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Trazodone increases extracellular serotonin levels in the frontal cortex of rats

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Abstract

The effects of the antidepressant drug, trazodone, on the extracellular 5-hydroxytryptamine (5-HT) levels in the frontal cortex of freely moving rats was investigated using microdialysis coupled to a high performance liquid chromatography (HPLC) detection method. Systemic administration of 1.25 and 2.5 mg/kg s.c. of trazodone was followed by a rise in the 5-HT level which reached a 5-fold peak over the basal level 5 h after injection, and a 3-fold peak after 1 h. Higher doses had no effect. The increase was prevented by pretreatment with fluoxetine (10 mg/kg s.c.), a 5-HT uptake inhibitor. Direct administration of trazodone (0.03, 0.1, 1, 2 μg/μl), by reverse dialysis into the frontal cortex, elicited a dose-dependent large increase in 5-HT levels. The increase was not prevented by systemic fluoxetine administration but was reduced by local perfusion of ketanserin (0.1 μg/μl) a 5-$HT_{2A/C}$ receptor antagonist. Trazodone s.c. administration for 7 days did not increase 5-HT basal levels but enhanced the effects of challenge doses of 2.5 and 5 mg/kg s.c. The present work demonstrated that trazodone increases the 5-HT extracellular level through a double mechanism which involves the 5-HT transporter and 5-$HT_{2A/C}$ receptors. This increase may trigger the chain of events which lead to the therapeutic effects, similar to the case of many other antidepressant drugs.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Microdialysis; Trazodone

1. Introduction

Trazodone was one of the first serotonergic compounds introduced for the treatment of depression. Developed and first marketed in Italy (Haria et al., 1994), at one time trazodone was the most widely used antidepressant drug in the US (Burke and Preskorn, 1995), and is still frequently prescribed for several mood disorders. Trazodone is characterized by a complex pharmacological profile showing extracellular 5-HT_{1A}, 5-HT_{2C}, 5-HT_{2A} receptor antagonist activity (Cusack et al., 1994; Owens et al., 1997). Furthermore, it is a selective inhibitor of extracellular 5-HT uptake, although weaker than the more recent selective serotonin reuptake inhibitors, such as fluoxetine (Owens et al., 1997). It has also been suggested that at least part of trazodone's clinical efficacy in depression is mediated by its metabolite *meta*-chlorophenyl-piperazine (mCPP) (Marek et al., 1992). mCPP shows affinity for several 5-HT receptors with agonistic activity on 5-HT_{2C} and antagonistic activity on 5-HT_{2A} receptors being predominant (Fiorella et al., 1995). Moreover, according to Hoyer (1988), mCPP also interacts with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors.

Although trazodone is frequently classified as a 5-HT uptake inhibitor, Marek et al. (1992), by comparing the experimental and clinical differences between the antidepressant activities of fluoxetine and trazodone, concluded that the antagonistic action at 5-$HT_{2A/2C}$ receptors is probably the most potent pharmacological effect of trazodone. In the last decade the selective serotonin reuptake inhibitors have become the most commonly prescribed drugs in the treatment of depression. It is believed that these drugs owe their therapeutic action to the increase in 5-HT concentration within the synaptic cleft, caused by the inhibition of its transport/reuptake, thus enhancing 5-HT transmission. At the same time the increased 5-HT levels activate the presynaptic autoinhibitory receptors reducing the firing rate of 5-HT neurons of the dorsal raphe nucleus (Chaput et al., 1986; Hajós et al., 1995). Electrophysiological and biochemical experiments have demonstrated desensitization of 5-HT presynaptic receptors during chronic treatment with these drugs (Blier and De Montigny, 1994),

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and it has been claimed that the time course of autoreceptor desensitization is comparable with the delay in the onset of the therapeutic action of selective serotonin reuptake inhibitors (Blier et al., 1988; De Montigny et al., 1990).

It is likely that an increase in 5-HT extracellular levels in the brain is a fundamental step for antidepressant activity. It has been shown that trazodone enhances electrically evoked 5-HT release from cortical slices preincubated with [^{3}H]5-HT (Groß et al., 1987). It has also been shown that the trazodone metabolite, mCPP, increases 5-HT extracellular levels in the ventromedial diencephalon of awake rats, and that the increase is blocked by pretreatment with fluoxetine, indicating an interaction with 5-HT transport (Baumann et al., 1993). Nevertheless, an extensive investigation of the effects of trazodone administration on brain extracellular 5-HT levels in vivo, reflecting the balance between 5-HT released from and taken up by neurons (Di Chiara, 1990), has never been reported. Therefore, the aim of the present research was to study, by means of the microdialysis technique, the effect of trazodone on 5-HT extracellular levels in the frontal cortex of freely moving rats.

