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# Effects of acute and chronic tianeptine administration on serotonin outflow in rats: comparison with paroxetine by using in vivo microdialysis

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**Abstract**

Using in vivo microdialysis, we compared the effects of tianeptine (an antidepressant drug which, in marked contrast with other antidepressants, is thought to increase the uptake of serotonin (5-hydroxytryptamine, 5-HT) on extracellular 5-HT concentrations ($[5\text{-HT}]_{ext}$) in the frontal cortex and raphe nuclei of freely moving rats with those of paroxetine, a potent selective serotonin reuptake inhibitor. A single paroxetine dose (1 mg/kg, i.p.) increased $[5\text{-HT}]_{ext}$ over baseline in the frontal cortex and raphe nuclei, respectively. A single administration of tianeptine (10 mg/kg, i.p.) did not change $[5\text{-HT}_{ext}]$ in the two brain regions studied. Repeated exposure to paroxetine (0.5 mg/kg) b.i.d. for 14 days induced a sixfold significant increase in basal $[5\text{-HT}]_{ext}$ in the raphe nuclei. Administration of tianeptine (5 mg/kg) b.i.d. for 14 days did not affect 5-HT baseline concentrations. In rats chronically treated with either paroxetine or tianeptine, drug challenge did not alter area under the curve values. Thus, our in vivo data indicate that tianeptine and paroxetine do not exert a similar in vivo effect on the serotonergic system in rat brain. 

*Keywords:* 5-HT (5-hydroxytryptamine, serotonin); Paroxetine; Tianeptine; Frontal cortex; Raphe nuclei; Microdialysis, in vivo

## 1. Introduction

Tianeptine 7-[(3-chloro-6,11-dihydro-5,5-dioxo-6-methyldibenzo[c,f][1,2] thiazepin-11-yl) amino] heptanoic acid, sodium salt, is an antidepressant drug with clinical efficacy (Cassano et al., 1996; Costa e Silva et al., 1997; Ginestet, 1997). In contrast with most antidepressant drugs which have been shown to inhibit the neuronal uptake of monoamines, tianeptine enhances [$^3$H]5-HT (5-hydroxytryptamine, 5-HT) uptake as suggested by studies performed ex vivo with cortical and hippocampal synaptosomes of rats treated either acutely or chronically with this drug (Mennini et al., 1987; Fattaccini et al., 1990). Using in vivo microdialysis, it has been shown that tianeptine, given either systemically as a single dose or as daily doses for 14 days, partially inhibits the $K^+$-induced increase in extracellular concentrations of 5-HT ($[5\text{-HT}]_{ext}$) in the ventral hippocampus of rats (Whitton et al., 1991) and the 5-hydroxytryptophan-induced increase in $[5\text{-HT}]_{ext}$ in the frontal cortex (Dalta and Curzon, 1993). Furthermore, the "5-HT syndrome" induced by administration of tryptophan and tranylcypromine to rats is significantly reduced by tianeptine pre-treatment (De Simoni et al., 1992).

At that time, some features restricted the use of the in vivo microdialysis technique such as the detection limit of 5-HT in the dialysate when using the high-performance liquid chromatography (HPLC) coupled with an electrochemical detector. Thus, previous studies were performed using the evoked-release of 5-HT (e.g., by local perfusion of KCl or administration of 5-hydroxytryptophan) and used the local perfusion of the brain region of interest with

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a medium containing 1 μM of citalopram, a serotonin selective reuptake inhibitor, to raise artificially basal [5-HT]$_{ext}$ up to a detectable level (Whitton et al., 1991). Although this allowed investigators to measure reliably tianeptine-induced decreases in [5-HT]$_{ext}$, the presence of a selective serotonin reuptake inhibitor probably modified the effects of various drugs on 5-HT release, as reported by many authors (Invernizzi et al., 1992; Kreiss et al., 1993). In support of this hypothesis, after infusion of a selective serotonin reuptake inhibitor into a serotoninergic nerve terminal region to block maximally the reuptake of 5-HT, the peripheral injection of a 5-HT reuptake blocker induced a decrease in [5-HT]$_{ext}$ (Rutter and Auerbach, 1993). Thus, the excess of endogenous [5-HT$_{ext}$] produced by the acute local blockade of the selective 5-HT transporter may activate 5-HT autoreceptors of the 5-HT$_{1A}$ and 5-HT$_{1B}$ sub-types, thus leading to a decrease in the firing rate of 5-HT neurones as well as in 5-HT release at nerve terminals (Artigas, 1993; Gardier et al., 1996).

Indirect measurement of 5-HT neurotransmission by means of 5-hydroxyindolacetic acid (5-HIAA) voltammetric measurements has also been performed in rats to study the influence of tianeptine on 5-HT catabolism, the intensity of which depends mainly upon the activity of monoamine oxidase type A (Marinesco et al., 1996). This study showed that tianeptine increased the availability of of serotonin to monoamine oxidase type A, which is synthesized within non-serotonergic neurons and glial cells.

Recent improvements in the microdialysis procedure, such as lowering the rate of perfusion of the microinjection pump, design of home-made concentric dialysis probes (with higher in vitro recovery), together with an improved detection limit of HPLC detectors, allow us to reconsider the effects of tianeptine on [5-HT]$_{ext}$ by using in vivo microdialysis approach in awake, freely moving rats. Thus, the effects of tianeptine and paroxetine were compared regarding changes in [5-HT]$_{ext}$ measured simultaneously in each animal in two different brain areas, a serotoninergic nerve terminal region, the frontal cortex, and nearby cell bodies and dendrites of serotoninergic neurones, the raphe nuclei. Following the daily administration of the drugs for 14 days, we measured the effects of a challenge dose of either paroxetine or tianeptine on [5-HT]$_{ext}$ in the frontal cortex and raphe nuclei of rats.

