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Long-term exposure of rats to tramadol alters brain dopamine and α_1-adrenoceptor function that may be related to antidepressant potency

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Abstract

The aim of the present study was to determine whether tramadol, which has a potential antidepressant efficacy, evokes, when administered repeatedly, changes similar to the alterations induced by conventional antidepressant drugs. Repeated administration of tramadol (20 mg/kg i.p. for 21 days) enhanced the D-amphetamine-induced locomotor hyperactivity and increased the density of α_1-adrenoceptors in the rat brain cortex, as measured by saturation analysis of [^{3}H]prazosin binding. Autoradiographic analysis of [^{3}H]7-OH-DPAT and [^{3}H]raclopride binding revealed a significant up-regulation of dopamine D2 and D3 receptors in the rat nucleus accumbens upon repeated treatment with tramadol. All the above-mentioned effects induced by repeated administration of tramadol resemble the effects induced by conventional antidepressants. However, tramadol when administered repeatedly did not increase the levels of mRNA encoding for brain-derived neurotrophic factor (BDNF) and its receptor, TrkB. This is what differs tramadol from conventional antidepressants, since neurotrophic effects of these drugs have recently been postulated.

Keywords: Tramadol; α_1-Adrenoceptors; Dopamine D2 and D3 receptors; BDNF; TrkB; Brain, rat

1. Introduction

Tramadol ((1*RS*,2*RS*)-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl)-cyclo-hexanol hydrochloride), used mainly for the treatment of moderate to severe pain (McClellan and Scott, 2003), has a relatively weak opioid receptor activity. Clinically active tramadol is a racemic mixture of two enantiomers that have two distinct but complementary mechanisms of action: the (+)tramadol is a selective agonist for μ-opioid receptor, it preferentially inhibits serotonin reuptake and enhances serotonin efflux in the brain, whereas the (−)enantiomer mainly inhibits noradrenaline reuptake (Scott and Perry, 2000; Frink et al., 1996). Since it has been suggested that both opioid and monoaminergic systems play a role in depressive disorders, therefore, tramadol has been studied in the forced swimming test in mice, a test developed to predict the antidepressant action of drugs. The studies of Rojas-Corrales et al. (1998) have shown that, indeed, tramadol displays an antidepressant-like effect in mice, mediated by the noradrenergic system rather than serotonergic or opioidergic pathways. This study has been further extended using helplessness model of depression in rats (Rojas-Corrales et al., 2002). In line with evidence that tramadol may be an antidepressant, another study was undertaken to examine neurochemical effects of long-term tramadol administration. This study revealed that specific frontocortical [^{3}H]dihydroalprenolol (β-adrenoceptor antagonist) and [^{3}H]ketanserin (serotonin 5-HT$_{2A}$ receptor antagonist) binding was lower in the group receiving tramadol for 21 days, as compared to the control group (Hopewood et al., 2001), indicating the similarity in the effects evoked by repeated administration of tramadol and majority of conventional antidepressants.

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Therefore, it seemed interesting to explore further the idea that tramadol administered repeatedly might induce other behavioral and biochemical alterations characteristic of conventional antidepressant drugs. Especially worth checking were noradrenergic and dopaminergic systems, since our previous results strongly indicated the role of α_1-adrenoceptor and dopamine D2/D3 receptors in the mechanism of action of antidepressant drugs.

For example, it has been shown that antidepressants administered repeatedly (but not at a single dose) potentiate behavioral effects (locomotor hyperactivity or exploration) evoked by dopamine stimulants such as D-amphetamine (Maj, 1990; Maj et al., 1984; Dziedzicka-Wasylewska et al., 2002), so a hypothesis has been formed that antidepressant drugs given repeatedly activate the dopaminergic system by increasing responses to stimulants. Further support for this concept has come from biochemical results indicating that repeated administration of antidepressant drugs increases the binding (density and affinity) of dopamine D2 and D3 receptors in respective brain structures (Maj et al., 1996; 1998; Rogoż and Dziedzicka-Wasylewska, 1999) as well as showing a rise in the concentration of mRNA encoding for dopamine D2 (Dziedzicka-Wasylewska et al., 1997) and D3 (Lammers et al., 2000) receptors.