2. Materials and methods

2.1. Animal housing and surgery

Male adult Wistar rats (Nossan, Italy) weighing 250–300 g were used. They were housed in groups of three with free access to food and water and kept on a 12-h light/dark cycle. All animal-use procedures conformed to the guidelines of the European Community's Council for Animal Experiments (DL 116/92). The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed in a Kopf stereotaxic frame and a guide cannula (CMA/11) was implanted in the frontal cortex with the following coordinates: AP 4.5 mm, L −2.8 and H −4.5 mm from the bregma (Paxinos and Watson, 1982).

2.2. Microdialysis procedure

Twenty-four hours after surgery each rat was placed in a Plexiglas cage and a pre-equilibrated Cuprophan CMA/11 microdialysis probe (2 mm long, 0.24 mm o.d.) was slowly lowered, via the guide cannula, into its frontal cortex. The probes were perfused at a constant flow rate of 1.3 μl/min (CMA/100 microinjection pump, Carnegie

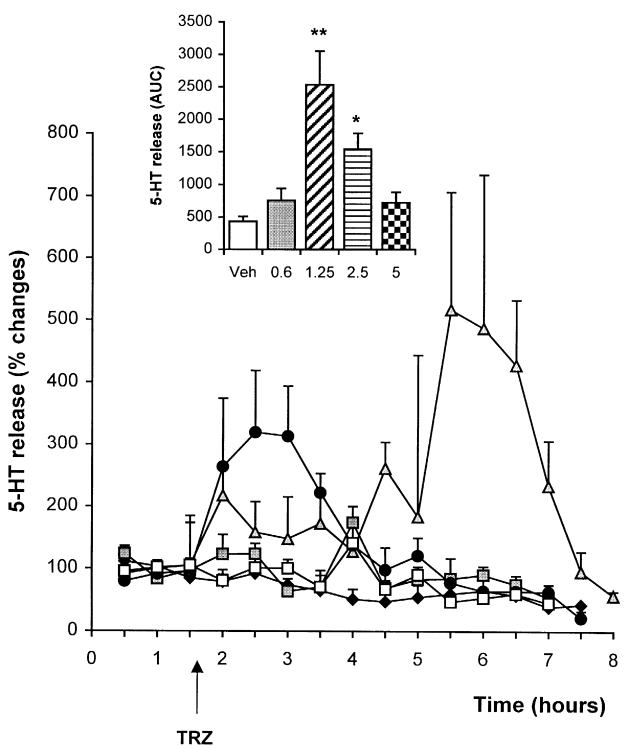

Fig. 1. Effect of systemic administration of trazodone on extracellular levels of 5-HT in the frontal cortex of rats. Trazodone or vehicle was administered after collection of three baseline samples, as shown by the arrow. 5-HT release is expressed as percent change over the mean of the first three samples (mean ± S.E.M.; $n = 4–8$ rats). ◆ Vehicle; ▨ trazodone 0.6 mg/kg; △ trazodone 1.25 mg/kg; ● trazodone 2.5 mg/kg; □ trazodone 5 mg/kg. Inset: the bars in the inset represent AUC calculated from 2 h to the end for each curve. For each period: vehicle, open bar; trazodone 0.6 mg/kg, grey bar; trazodone 1.25 mg/kg, diagonal striped bar; trazodone 2.5 mg/kg, horizontal striped bar; trazodone 5 mg/kg, checked bar. Significance of differences between experimental groups was calculated by one-way ANOVA ($F(4, 31) = 7.66$; $P < 0.0002$) followed by Fisher LSD test (**at least $P < 0.05$ vs. all other groups; *at least $P < 0.05$ vs. vehicle and 1.25).

Medicine, Sweden) with Ringer solution (composition in mM: NaCl 147, $CaCl_2$ 1.2, KCl 4.0, pH 7.0). After a 2-h settling period, 30-min dialysate samples were collected. Three basal samples, the mean of which was taken as basal extracellular level, were collected before drug administration.

2.3. Histological control

At the end of the experiment, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and killed by decapitation. The brain was rapidly removed and placed in a vial containing 10 ml 9% phosphate-buffered formaldehyde solution. Coronal 50-μm-thick slices were cut using a freezing microtome. The slices were examined under a light microscope to verify the placement of the fiber. Data obtained from rats in which the dialysis membrane was not correctly positioned were discarded (approximately 5%).