## 2. Materials and methods

### 2.1. Surgery

Microdialysis experiments were carried out with male Sprague–Dawley rats (200–300 g, Charles River, France). Animals were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. The size of the dialysis membranes was 3.5 mm long × 0.30 mm OD for the two regions studied; the membranes were made of a regenerated cellulose cuprophane-like material. Rats were implanted with two concentric probes following coordinates (in mm) taken from bregma and skull surface (Paxinos and Watson, 1986): one in the right frontal cortex (A +3.5, L +2.5, V −4) and the second in the dorsal and median raphe nuclei (A −7.8, L −0.4, V −9). In this latter case, we placed the probe very close to the dorsal and median raphe nuclei, as described by Adell and Artigas (1991). Therefore, mechanical lesioning of both nuclei and perforation of the cerebral aqueduct were prevented. The probes were then cemented in place.

### 2.2. Dialysis procedure

The animals were allowed to recover from surgery for approximately 20 h, and then the probes were continuously perfused with an artificial cerebrospinal fluid (composition in mM: NaCl 147, KCl 3.5, $CaCl_2$ 1.0, $MgCl_2$ 1.2, $NaH_2PO_4$ 1.0, $NaHCO_3$ 25.0, pH 7.4 ± 0.2) at a flow rate of 1.3 μl/min, using a CMA/100 Microinjection Pump (Carnegie Medicin, Stockholm, Sweden). Dialysate samples were collected every 15 min in small Eppendorf tubes. The HPLC analysis of 5-HT was carried out as described (Malagié et al., 1995), except that the electrochemical detector was a Hewlett Packard 1049 (Les Ulis, France). The limit of sensitivity for 5-HT was typically ≈ 0.5 fmol/sample.

### 2.3. Drugs and treatment

Paroxetine hydrochloride (SmithKline Beecham, UK) and tianeptine (Servier, France) were dissolved in NaCl 0.9% and administered in a volume of 2 ml/kg intraperitoneally (i.p.). Controls received an appropriate volume of NaCl 0.9% (2 ml/kg, by the i.p. route) twice daily for 14 days, and then received one injection of NaCl 0.9% as a challenge dose the day of the microdialysis experiment (Group 1). In two groups of rats (Group 2 and Group 3), either NaCl 0.9% or paroxetine (0.5 mg/kg) was injected i.p. twice daily for 14 days. In two other groups of rats (Group 4 and Group 5), either NaCl 0.9% or tianeptine (5 mg/kg) was injected i.p. twice daily for 14 days. Rats received drug treatments twice daily for 14 days (9 a.m. and 6 p.m.), and then their corresponding challenge dose 16 h after the last drug administration. This schedule was chosen to optimize the delay between the last drug injection and the start of dialysate collection. At this time, four to five basal fractions were collected to obtain basal extracellular 5-HT values (means ± S.E.M.). Then, rats from the four drug-treated groups received the challenge dose of either paroxetine (1 mg/kg, i.p.; Group 2 and Group 3) or tianeptine (10 mg/kg, i.p.; Group 4 and Group 5). Response to drug administration was determined over a 3-h period. At the end of the experiments, placement of microdialysis probes was verified histologically.

### 2.4. Data analysis and statistics

Statistical analyses were performed by using the computer software StatView 4.02 (Abacus Concepts, Berkeley, CA, USA). Data (not corrected for in vitro recovery) are expressed as a percentage of the basal value (means ± S.E.M.). To compare $[5\text{-HT}]_{ext}$ to the respective basal value in each group of treated animals, statistical analysis was carried out using a 1-way analysis of variance (ANOVA) for repeated measures on time, followed by Fisher Protected Least Significance Difference (PLSD) post-hoc test. Furthermore, using percentage data, net changes in dialysate 5-HT were determined by means of the area under the curve (AUC) values calculated for the amount of 5-HT outflow collected during the 0–150 min period. Statistical comparisons of these AUCs calculated for the two brain regions studied following administration of either paroxetine or tianeptine were made by applying a 2-way ANOVA with [A] the drug treatment and [B] the brain region as main factors. Significance level was set at $P < 0.05$.

## 3. Results

### 3.1. Effect of chronic treatment with paroxetine or tianeptine on basal [5-$HT_{ext}$]

In the frontal cortex, basal $[5\text{-HT}]_{ext}$ concentrations from animals receiving a single dose of either NaCl 0.9%, paroxetine or tianeptine were not significantly different from those of animals receiving chronic treatment with either paroxetine or tianeptine for 14 days ($F(4,67) = 2.3$, $P = 0.07$; Table 1). In the raphe nuclei, a 14-day treatment with paroxetine 0.5 mg/kg b.i.d. induced a sixfold increase in basal $[5\text{-HT}]_{ext}$ when compared to baseline in control saline-treated rats [$F(4,73) = 39.2$, $P < 0.001$], while a chronic treatment with tianeptine 5 mg/kg b.i.d. did not alter basal $[5\text{-HT}_{ext}]$ concentrations.

### 3.2. Effect of a challenge dose of paroxetine on 5-HT extracellular concentrations in naive rats and in rats chronically treated with paroxetine

Administration of a single dose of paroxetine (1 mg/kg i.p.) in naive rats induced a significant increase in $[5\text{-HT}]_{ext}$ in both the frontal cortex [$F(12,72) = 8.9$, $P < 0.001$, Fig. 1] and raphe nuclei [$F(12,84) = 11.2$, $P < 0.001$, Fig. 2]. The maximal increases were to $+329 \pm 50\%$ and $+294 \pm 27\%$ of the respective basal value (100%) in the frontal cortex and raphe nuclei, respectively. The corresponding AUC values calculated for 5-HT outflow collected during the 0–150 min period were $263 \pm 40$ and $261 \pm 29$, respectively (Fig. 5). A challenge dose of paroxetine (1 mg/kg i.p.) in rats chronically treated with paroxetine (0.5 mg/kg, i.p. twice daily for 14 days) did not change $[5\text{-HT}]_{ext}$ in the frontal cortex [$F(12,60) = 1.1$, $P = 0.39$, Fig. 1] while it decreased $[5\text{-HT}]_{ext}$ in the raphe nuclei [$F(12,72) = 6.9$, $P < 0.001$, Fig. 2]. In this latter region, the maximal decrease was to $-78 \pm 5\%$ of the respective basal value (100%) at $t = 150$ min following the paroxetine challenge. The corresponding AUC values were $130 \pm 16$ and $91 \pm 4$ in the frontal cortex and raphe nuclei, respectively (Fig. 5).

