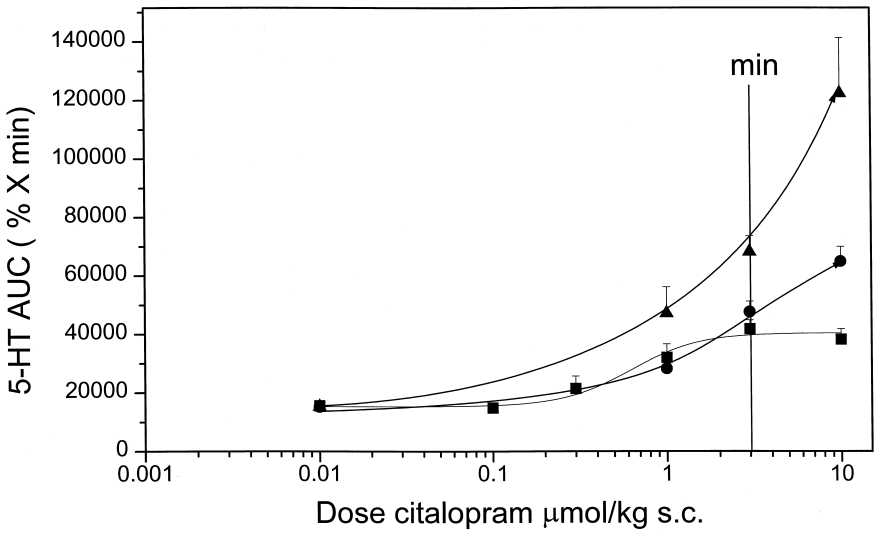

Fig. 8. Effects of citalopram 10 μmol/kg, alone, or combined with WAY 100635 1.0 μmol/kg or GR 127935 1.0 μmol/kg. Data are presented as AUC from $t = 0$–150 min. Citalopram (■), citalopram combined with WAY 100635 (●), citalopram combined with GR127935 (▲). Min denotes entrance of plasma citalopram levels in the therapeutic window.

($F(1,107) = 2.73$, $P < 0.05$), citalopram 10 μmol/kg s.c. ($F(1,118) = 14.6$, $P < 0.05$) (Figs. 5 and 6, respectively). Conversion of these data into AUCs (0–150 min) clearly shows that augmentation by the 5-HT_{1B} receptor antagonist is already evident at low doses of the selective serotonin reuptake inhibitor, while under the same conditions the 5-HT_{1A} receptor antagonist is devoid of effect (Fig. 8).

4. Discussion

The present study clearly demonstrates that in contrast to a 5-HT_{1B} receptor antagonist, augmentation by a 5-HT_{1A} receptor antagonist critically depends on the dose of citalopram. Several microdialysis studies have investigated the acute effects of selective serotonin reuptake inhibitors on extracellular 5-HT levels, but none of them has related these data to plasma concentrations of the selective serotonin reuptake inhibitor. To our knowledge, this is the first microdialysis study is the first in which measurement of pharmacodynamic effects of a selective serotonin reuptake inhibitor is combined with an estimation of some of its pharmacokinetic parameters.

4.1. Pharmacokinetic experiments

Restrictions imposed by the detection limit of the citalopram assay and the maximal amount of plasma that could reasonably be taken from the canula in the jugular vein made it necessary to use cardiac puncture (5 ml) for the lowest dose of citalopram. Concentration of these samples enabled us to estimate plasma levels even at the lowest dose of citalopram (0.1 μmol/kg). Due to large variation of citalopram plasma levels 15 min after injection, blood samples were taken 30 min after administration. Citalopram plasma levels measured at this time point displayed a linear relationship with the corresponding doses of 0.1, 3.0 and 10.0 μmol/kg. In principle, the plasma levels of all other doses used in the present study can be estimated from this linear relation.

Evaluation of treatment studies with citalopram in major depression indicates that clinically effective plasma levels range between 0.12 and 0.88 μM (Baumann, 1992). In the present study in rats, similar plasma levels of citalopram were obtained only at doses of 3 and 10 μmol/kg. At the highest dose of citalopram, plasma levels were maintained within this range for at least 4 h. Due to rapid elimination in rats, plasma levels of the lower dose already dropped below 0.12 μM after 45 min. The pharmacokinetic parameters after 10 μmol/kg citalopram are somewhat different from the data initially published by Overø et al., and indicate a faster elimination in the present study (Fredricson Overø, 1982). Different rat strains, route of administration and protocols may be responsible for this discrepancy (Fredricson Overø, 1982).

Although it is realized that pharmacokinetics and pharmacodynamics differ between rats and humans, we used the reported clinically effective plasma levels as a starting point for our experiments.

In vivo citalopram is metabolized into several metabolites, of which the desmethyl and the didesmethyl analogues are the most prominent (Øyehaug et al., 1982).

Plasma levels of the metabolites, in depressive patients treated with citalopram under steady state conditions, have

been reported to be at about two to three times lower as with the parent compound (Baumann, 1992). Compared to citalopram, the metabolites have a 10 times lower affinity for the 5-HT reuptake site. Arguably, their pharmacological effects, if any, will not be predominant (Hyttel, 1994). In the present study, we could not detect any of the citalopram metabolites.