2.4. In vitro recovery experiments

In order to evaluate 5-HT recovery through the dialysis probe, in vitro recovery experiments were performed. The dialysis fibers were immersed in Ringer solution containing the concentration of 5-HT expected in the frontal cortex. The fiber was perfused at a rate of 1.3 μl/min at room temperature, and samples were collected at 30-min intervals. The 5-HT content in the outer solution and in the dialysate samples was measured by high pressure liquid chromatography (HPLC). The recovery of 5-HT in the dialysate was 47% of the amount in the outer solution.

2.5. Assay of 5-HT in the perfusate

The samples, frozen at −70°C immediately after collection, were analyzed by HPLC using a reverse-phase column with 3-μm particle size (Ultrasphere C18 ODS; 0.46 × 7.5 cm; Beckman, CA) thermostated at 32°C. The mobile phase was 0.14 M NaH_2PO_4, 2 mM octyl sodium sulphate, 0.5 mM EDTA (pH of the finished mobile phase 2.8, adjusted with phosphoric acid) and 21% methanol (Adell and Artigas, 1991). 5-HT quantification was carried out using a coulometric detector (ESA 5014, Coulochem II) with a dual electrode analytical cell (model 5014B); the first electrode was set at 0 mV and the second at 275 mV. The mobile phase was delivered at a flow rate of 0.8 ml/min. The minimal detectable amount of 5-HT was 2 fmol (signal/noise ratio = 3).

2.6. Drugs

Trazodone hydrocloride and fluoxetine HCl were supplied by ACRAF Angelini Research Laboratories, Pomezia, Italy; ketanserin tartrate was a gift from Prof. Renato Corradetti. The drugs, freshly dissolved in saline, were injected s.c. in a volume of 1 ml/kg body weight or dissolved in Ringer solution administered locally by reverse dialysis through the microdialysis probes at the concentrations stated in Results.

2.7. Statistical analysis

The changes in 5-HT release were expressed as percent variations over the mean of the first three samples taken as baseline release. Differences between experimental groups were evaluated, as shown in the figure insets, by comparing areas under the curve (AUC). Statistical analysis was performed using the NCSS 5.0 program. Significance was evaluated by one way analysis of variance (ANOVA) followed by least significant differences (LSD) Fisher post hoc analysis, or by Student's *t*-test, as appropriate. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effect of systemic administration of trazodone on 5-HT extracellular levels

The basal extracellular level of 5-HT in the cortex of freely moving rats was 0.28 ± 0.04 fmol/μl (mean ±

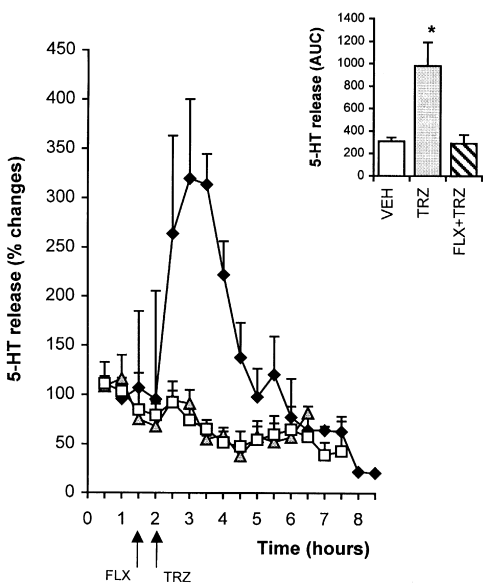

Fig. 2. Effect of pretreatment with fluoxetine (FLX; 10 mg/kg) on the increase in extracellular 5-HT levels induced by systemic administration of trazodone (TRZ; 2.5 mg/kg) in the frontal cortex of rats. After collection of three baseline samples, fluoxetine was administered 30 min before trazodone injection, as shown by the arrows. 5-HT release is expressed as percent change over the mean of the first three samples (mean ± S.E.M.; $n = 5–6$ rats). □ Vehicle; ◆ trazodone 2.5 mg/kg; σ fluoxetine + trazodone 2.5 mg/kg. Inset: the bars represent AUC calculated from 2 to 4.5 h for each curve. Vehicle, open bar; trazodone, grey bar; fluoxetine + trazodone, striped bar. Significance of differences between experimental groups was calculated by one-way ANOVA ($F(2, 19) = 8.51$; $P < 0.002$) followed by Fisher LSD test (* at least $P < 0.05$ vs. other groups).