### 3.3. Effect of a challenge dose of tianeptine on 5-HT extracellular concentrations in naive rats and in rats chronically treated with tianeptine

Administration of a single dose of tianeptine (10 mg/kg, i.p.) either in naive or in chronically treated rats did not change $[5\text{-HT}]_{ext}$ in either the frontal cortex [$F(12,84) = 1.7$, $P = 0.09$ and $F(12,72) = 0.8$, $P = 0.67$, respectively, Fig. 3] or the raphe nuclei [$F(12,84) = 2.0$, $P = 0.03$ and $F(12,96) = 1.6$, $P = 0.09$, respectively, Fig. 4]. The corre-

Table 1
Basal extracellular concentrations of serotonin (5-HT) in fmol/20 μl measured in the frontal cortex and the raphe nuclei of rats following treatment with either paroxetine or tianeptine. Each value is the mean ± S.E.M. of several determinations indicated in parentheses

| Treatment | Frontal cortex | Raphe nuclei |
|---|---|---|
| *Group 1:* Chronic NaCl 0.9% (b.i.d. × 14 days) followed by an acute NaCl 0.9% challenge | 2.85 ± 0.35 ($n = 14$) | 8.72 ± 1.72 ($n = 14$) |
| *Group 2:* Chronic NaCl 0.9% (b.i.d. × 14 days) followed by a paroxetine challenge (1 mg/kg) | 3.64 ± 0.49 ($n = 14$) | 8.25 ± 0.83 ($n = 16$) |
| *Group 3:* Chronic paroxetine (0.5 mg/kg b.i.d. × 14 days) followed by a paroxetine challenge (1 mg/kg) | 4.08 ± 0.61 ($n = 12$) | 46.87 ± 5.81 ($n = 14$)[a] |
| *Group 4:* Chronic NaCl 0.9% (b.i.d. × 14 days) followed by a tianeptine challenge (10 mg/kg) | 2.06 ± 0.31 ($n = 16$) | 11.52 ± 1.92 ($n = 16$) |
| *Group 5:* Chronic tianeptine (5 mg/kg b.i.d. × 14 days) followed by a tianeptine challenge (10 mg/kg) | 3.08 ± 0.74 ($n = 14$) | 6.83 ± 0.6 ($n = 18$) |

[a] $P < 0.001$, one-way ANOVA followed by Fisher PLSD multiple comparison test when compared to 5-HT extracellular level of the control group in the same brain area.