4.2. Pharmacological experiments

In the present study, systemic administration of citalopram induced a dose dependent increase of extracellular 5-HT levels in the ventral hippocampus of the rat. This observation is in agreement with several other studies (Bel and Artigas, 1992; Hjorth et al., 1997; Invernizzi et al., 1997; Romero and Artigas, 1997; Gundlah et al., 1998), review by Fuller (1994). At higher doses, the effect of citalopram reached a plateau value. Suprisingly, citalopram plasma concentrations at this plateau value were in the same range as reported for clinically effective plasma levels (Baumann, 1992).

The relation between dose of the selective serotonin reuptake inhibitor and its effect on extracellular 5-HT levels has not very well been investigated. Hjorth et al., using doses of citalopram comparable to those in the present study, reported that increasing the citalopram dose from 0.5 to 5 mg/kg (1.25 and 12.5 μmol/kg, respectively) did not proportionally increase its effect on 5-HT levels in the ventral hippocampus (Hjorth et al., 1997). The latter indicates that plateau values had been reached somewhere between these doses, which is in agreement with our data.

In the present study, administration of 0.1 μmol/kg citalopram had no effect on 5-HT levels in the ventral hippocampus. The next dose of 0.3 μmol/kg transiently increased extracellular 5-HT. Plasma level of citalopram ($t = 30$ min) at the lowest dose was approximately 10 nM. This plasma value should be kept in mind when performing chronic experiments with citalopram. If experiments after cessation of chronic treatment are performed when plasma levels are still above this value, effects of pharmacological probes may be interfered by residual effects of the selective serotonin reuptake inhibitor.

Activation of somatodendritic 5-HT_{1A} autoreceptors reduces neuronal firing of 5-HT raphe nuclei cells (Arborelius et al., 1994). Selective serotonin reuptake inhibitors have been shown to increase extracellular 5-HT in both terminal and cell body areas. The increase in extracellular 5-HT in the cell body region activates 5-HT_{1A} autoreceptors, thereby decreasing 5-HT neuronal firing (Chaput et al., 1986). The latter process counteracts the primary effect of the selective serotonin reuptake inhibitor. In theory, co-administration of a 5-HT_{1A} receptor antagonist should prevent this secondary effect of the selective serotonin reuptake inhibitor. Indeed, several studies have shown that co-administration of a 5-HT_{1A} receptor antagonist potentiates the effect of a selective serotonin reuptake inhibitor on extracellular 5-HT (Hjorth et al., 1996; Gobert et al., 1997; Gundlah et al., 1997). The present study corroborates and extends these findings. The 5-HT_{1A} receptor antagonist WAY 100635 potentiated the effect of citalopram on extracellular 5-HT, but the effect appeared critically dependent on the plasma level of the selective serotonin reuptake inhibitor. Apparently, a certain level of feedback regulation is necessary to show the effect of a 5-HT_{1A} receptor antagonist. This finding supports the study by Hjorth et al. (1997), wherein WAY 100635 augmented the effect of a high, but not a low dose of citalopram.

In addition to the dependence of augmentation on the selective serotonin reuptake inhibitor plasma level, Hjorth et al. also showed that this effect is critically dependent on the dose of 5-HT_{1A} antagonist (Hjorth et al., 1997). In order to eliminate the possibility that augmentation remained absent due to insufficient presence of 5-HT_{1A} receptor antagonist, a supramaximal dose was administered. Co-administration of an even higher amount of Way 100635 (10 μmol/kg) revealed that the augmentation was maximal with regards to the dose of 5-HT_{1A} antagonist administered (data not shown).

In addition to the somatodendritic 5-HT_{1A} autoreceptor mediated feedback, 5-HT release is also controlled by 5-HT_{1B} terminal autoreceptors. Accordingly, simultaneous systemic administration of the putative 5-HT_{1B} receptor antagonist GR 127935 and an selective serotonin reuptake inhibitor has been shown to augment the effect of the latter on extracellular 5-HT (Rollema et al., 1996; Gobert et al., 1997; Sharp et al., 1997).

In contrast with WAY 100635, augmentation by GR 127935 appeared independent of the dose of citalopram. Apparently the level of feedback regulation is less critical for 5-HT_{1B} receptor antagonists.

Notably, GR 127935 also has moderate to high affinity for 5-HT_{1D} receptors, suggesting that there is room for at least partial involvement of somatodendritic 5-HT_{1D} autoreceptors in the observed effects (Sprouse et al., 1997). Arguably, blockade of the somatodendritic 5-HT_{1D} autoreceptors further increases 5-HT levels in the cell body region. Theoretically, this will lead to an increased activation of 5-HT_{1A} autoreceptors in the same area, thereby decreasing firing rate and terminal 5-HT release. The latter effect would counteract the effect of GR 127935 through terminal 5-HT_{1B} autoreceptors. Anyhow, the strong potentiation of the effect of citalopram by GR 127935 suggests that an effect through 5-HT_{1D} receptors, if any, is of minor importance at this dose of antagonist.