S.E.M.; $n = 46$). The effect of acute administration of trazodone (0.6–5 mg/kg, s.c.) on the extracellular 5-HT level is shown in Fig. 1. Vehicle-treated rats (saline, 1 ml/kg, s.c.) were used as controls. Trazodone at the dose of 0.6 mg/kg s.c. did not modify 5-HT levels, while at the dose of 1.25 mg/kg it induced a rapid increase in extracellular 5-HT levels which rose stepwise, beginning immediately after the injection and peaked (about 5-fold the basal level) 4 h later, ending 6 h after injection. The administration of 2.5 mg/kg was followed by a single large peak (3-fold the basal level) with a gradual return to the basal level within 4 h. At the dose of 5 mg/kg trazodone had no effect.

As shown in the inset, the dose–effect relationship showed a bell-shaped trend. The columns in the inset indicate the AUC variations calculated between 2 h and the end of the experiment. Statistical analysis with a one-way ANOVA showed a significant difference between treatments. The maximal increase in 5-HT release was evoked by trazodone 1.25 mg/kg. The Fisher LSD post-comparison test (*at least $P < 0.05$ vs. controls) showed that it was significantly different vs. all groups. Also the increase following trazodone 2.5 mg/kg, was significantly different vs. control and the dose of 1.25 mg/kg. Acute treatments with trazodone at the high dose of 50 mg/kg induced no modification of 5-HT levels in the frontal cortex of awake rats (data not shown).

None of the doses, except 50 mg/kg, induced gross behavioral changes.

3.2. Effect of fluoxetine pretreatment

In order to ascertain whether a selective 5-HT uptake inhibitor influences the effect of trazodone on extracellular 5-HT levels, fluoxetine (10 mg/kg, s.c.) was administered 30 min before the injection of trazodone (2.5 mg/kg, s.c.). The results are shown in Fig. 2. Trazodone administration was followed by a 200% increase in 5-HT extracellular levels, which returned to basal levels within 2.5 h. Fluoxetine administered 30 min before trazodone completely prevented the increase caused by trazodone. Statistical analysis with a one-way ANOVA followed by Fisher LSD post-comparison test (*at least $P < 0.05$ vs. controls) confirmed that the AUC calculated in the presence of fluoxetine was not different from that for the controls.

3.3. Effect of local administration of trazodone on cortical 5-HT extracellular levels

In order to investigate whether trazodone enhanced extracellular levels of 5-HT by acting directly on serotoninergic nerve endings in the frontal cortex, the drug was administered locally by reverse dialysis. Fig. 3 shows that perfusion of trazodone (0.03–2 μg/μl) through the dialy-