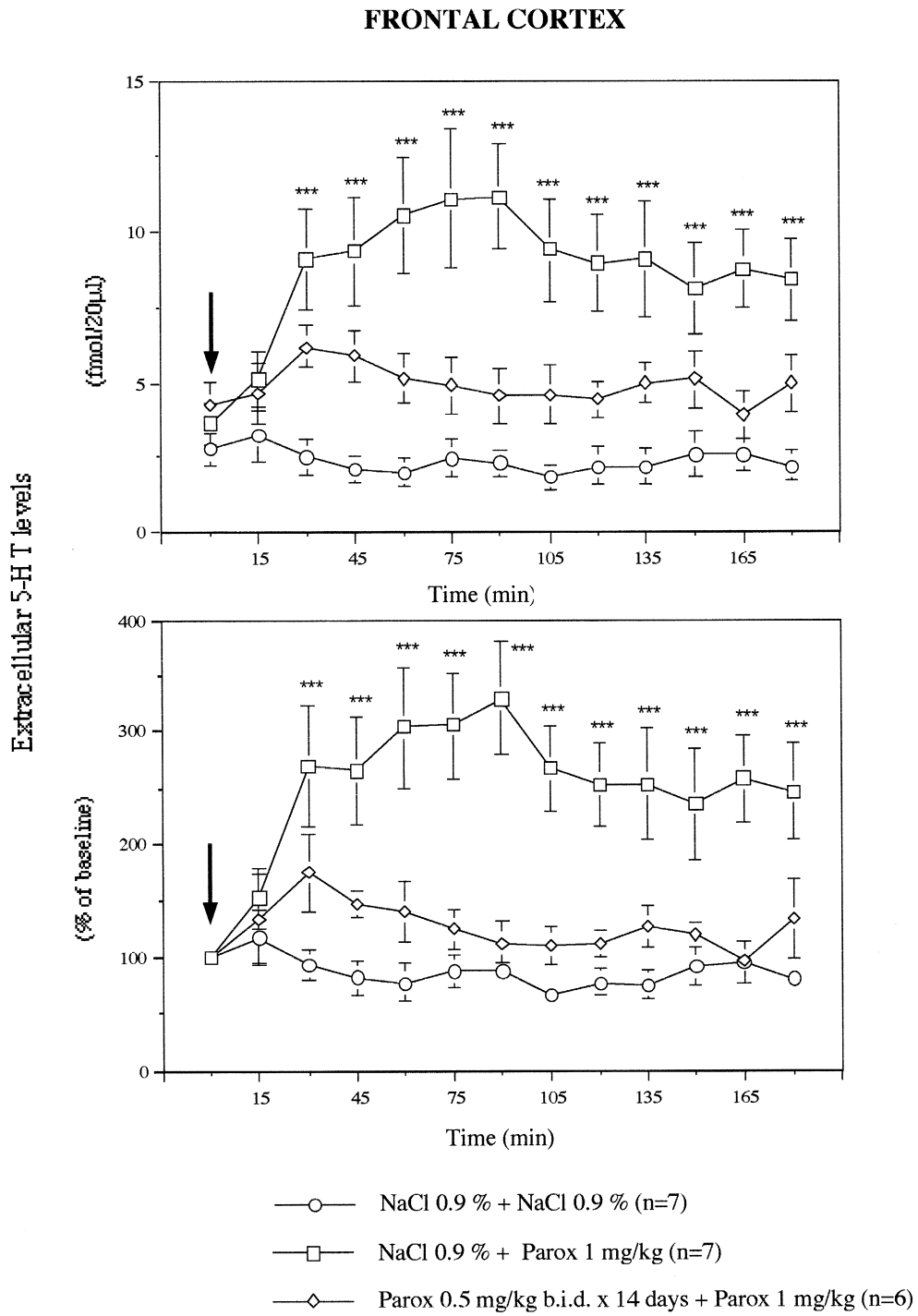


Fig. 1. Effect of a challenge dose of paroxetine (1 mg/kg) in naive rats (Group NaCl 0.9% b.i.d. for 14 days + paroxetine 1 mg/kg as a challenge dose; —□—) and in rats chronically treated with paroxetine (Group paroxetine 0.5 mg/kg b.i.d. for 14 days + paroxetine 1 mg/kg challenge; —◇—) on extracellular 5-HT concentrations measured in the frontal cortex of freely moving rats. Controls receiving NaCl 0.9% (2 ml/kg, by the i.p. route) twice daily for 14 days, followed by one injection of NaCl 0.9% as a challenge dose the day of the microdialysis experiment are also represented (Group NaCl 0.9% b.i.d. for 14 days + a NaCl 0.9% challenge; —○—). Data are expressed in fmol/20 µl (upper panel) or as percentage of baseline concentrations (lower panel). Each point represents the mean ± S.E.M. for 6–7 animals. The challenge dose of paroxetine was administered at $t = 0$ (arrow). $^{***}P < 0.001$, ANOVA for repeated measures followed by Fisher PLSD multiple comparison test when compared to the respective basal value.

sponding AUC values were 123 ± 16 and 92 ± 7 in the frontal cortex and 106 ± 6 and 123 ± 5 in the raphe nuclei, in naive and in chronically treated rats, respectively (Fig. 5). In the raphe nuclei, a challenge dose of tianeptine (10 mg/kg i.p.) induced a slight but not statistically significant increase in $[5\text{-HT}]_{ext}$ in chronically treated rats; the maxi-