Arguably, pharmacokinetic interactions between citalopram and the 5-HT receptor antagonists could also play a role. However, citalopram concentrations, measured locally in the brain by means of microdialysis, were not affected by co-administration of either WAY 100635 or GR 127935, which contradicts any putative influence of the antagonists on the bio-availability of citalopram (data

not shown). In theory, citalopram could also influence the availability of the 5-HT antagonists in the brain. For this reason a supra maximal antagonist dose of WAY 100635 was chosen to minimize the risk that an increased availability of the antagonist would further enhance the effect on 5-HT levels. Although no effort was made to relate the effects of GR 127935 to its dose, it can be stated that the observed effects of the compound would be even stronger at higher doses, thereby decreasing the necessity to the use of a higher dose in the present study. Moreover, citalopram has been shown to lack important pharmacokinetic interactions through inhibition of cytochrome p450 *iso*-enzymes, which renders increase of availability of the antagonist rather unlikely (Sproule et al., 1997).

4.3. Pharmacodynamic and pharmacokinetic considerations

In the present study, potentiation of citalopram's effect on extracellular 5-HT by the 5-HT_{1A} receptor antagonist WAY 100635 appeared critically dependent on the plasma levels of the selective serotonin reuptake inhibitor. It is of note that augmentation could only be observed when the effect of citalopram had reached its plateau value. Much to our surprise, the corresponding citalopram plasma levels were similar to clinically effective plasma levels of the drug. Despite the fact that it is difficult or even impossible to extrapolate data obtained from rats to humans, this still may have implications for selective serotonin reuptake inhibitor augmentation studies with 5-HT_{1A} receptor antagonists in depressed patients.

For example, pindolol augmentation studies in major depression have yielded mixed results (McAskill et al., 1998), which may be partly due to differences in selective serotonin reuptake inhibitor plasma levels between patients. It can be speculated that patients with high selective serotonin reuptake inhibitor plasma concentrations would benefit more from the addition of pindolol than patients with lower plasma values.

Selective 5-HT_{1B} receptor antagonists may have great potential in selective serotonin reuptake inhibitor augmentation strategies. The present study indicates that this type of augmentation is more prominent than through 5-HT_{1A} receptors and is not restricted to clinically effective plasma levels of the selective serotonin reuptake inhibitor. It can be argued that they may be superior to 5-HT_{1A} receptor antagonists in hastening response to antidepressant treatment. Moreover, they may be beneficial for patients with low selective serotonin reuptake inhibitor plasma levels, viz. rapid metabolizers. An additional argument for this combination could be the use of a lower dose of the selective serotonin reuptake inhibitor, which may diminish side effects.

In view of the latter considerations, we expect that pharmacokinetics will become increasingly important in future clinical studies.

Assessment of pharmacokinetic parameters is also indispensable in pre-clinical studies. For example, desensitization of somatodendritic 5-HT_{1A} autoreceptors is believed to be the result of chronic activation of this receptor type. It can be argued that selective serotonin reuptake inhibitor plasma concentrations should be kept within the therapeutic window to insure permanent activation. Evaluation of chronic treatment studies suggests a relation between the latter criterion and desensitization of 5-HT_{1A} autoreceptors (Chaput et al., 1986; Moret and Briley, 1990, 1996; Invernizzi et al., 1994). Authors who failed to maintain selective serotonin reuptake inhibitor levels within the therapeutic window, consequently failed to observe effects upon chronic treatment (Hjorth and Auerbach, 1994; Auerbach and Hjorth, 1995; Bosker et al., 1995a,b; Gundlah et al., 1997).

One might suppose that the relation found in the present study between 5-HT_{1A} and 5-HT_{1B} receptor antagonist's effects and selective serotonin reuptake inhibitor plasma levels may be indicative for the occurrence of desensitization processes. Following this reasoning, 5-HT_{1B} receptors would desensitize irrespective of selective serotonin reuptake inhibitor plasma levels. This happens not to be the case. Desensitization of terminal 5-HT_{1B} receptors is even more controversial than is the case with 5-HT_{1A} autoreceptors (Chaput et al., 1986; Auerbach and Hjorth, 1995; Bosker et al., 1995a,b). Apparently, differences in animal strain, selective serotonin reuptake inhibitor and brain area also play an important role in explaining discrepant findings.

4.4. Conclusion

The effect of citalopram on extracellular 5-HT is potentiated by a 5-HT_{1A} and a 5-HT_{1B} receptor antagonist, albeit in a different dose range of the selective serotonin reuptake inhibitor. Moreover, the study indicates that assessment of selective serotonin reuptake inhibitor pharmacokinetics could be important for evaluating their pharmacological effects in relation to the clinical effects. It should be realized that extrapolation to human studies should be done carefully, taking into account differences in brain selective serotonin reuptake inhibitor concentrations, receptor phamacology, and neuroanatomy.

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