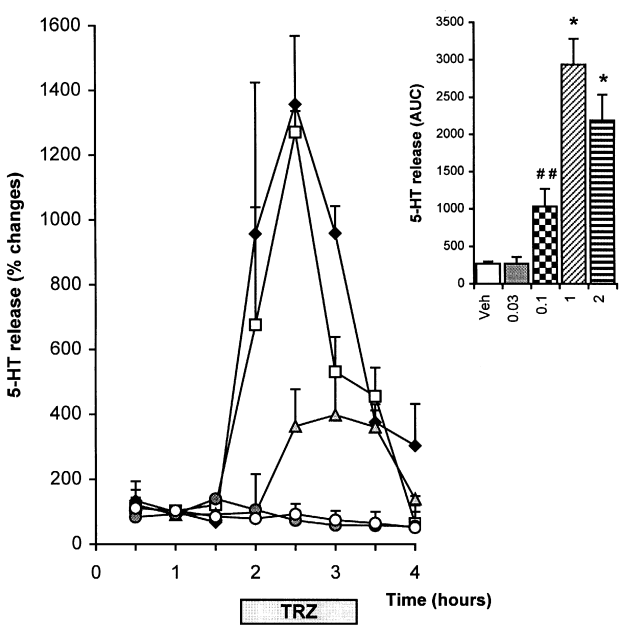

Fig. 3. Effect of local administration of trazodone on extracellular levels of 5-HT in the frontal cortex of rats. Trazodone (TRZ; 0.03–2 μg/μl) was dissolved in Ringer solution and administered through the membrane after collection of three baseline samples, as shown by the horizontal bar under the abscissa. 5-HT release is expressed as percent change over the mean of the first three samples (mean ± S.E.M.; $n = 3$–8 rats). ○ Vehicle; ● trazodone 0.03 μg/μl; ▲ trazodone 0.1 μg/μl; ♦ trazodone 1 μg/μl; □ trazodone 2 μg/μl. Inset: the bars represent AUC calculated from 2 to 3.5 h for each curve. Vehicle, open bar; trazodone 0.03 μg/μl, grey bar; trazodone 0.1 μg/μl checked bar; trazodone 1 μg/μl, diagonal striped bar; trazodone 2 μg/μl, horizontal striped bar. Significance of differences between experimental groups was calculated using one-way ANOVA ($F(4, 20) = 26.12$; $P < 0.0001$) followed by Fisher LSD test (*at least $P < 0.05$ vs. vehicle, 0.03 and 0.1; $^{\#\#}$at least $P < 0.05$ vs. all other groups).

sis membrane for 90 min increased extracellular 5-HT levels in a dose-dependent manner. Perfusion of 0.1 μg/μl trazodone was accompanied by a 4-fold increase in 5-HT levels, which peaked 1 h after beginning of perfusion and returned to their basal values within 1 h after the end of the perfusion. Perfusion with 1 and 2 μg/μl brought about much larger increases, about 12-fold, which showed a rapid onset and returned to the basal levels within 2 h. No effect was observed after the perfusion with 0.03 μg/μl. From the AUC shown in the inset it appears that the maximum increase was seen with 1 μg/μl trazodone.

In order to ascertain whether the effect of local administration of trazodone on extracellular 5-HT levels was influenced by systemic administration of a selective 5-HT uptake inhibitor (Fig. 4), fluoxetine (10 mg/kg, s.c.) was injected 30 min before trazodone (0.1 μg/μl). Contrary to what was observed with systemically administered trazodone, fluoxetine did not block the 5-HT increase brought about by local administration of trazodone. As indicated by the comparison between AUCs, shown in the inset, there was no significant difference between trazodone and trazodone plus fluoxetine.

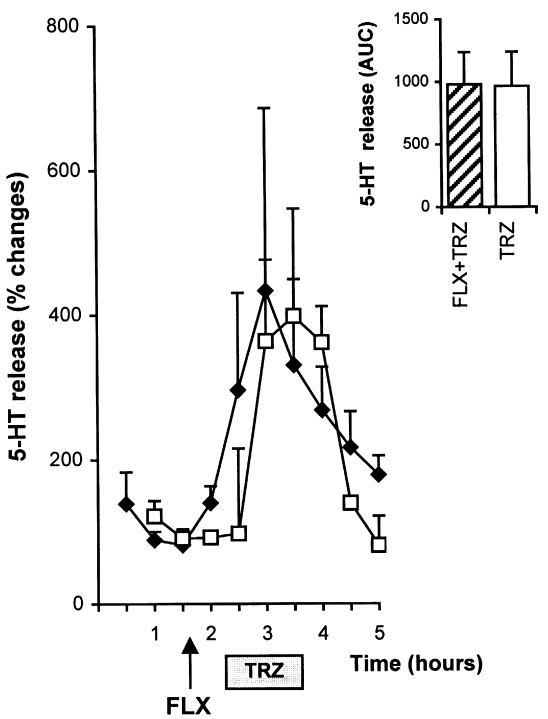

Fig. 4. Effect of pretreatment with fluoxetine (FLX; 10 mg/kg s.c.) on the increase of extracellular 5-HT levels induced by local administration of trazodone (TRZ; 0.1 μg/μl) in the frontal cortex of rats. After collection of three baseline samples, fluoxetine was administered 30 min before each trazodone administration, as shown by the arrow and the bar. 5-HT release is expressed as percent change over the mean of the first three samples (mean ± S.E.M.; $n = 4$ independent experiments). □ Trazodone 0.1 μg/μl; ♦ fluoxetine 10 mg/kg + trazodone 0.1 μg/μl. Inset: the bars represent AUC calculated from 2.5 to the end of experiment for each curve. Trazodone, open bar; fluoxetine + trazodone, striped bar.

As shown in Fig. 5, the increase induced by local perfusion of trazodone at the dose of 0.1 μg/μl, was significantly reduced by local perfusion of ketanserin (0.1 μg/μl), a $5\text{-HT}_{2A/2C}$ receptor antagonist, starting 1 h before trazodone and continuing throughout trazodone administration. The inset shows that the AUC calculated in the presence of ketanserin and trazodone together is not statistically different from that of the controls. Perfusion with ketanserin alone was accompanied by an initial, short-lasting, statistically significant, increase in 5-HT extracellular levels followed by a gradual long-lasting decrease.