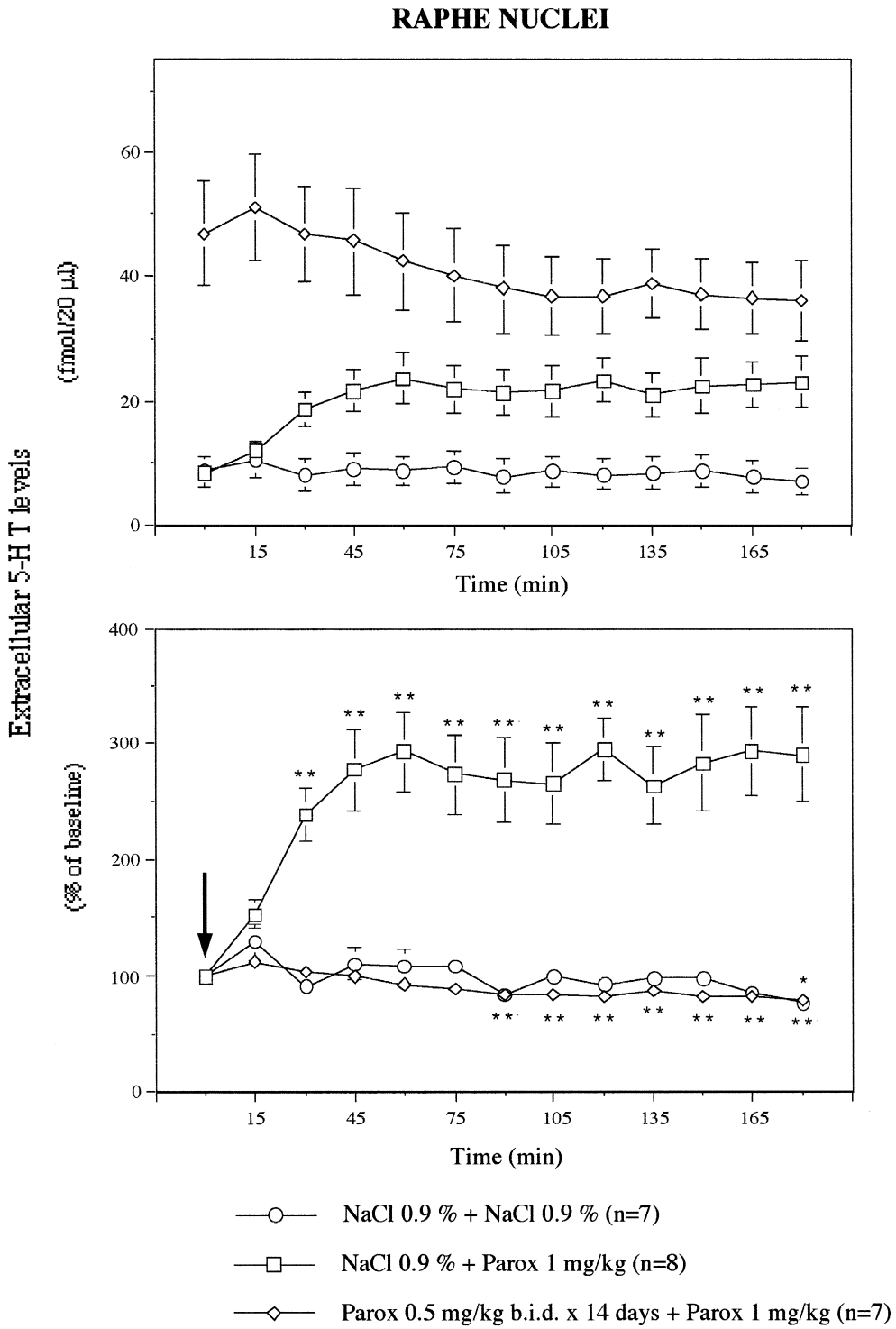


Fig. 2. Effect of a challenge dose of paroxetine (1 mg/kg) in naive rats (Group NaCl 0.9% b.i.d. for 14 days + paroxetine 1 mg/kg as a challenge dose; —□—) and in rats chronically treated with paroxetine (Group paroxetine 0.5 mg/kg b.i.d. for 14 days + paroxetine 1 mg/kg challenge; —◇—) on extracellular 5-HT concentrations measured in the raphe nuclei of freely moving rats. Controls receiving NaCl 0.9% (2 ml/kg, by the i.p. route) twice daily for 14 days, followed by one injection of NaCl 0.9% as a challenge dose the day of the microdialysis experiment are also represented (Group NaCl 0.9% b.i.d. for 14 days + a NaCl 0.9% challenge; —○—). Data are expressed in fmol/20 µl (upper panel) or as percentage of baseline concentrations (lower panel). Each point represents the mean ± S.E.M. for 7–8 animals. The challenge dose of paroxetine was administered at $t = 0$ (arrow). $^{*}P < 0.05$, $^{***}P < 0.001$, ANOVA for repeated measures followed by Fisher PLSD multiple comparison test when compared to the respective basal value.

mal increase was to $+150 \pm 19\%$ of the basal value (100%) at $t = 30$ min following the tianeptine challenge.

To summarize the data we obtained in the five different groups of rats, AUC values were calculated (Fig. 5). A

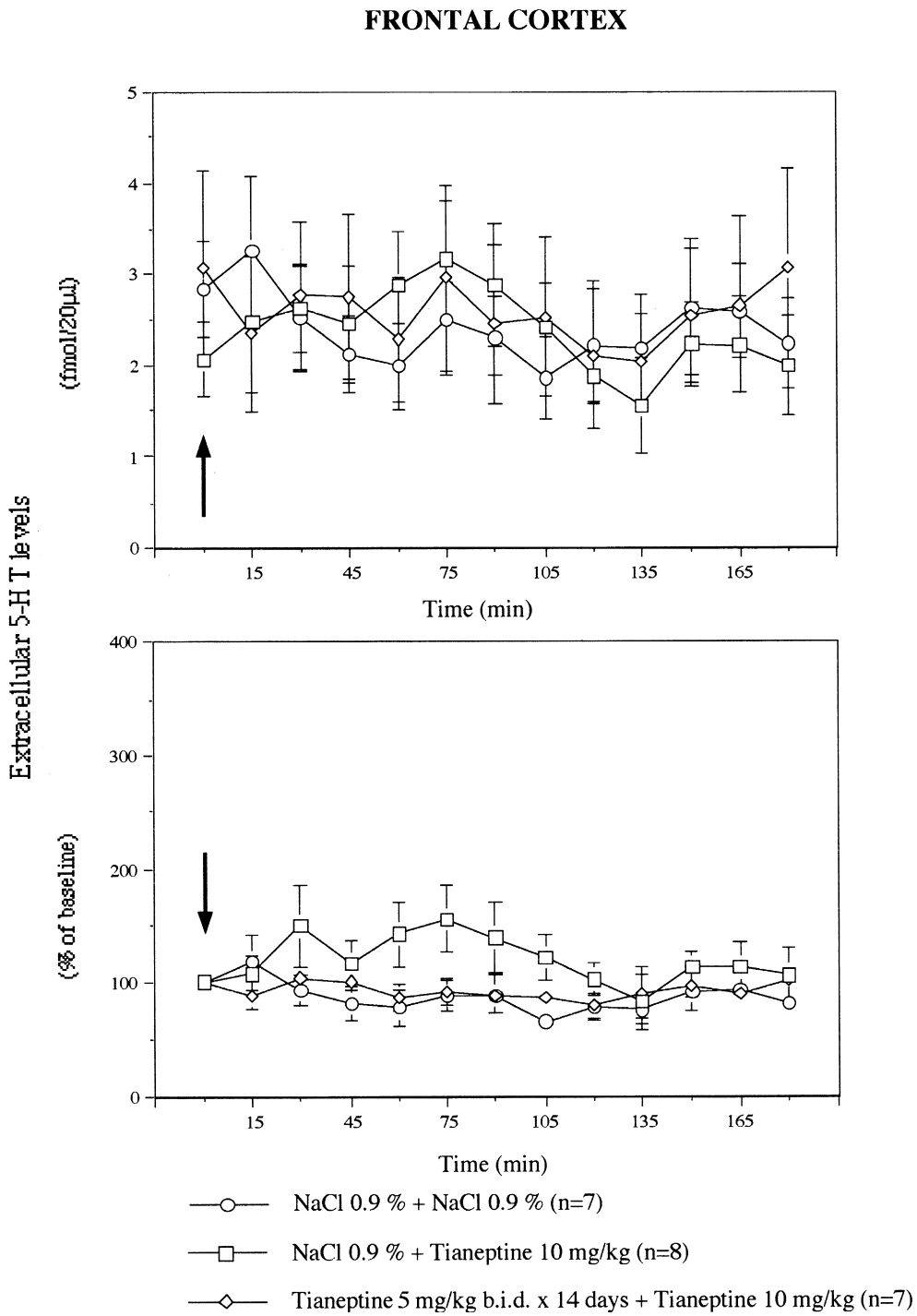


Fig. 3. Effect of a challenge dose of tianeptine (10 mg/kg) in naive rats (Group NaCl 0.9% b.i.d. for 14 days + tianeptine 10 mg/kg as a challenge dose; —□—) and in rats chronically treated with tianeptine (Group tianeptine 5 mg/kg b.i.d. for 14 days + tianeptine 10 mg/kg challenge; —◇—) on extracellular 5-HT concentrations measured in the frontal cortex of freely moving rats. Controls receiving NaCl 0.9% (2 ml/kg, by the i.p. route) twice daily for 14 days, followed by one injection of NaCl 0.9% as a challenge dose the day of the microdialysis experiment are also represented (Group NaCl 0.9% b.i.d. for 14 days + a NaCl 0.9% challenge; —○—). Data are expressed in fmol/20 µl (upper panel) or as percentage of baseline concentrations (lower panel). Each point represents the mean ± S.E.M. for 7–8 animals. The challenge dose of paroxetine was administered at $t = 0$ (arrow).