Another line of evidence points to the action of antidepressant drugs within the noradrenergic system. Various antidepressants administered repeatedly increase the responsiveness of the α_1-adrenoceptors, as manifested by the potentiation of the behavioral hyperexploration evoked by α_1-adrenoceptor agonists (phenylephrine or methoxamine) (Maj et al., 1985; Rogóż et al., 2001). Moreover, antidepressants administered repeatedly increase the binding to α_1-adrenoceptors in different brain regions, in particular the affinity of these receptors for their agonists (i.e. also to noradrenaline, the endogenous neuromediator) (Nowak and Przegaliński, 1988; Menkes et al., 1983).

Recently, much attention has been paid to the characterization of postreceptor adaptations that occur in response to antidepressant treatment. This has led to the discovery that repeated administration of antidepressants up-regulates the expression of brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, in the hippocampus (Nibuya et al., 1995).

Therefore, the present study was designed to determine whether tramadol, which has a potential antidepressant efficacy, evokes, when administered repeatedly, changes similar to the above-mentioned alterations induced by conventional antidepressant drugs.

2. Materials and methods

2.1. Animals

The experiments were carried out on rats (male Wistar, ca. 80 days old, weighing 220–230 g). The animals had free access to food and water before the experiment and were kept at a constant room temperature (22±1 °C), under a 12-h light/dark cycle (light on at 7 a.m.). Experimental protocols were approved by the local Ethics Committee and met guidelines of the responsible agency of the Institute of Pharmacology.

2.2. Drug administration

Tramadol (20 mg/kg; Pliva, Poland) dissolved in saline was administered i.p. once (acute treatment) or repeatedly (once daily for 21 days). All animals were handled in the same manner once daily for 21 days. Control animals received vehicle for the whole experimental period while repeatedly treated animals received the drug. The animals treated acutely received saline for 20 days and, on day 21, they received the drug. Using this experimental paradigm, we avoided the effect of a single i.p. injection, which inevitably, as a stressful event for an animal, may mask or change the actual effect of acute administration of the studied drug. Moreover, all groups of animals, treated acutely or repeatedly, were taken for behavioral experiment or decapitated for biochemical assay at the same time.

2.3. Tissue preparation

The rats used for biochemical experiments were sacrificed at 24 h after a single (acute treatment) or the last dose (repeated treatment) of tramadol. The tissue (cortex for α_1-adrenoceptor binding) was dissected out, frozen on dry ice and stored until used for binding experiments. Brains used for autoradiographic analysis or in situ hybridization were rapidly dissected and frozen by immersion in cold heptane in a dry-ice bath and stored at −70 °C until sectioned. Consecutive coronal sections (12 μm) were cut at −19 °C using Jung CM 3000 cryostat microtome (Leica). The identification and nomenclature of the brain structures was based on the Paxinos and Watson Rat Brain Atlas (1998).

2.4. Procedures

2.4.1. Behavioral studies

2.4.1.1. D-Amphetamine-induced locomotor hyperactivity in rats. Locomotor activity was measured in actometers (Opto-Varimex, Columbus Instruments, OH, USA), starting at 24 h after single (acute experiment) or last (repeated treatment) administration of tramadol or saline. Locomotor activity was measured 30 min after D-amphetamine (0.5 mg/kg s.c.; Sigma, USA) administration, and lasted for 1 h. Each group consisted of seven rats.

2.4.2. Biochemical studies

2.4.2.1. α_1-Adrenoceptor binding in the rat brain cortex. The experiment was carried out according to the method used previously (Rogóż et al., 2001). The tissue was

homogenized for 15 s in 10 ml of an ice-cold Tris–HCl buffer (50 mM, pH 7.4) using Ultra-Turrax homogenizer. The homogenates were centrifuged at 30,000×*g* for 10 min. That step was repeated twice. Final pellets were resuspended in Tris–HCl buffer to achieve a final concentration of 10 mg wet tissue/ml. Saturation isotherms were generated using seven concentrations (0.01–2 nM) of [^{3}H]prazosin (specific activity 19.5 Ci/mmol, Amersham). The non-specific binding was defined in the presence of 10 μM phentolamine. The 50 μl of phentolamine (non-specific) or Tris–HCl buffer (total) and 50 μl of [^{3}H]prazosin were added to a final volume of 900 μl of tissue suspension. The affinity of α_1-adrenoceptors for an agonist was estimated by studying the ability of various concentrations of phenylephrine (0.1 nM–1 mM) to compete for [^{3}H]prazosin binding sites. A total of 50 μl of phenylephrine and 50 μl of [^{3}H]prazosin (final concentration: 0.3 nM) were added to a volume of 900 μl of tissue suspension.