3.4. Effect of repeated administration of trazodone on extracellular 5-HT levels

Trazodone was administered for 7 days at the doses of 2.5 or 5 mg/kg (once daily, s.c.). The basal 5-HT levels measured 24 h after the last injection were 0.199 ± 0.056 and 0.232 ± 0.209 fmol/μl in the rats treated with 2.5 and 5 mg/kg trazodone, respectively ($n = 5$ per dose). These values were not different from those for the saline-treated rats (0.228 ± 0.085 fmol/μl). Even when trazodone (5 mg/kg s.c.) was administered daily for 15 days, the cortical extracellular levels of 5-HT, 24 h after the last injection, did not increase (0.196 ± 0.055 fmol/μl; $n = 5$). However, a challenge administration, 24 h after the last of seven daily treatments, elicited a large increase in 5-HT extracellular levels as shown in Fig. 6. The injection of trazodone 2.5 mg/kg induced an increase in 5-HT that lasted for about 6 h, with large interindividual variability, and a peak 3-fold higher than the basal levels. The dose of 5 mg/kg, which had no effect when given alone (see Fig. 1), caused a short-lasting 2-fold increase 4 h after the injection. As shown in the inset, the increase induced by trazodone was statistically significant vs. the controls.

4. Discussion

The present experiments demonstrated for the first time that systemic administration of trazodone is followed by an increase in extracellular 5-HT levels in the frontal cortex. However, this increase could be observed only within a limited range of doses, and the dose–effect relationship showed a narrow bell-shaped curve. Moreover, the time course of the increase differs between the two effective doses tested, since after 1.25 mg/kg a delayed increase lasting up to 7 h was observed, while after 2.5 mg/kg the increase occurred within 1 h and faded within 2 h. The lack of effect of 5 mg/kg trazodone 5-HT levels was also observed by Cheng et al. (1999) in the striatum.

Since trazodone yields several metabolites (see references in Haria et al., 1994), the possibility exists that the changes in 5-HT extracellular levels depend on the interac-

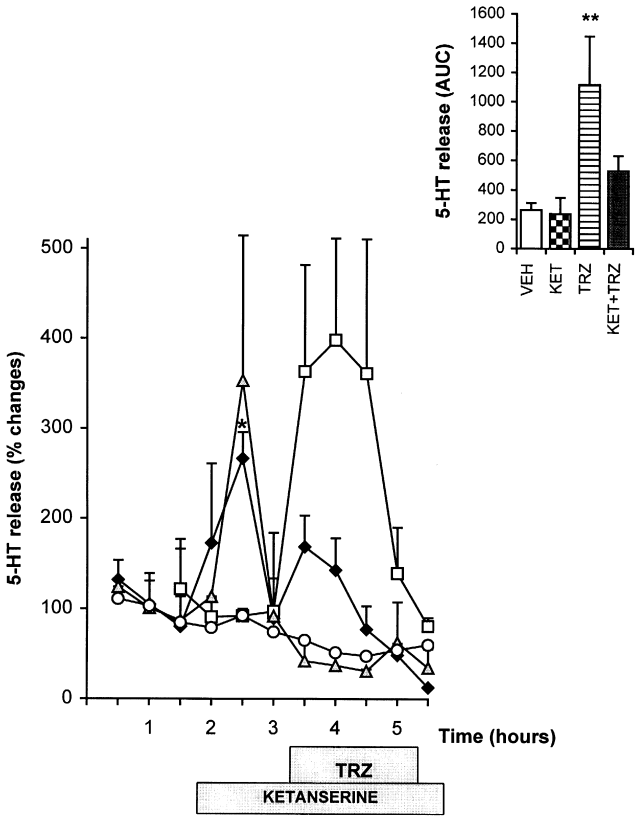

Fig. 5. Effect of local perfusion with ketanserin (KET; 0.1 μg/μl) on the increase of extracellular 5-HT levels induced by local administration of trazodone (TRZ; 0.1 μg/μl) in the frontal cortex of rats. Drugs were dissolved in Ringer solution and administered through the membrane after collection of three baseline samples, as shown by the horizontal bar under the abscissa. 5-HT release is expressed as percent change over the mean of the first three samples (mean ± S.E.M.; $n = 3–9$ rats). ○ Vehicle; ▽ ketanserin 0.1 μg/μl; □ trazodone 0.1 μg/μl; ◆ ketanserin 0.1 μg/μl + trazodone 0.1 μg/μl. Significance of differences between experimental groups was calculated using one-way ANOVA ($F(3, 16) = 4.38$; $P < 0.05$) followed by Fisher LSD test (*at least $P < 0.05$ vs. trazodone and controls). Inset: the bars represent AUC calculated from 3 to 5.5 h for each curve. Vehicle open bar; ketanserin 0.1 μg/μl, checked bar; trazodone 0.1 μg/μl, striped bar; trazodone 0.1 μg/μl + ketanserin 0.1 μg/μl, grey bar. Significance of differences between experimental groups was calculated using one-way ANOVA ($F(3, 19) = 6.67$; $P < 0.004$) followed by Fisher LSD test (**at least $P < 0.05$ vs. other groups).