two-way ANOVA (drug treatment × brain region) on the AUC values revealed a significant effect of "drug treatment" [$F(4,65) = 31.2$, $P < 0.001$], but no significant effect of "brain region" [$F(1,65) = 0.1$, $P = 0.75$]. The absence of a significant interaction between the "treatment" factor and the "brain region" factor [$F(4,65) = 1.1$, $P = 0.37$] led us to perform further statistical analyses on the "treatment" factor only. Thus, a one-way ANOVA fol-

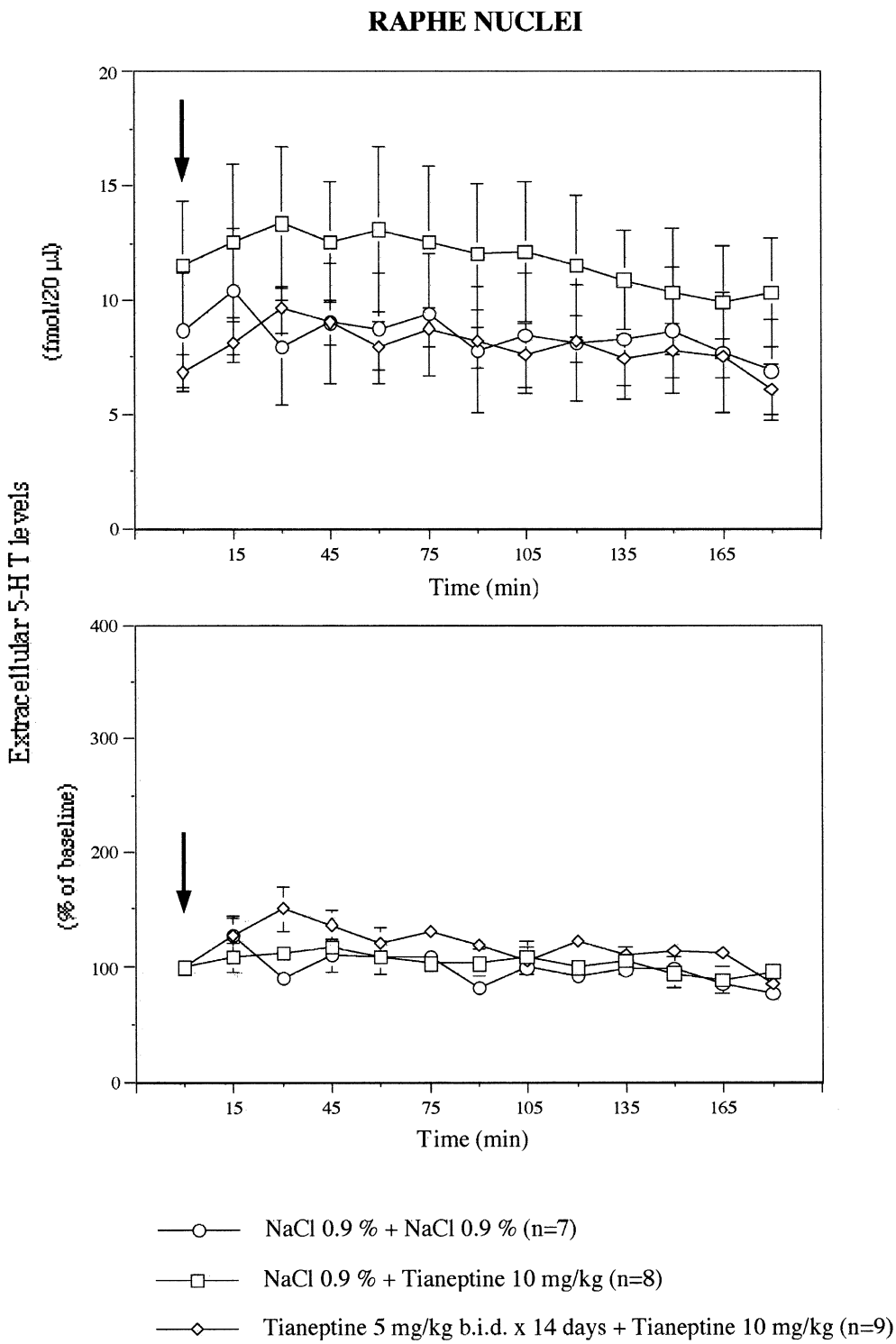


Fig. 4. Effect of a challenge dose of tianeptine (10 mg/kg) in naive rats (Group NaCl 0.9% b.i.d. for 14 days + tianeptine 10 mg/kg as a challenge dose; —□—) and in rats chronically treated with tianeptine (Group tianeptine 5 mg/kg b.i.d. for 14 days + tianeptine 10 mg/kg challenge; —◇—) on extracellular 5-HT concentrations measured in the raphe nuclei of freely moving rats. Controls receiving NaCl 0.9% (2 ml/kg, by the i.p. route) twice daily for 14 days, followed by one injection of NaCl 0.9% as a challenge dose the day of the microdialysis experiment are also represented (Group NaCl 0.9% b.i.d. for 14 days + a NaCl 0.9% challenge; —○—). Data are expressed in fmol/20 µl (upper panel) or as percentage of baseline concentrations (lower panel). Each point represents the mean ± S.E.M. for 7–9 animals. The challenge dose of paroxetine was administered at $t = 0$ (arrow).

lowed by post-hoc comparisons revealed that, in the frontal cortex [$F(4,35) = 11.6$, $P < 0.001$, Fig. 5A] and in the raphe nuclei [$F(4,38) = 24.5$, $P < 0.001$, Fig. 5B], the AUC values for naive rats treated with paroxetine 1 mg/kg

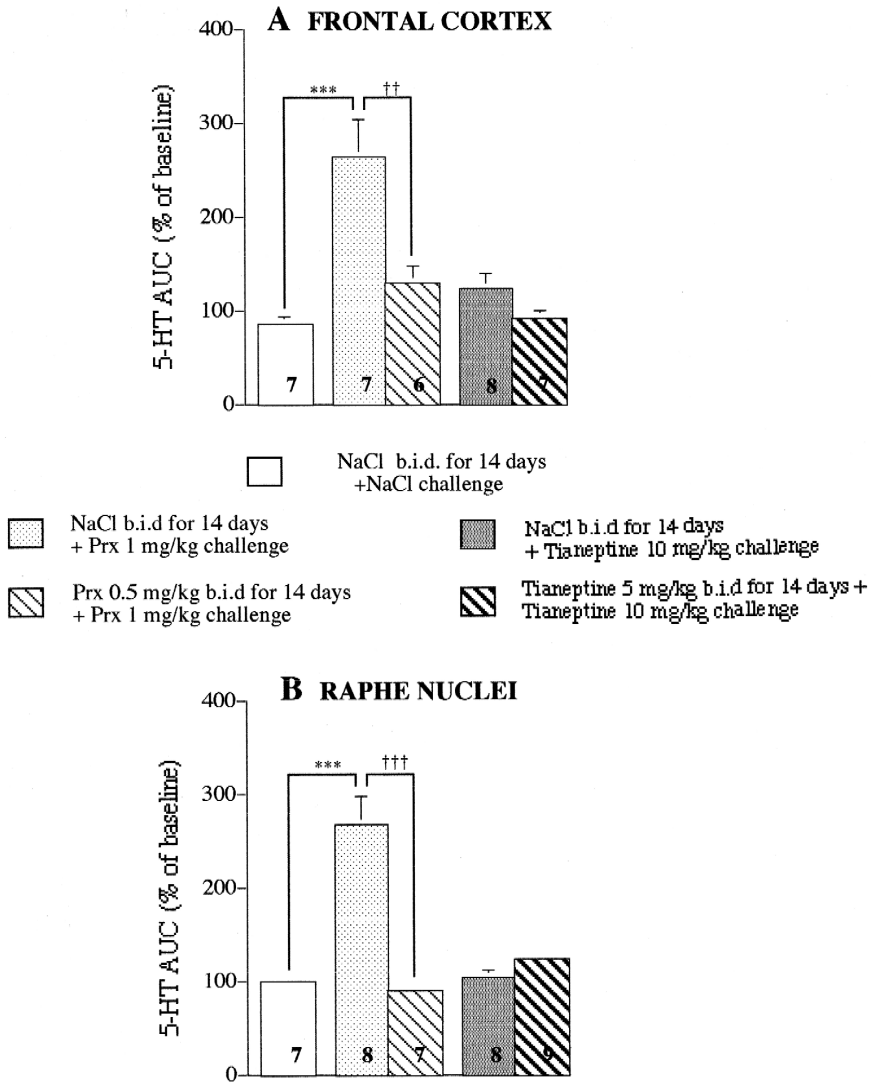


Fig. 5. Area under the curve (AUC) values (0–150 min) calculated for 5-HT outflow collected during the 0–150 min period in the five groups of rats described above, which were treated with either saline, paroxetine (Prx) or tianeptine. $^{***}P < 0.001$, one-way ANOVA followed by Fisher PLSD multiple comparison test when AUC values were significantly different from those of the control group in the same brain area; $††P < 0.01$, $†††P < 0.001$ versus the acutely treated group in the same brain area.

(a single dose) were significantly higher than in those for control rats. By contrast, a challenge dose of paroxetine administered to rats chronically treated with this selective serotonin reuptake inhibitor did not modify $[5\text{-HT}]_{ext}$ in the two brain areas studied. Furthermore, whatever the pretreatment, no significant difference was found between the AUC values calculated for the two brain areas after a challenge dose of tianeptine (Fig. 5A and B).

## 4. Discussion

The present results demonstrate that paroxetine and tianeptine do not induce similar changes in $[5\text{-HT}]_{ext}$ in frontal cortex and raphe nuclei, likely reflecting differences in their mechanism of action in rat brain. Indeed, a single administration of a low dose of paroxetine (1 mg/kg) induced a large increase in $[5\text{-HT}]_{ext}$ in both the frontal cortex and raphe nuclei, while in these brain areas, $[5\text{-HT}]_{ext}$ was not modified following a single administration of tianeptine (10 mg/kg). Earlier ex vivo studies with rat cortical and hippocampal synaptosomes have suggested that tianeptine is a 5-HT reuptake enhancer (Mennini et al., 1987; Fattaccini et al., 1990), but, in our in vivo model, tianeptine failed to induce a decrease in $[5\text{-HT}]_{ext}$ in the frontal cortex following single systemic administration. Thus, our data are in marked contrast with those of Curzon's group showing that tianeptine can reverse stimulus-evoked increases in $[5\text{-HT}]_{ext}$ (Whitton et al., 1991; Dalta and Curzon, 1993). Tianeptine also prevented the increase in plasma 5-HT concentrations induced by citalopram and paroxetine in rats, therefore suggesting that