tion between the effects of the intact drug and those of its metabolites. mCPP, a major metabolite, administered i.v. at doses of 1 and 2 mg/kg dependently increases extracellular 5-HT levels in rat diencephalon and the increase is prevented by fluoxetine (Baumann et al., 1993), as was observed in our experiments with trazodone. This finding suggests a presynaptic effect involving the 5-HT transporter. Pettibone and Williams (1984) demonstrated that mCPP increases the hypothalamic 5-HT efflux in vitro via a non-exocytotic, Ca^{2+}-independent, release process which is blocked by 5-HT uptake inhibitors.

It has been shown (Cheng et al., 1999) that mCPP represents less than 5% of the trazodone concentration in striatal extracellular fluids. Moreover, the local administration of trazodone is followed by a rapid increase of cortical 5-HT while trazodone metabolism by brain tissue is presumably slow and limited (Cheng et al., 1999). Therefore it may be assumed that trazodone influences 5-HT extracellular levels both directly and, indirectly, through its metabolites.

Two questions arise at this point: through which mechanism does the increase occur, and why are the larger doses ineffective. The blockade of the trazodone systemic effect by fluoxetine observed in our experiments, and by Baumann et al. (1993) using mCPP, supports a presynaptic mechanism involving the 5-HT transporter. Conversely, the mechanism underlying the large increase in 5-HT extracellular level after local administration of trazodone is presumably different. In this case fluoxetine does not block the increase, in agreement with the results of the in vitro experiments of Groß et al. (1987). These authors showed that trazodone enhances electrically evoked [^{3}H]5-HT from cortical slices and that the increase was not modified by the inhibitor of 5-HT uptake, 6-nitroquipazine. Furthermore, in our experiments, the effect of the local administration of trazodone was prevented by ketanserin a selective 5-HT_{2A} receptor antagonist (IUPHAR Receptor Compendium, 1998), with functional properties of an inverse agonist (Feldman et al., 1997) but also endowed with activity toward the 5-HT_{2C} subtype (Baxter et al., 1995).