tianeptine can interact with the 5-HT transporter under certain conditions (Ortiz et al., 1991). Conversely, our results agree with those of the literature suggesting that paroxetine, our reference selective serotonin reuptake inhibitor, behaves as a 5-HT reuptake blocker in vivo in rats (Hyttel, 1982; Thomas et al., 1987). Indeed, many microdialysis experiments have demonstrated that a single exposure to a selective serotonin reuptake inhibitor, including paroxetine, increases $[5\text{-HT}]_{ext}$ preferentially near the cell bodies and dendrites of serotoninergic neurones in the raphe nuclei compared to brain regions innervated by serotonergic nerve terminals such as the frontal cortex (fluvoxamine: Bel and Artigas, 1992; citalopram: Invernizzi et al., 1992; fluoxetine: Malagié et al., 1995; paroxetine: Gartside et al., 1995). The explanation widely given for this phenomenon is that serotonin autoreceptors limit the intrasynaptic availability of 5-HT at nerve terminals. Somatodendritic $5\text{-HT}_{1A}$ and terminal $5\text{-HT}_{1B}$ autoreceptors exert an inhibitory control on nerve terminal release of 5-HT (Artigas, 1993; Malagié et al., 1996; Roberts et al., 1998). Among these microdialysis studies, some have been performed in the frontal cortex and raphe nuclei of rats that are either awake (Bel and Artigas, 1992; Invernizzi et al., 1992) or anaesthetized (the other studies). In the present study performed with awake, freely moving rats, a single dose of paroxetine induced similar increases in $[5\text{-HT}]_{ext}$ in both the frontal cortex and raphe nuclei. The reason we failed to find a selective serotonin reuptake inhibitor-induced preferential effect in the raphe nuclei is unclear. One possibility could be that at a 1 mg/kg dose, paroxetine in rats has already had its maximal effect on $[5\text{-HT}]_{ext}$ in both brain regions since paroxetine is the most potent selective serotonin reuptake inhibitor available so far (Thomas et al., 1987). It should be noticed that Gartside et al. (1995) administered paroxetine to rats at doses of 0.8 and 2.4 mg/kg intravenously.

In vivo microdialysis experiments were also performed following repeated exposure to these drugs for 14 days. We found a sixfold increase in basal $[5\text{-HT}]_{ext}$ in the raphe nuclei following chronic treatment with paroxetine. So far, only increases in basal $[5\text{-HT}]_{ext}$ measured in serotoninergic nerve terminal areas have been found after chronic treatment with citalopram (Arborelius et al., 1996; Moret and Briley, 1996), fluoxetine (Rutter et al., 1994; Kreiss and Lucki, 1995; Invernizzi et al., 1996), fluvoxamine (Bel and Artigas, 1993) or paroxetine (Sayer et al., 1999). A two fold increase in basal $[5\text{-HT}]_{ext}$ in the raphe nuclei has also been observed in rats repeatedly treated with tranylcypromine, a monoamine oxidase inhibitor (Ferrer and Artigas, 1994). These discrepancies could be partly explained by the design of the experimental protocols. Indeed, some authors used osmotic minipumps for the long-term delivery of the selective serotonin reuptake inhibitor (Bel and Artigas, 1993; Ferrer and Artigas, 1994), while others repeatedly administered selective serotonin reuptake inhibitor to rats either b.i.d. or daily. Another difference between these microdialysis studies comes from the absence (Bel and Artigas, 1993; Moret and Briley, 1996) or the presence of a washout period between the last drug injection and the start of dialysate collection.

A challenge dose of paroxetine administered to chronically treated rats failed to increase $[5\text{-HT}]_{ext}$ in both the frontal cortex and raphe nuclei. This suggests that the prolonged administration of paroxetine induces adaptive changes either in a serotoninergic nerve terminal cortical region or in the raphe nuclei region. Indeed, extracellular unitary recordings and $[^3H]$-paroxetine binding assays have shown that repeated administration of paroxetine results in adaptive changes of the 5-HT transporter to its sustained occupation, such as a decrease in the number of 5-HT carriers (down regulation) in the latter two brain regions (Piñeyro et al., 1994). In addition, Lesch et al. (1993) found decreased 5-HT transporter mRNA concentrations in the raphe nuclei following long-term administration of fluoxetine to rats. This suggests that antidepressants regulate the 5-HT transporter at the level of gene expression. Thus, it is likely that repeated administration of selective serotonin reuptake inhibitor and long-term blockade of 5-HT reuptake lead to negative feedback mechanisms modifying the subsequent release/reuptake of 5-HT nearby cell bodies and dendrites of serotoninergic neurones in the raphe nuclei as well as in brain regions innervated by serotoninergic nerve terminals. Indeed, it has been recently demonstrated that chronic treatment with fluoxetine induced desensitization of presynaptic $5\text{-HT}_{1A}$ receptors but not of postsynaptic ones, thus suggesting that these adaptive changes in 5-HT neurotransmission exhibit marked regional differences (Le Poul et al., 2000). Furthermore, long-term blockade of 5-HT reuptake by selective serotonin reuptake inhibitor results in the desensitization of somatodendritic $5\text{-HT}_{1A}$ autoreceptors located on 5-HT neurones in the dorsal raphe nuclei (Chaput et al., 1986; Le Poul et al., 1995).

In the present microdialysis study, in long-term paroxetine-treated rats, we observed a blunted effect of a challenge dose of paroxetine in increasing dialysate 5-HT concentrations. Similarly, a challenge dose of tianeptine administered to chronically treated rats failed to increase $[5\text{-HT}]_{ext}$ in both the frontal cortex and raphe nuclei. However, that paroxetine and tianeptine induced similar effects on $[5\text{-HT}]_{ext}$ following their repeated administration does not mean a common origin for these two phenomena. A challenge dose of tianeptine administered either in naive rats or in tianeptine chronically treated rats gave similar results, i.e. it did not modify $[5\text{-HT}]_{ext}$ in the frontal cortex and raphe nuclei. Thus, although a challenge dose of either paroxetine or tianeptine did not induce any significant effect on $[5\text{-HT}]_{ext}$ in the frontal cortex and raphe nuclei when given chronically (twice daily for 14 days), we can conclude from data obtained following a single administration of these drugs that they do not have a similar in vivo effect on the serotonergic system in rat brain.

In summary, in the present study, by using in vivo microdialysis in freely moving rats, we did not find any evidence for the involvement of either blockade (in marked contrast with paroxetine) or activation of the selective 5-HT transporter in the effects of tianeptine in the frontal cortex or raphe nuclei of rats following either administration of a single dose or repeated exposure. Thus, our data suggest that activation of the 5-HT reuptake mechanism (i.e., the selective 5-HT transporter) is unlikely to account for the antidepressant effect of tianeptine. Paroxetine and tianeptine may exert their antidepressant properties in rats through different sites of action.

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