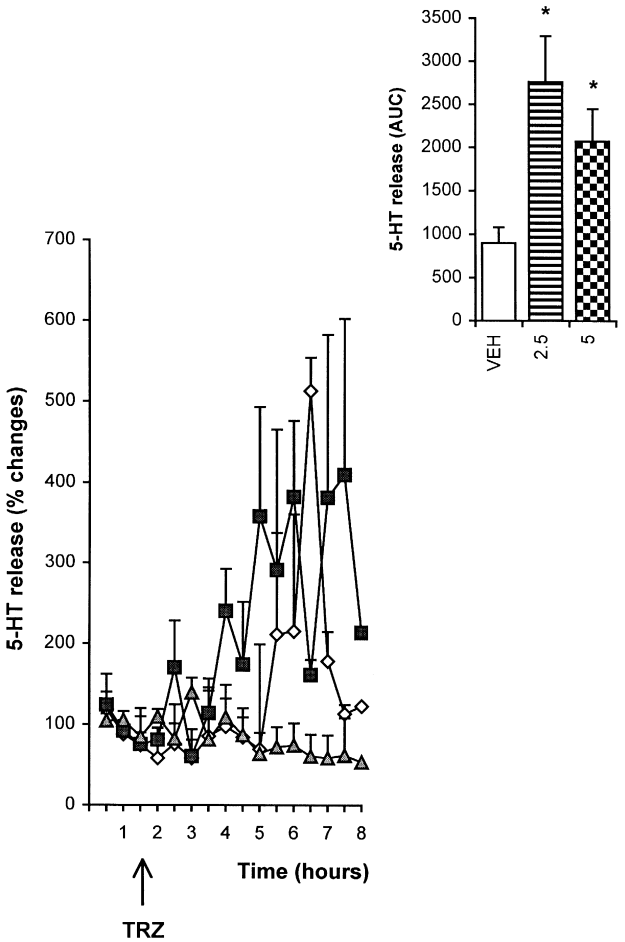

Fig. 6. Effect of 7 days repeated administration of trazodone (TRZ; 2.5 and 5 mg/kg) on extracellular 5-HT levels in the frontal cortex of rats. Trazodone or vehicle was administered after collection of three baseline samples, as shown by the arrow. 5-HT release is expressed as percent change over the mean of the first three samples (mean ± S.E.M.; $n = 4–5$ rats). ▲ Saline-treated rats; ■ trazodone 2.5 mg/kg; ◇ trazodone 5 mg/kg. Inset: the bars represent AUC calculated from 2 h to the end of the experiment for each curve. Vehicle, open bar; trazodone 2.5 mg/kg, striped bar; trazodone 5 mg/kg, checked bar. Significance of differences between the experimental groups was calculated using one-way ANOVA ($F(2, 10) = 6.95$; $P < 0.01$) followed by Fisher LSD test (*at least $P < 0.05$ vs. control).

A presynaptic location of 5-HT_2 type receptors on serotonergic cortical fibers has not yet been reported. According to Willins et al. (1997), apical dendrites of pyramidal cells and parvalbumin-positive interneurons in the rat cortex show very dense 5-HT_{2A} immunoreactivity. Evidence that cortical interneurons receive a dense serotonergic innervation has been presented by Smiley and Goldman-Rakic (1996). It has been shown that 5-HT facilitates the release of γ-aminobutyric acid in the cortex through 5-HT_{2A} receptors (Cozzi and Nichols, 1996). On the basis of this information, the hypothesis can be put forward that trazodone administered locally enhances 5-HT extracellular levels through a loop involving cortical interneurons regulated by 5-HT_2 subtype receptors.

However, since a 5-HT increase induced by systemic administration of trazodone was completely blocked by fluoxetine, it appears that the main mechanism of action of trazodone is located presynaptically and involves the 5-HT transporter. Once daily administration of trazodone for 7 or 14 days did not result in an increase in the basal 5-HT levels, as shown by our finding that 24 h after the last administration there was no difference in 5-HT levels between control and treated rats. An increase in 5-HT extracellular basal levels in the hippocampus and striatum was detected in rats treated with fluoxetine for 14 days 48 h after the last administration (Kreiss and Lucki, 1995), and in the frontal cortex 12 h after the last injection in rats treated for 14 days with citalopram (Arborelius et al., 1996). Conversely, others have failed to detect changes in 5-HT concentrations after chronic administration of reuptake inhibition (Hjorth and Auerbach, 1994; Invernizzi et al., 1994; Bosker et al., 1995a,b). The different effects can be explained by the presence or the absence of the drugs during the microdialysis experiment, due to the different ways of administration (Arborelius et al., 1996).

According to Yamato et al. (1974) trazodone shows, in rats, a bi-exponential half-life whose slower component is 1.5 h. Therefore, the plasma trazodone concentration 24 h

after a single 4-mg/kg oral treatment was around 0.05 μg/μl.

Presumably, in our experiments, the brain trazodone concentration 24 h after the last administration was too low for a reuptake inhibition strong and prolonged enough to maintain elevated 5-HT extracellular levels. Nevertheless, 24 h after the last of seven daily administrations, the increase in 5-HT levels following the injection of 2.5 mg/kg lasted longer than the increase observed after a single administration, while after the administration of 5 mg/kg a tendency to an increase was observed but with a large variability. It appears from these findings that repeated trazodone administration sensitizes the cortical serotoninergic system.

No satisfactory explanation can be offered for the narrow bell-shaped dose–effect relationship. A hypotensive effect of trazodone can be ruled out since a moderate decrease in blood pressure has been reported only after i.v. administration (Silvestrini et al., 1968). The possibility that higher doses of trazodone or its metabolites could activate inhibitory autoreceptors of the 5-HT_1 subtype or other receptors could be envisaged. This activation seems to disappear after repeated administration, as shown by the longer duration of the increase in 5-HT levels after subchronic administration and by the appearance of an increase after 5 mg/kg administration also.

In conclusion, the present work demonstrated that trazodone enhances 5-HT extracellular levels. Through this action, it may trigger the chain of events leading to therapeutic effects similarly to many other antidepressant drugs. The mechanisms through which the increase in 5-HT extracellular level occurs still needs to be fully explained. However they involve the 5-HT transporter and the activation of one or more 5-HT receptor subtypes.

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