Afterwards, the samples were incubated at 25 °C for 25 min, followed by a 15-min ice-cold bath. The bound ligand was separated by vacuum filtration through Whatman GF/C filters and washed three times with 5 ml of ice-cold Tris–HCl buffer. Radioactivity was measured with Beckman LS 6500 scintillation counter. All assays were performed in duplicate. The data were analyzed using iterative fitting routines (Graph PAD Prism 2.0). Each group consisted of seven rats.

2.4.2.2. Autoradiography of dopamine D2 and D3 receptors. Receptor binding with [^{3}H]raclopride (NEN Du Pont, specific activity: 44.50 Ci/mmol) was visualized using the procedure described earlier (Rogoż and Dziedzicka-Wasylewska, 1999). Briefly, the sections were first preincubated for 10 min at room temperature in 50 mM Tris–HCl buffer (pH 7.4) containing the following ion solution: 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 0.1% ascorbic acid. Sections were then incubated for 90 min at room temperature in the same buffer with 5.7 nM radioligand. To determine the non-specific binding, parallel sections were incubated in the presence of 1 μM (+)butaclamol. Following incubation period, tissue sections were washed four times in ice-cold 50 mM Tris–HCl buffer (pH 7.4), twice in distilled water and then dried with cool air.

Dopamine D3 receptors were labeled with [^{3}H]7-OH-DPAT (2-(*N*,*N*-di[2,3(*n*)-[^{3}H]propylamino)-7-hydroxy-1,2,3,4-tetrahydronaphtalene; Amersham, specific activity: 155 Ci/mmol), as described previously (Maj et al., 1998). Briefly, the tissue sections were first preincubated for 10 min at room temperature in 50 mM HEPES/NaOH buffer (pH 7.5), containing 1 mM EDTA and 0.1% bovine serum albumin. The sections were then incubated in the buffer described above with 0.5–1 nM of [^{3}H]7-OH-DPAT. To determine non-specific binding, parallel sections were incubated in the presence of 10 μM dopamine. Following the incubation, the tissue sections were washed four times in ice-cold 50 mM HEPES/NaOH buffer (pH 7.5), containing 100 mM NaCl, rinsed twice in distilled water and then dried in cool air.

The representative autoradiograms are presented in Fig. 1.

2.4.2.3. Measurements of mRNA encoding BDNF and TrkB—in situ hybridization. The sections were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) for 15 min, rinsed in PBS and treated with 0.25% acetic anhydride in 0.1 M triethanoloamine (pH 8.0) for 10 min to reduce nonspecific hybridization of the probes. The sections were washed twice for 5 min in 2×SSC (300 mM NaCl/30 mM sodium citrate, pH 7.0). Following dehydration in increasing concentration of ethanol (70–100%), the sections were delipidated in chloroform for 5 min, rinsed in ethanol and dried. The oligonucleotide probes complementary to bp: 157–204 of rat BDNF mRNA (Maisonpierre et al., 1990) and to bp: 1879–1920 of rat TrkB full-length receptor mRNA (intracellular domain) (Middlemas et al., 1991) were used. The oligonucleotide probes were labeled at the 3′ end with [^{35}S]dATP (ICN) using terminal transferase (Roche). The sections were hybridized overnight at 42 °C with 0.5–1×10^6 dpm of the labeled probe in 50 μl of the hybridization solution (50% formamide, 4×SSC, 1×Denhardt's solution, 10% dextran sulfate, 0.5 mg/ml herring sperm DNA, 0.25 mg/ml yeast tRNA, 25 mM sodium phosphate (pH 7.0) and 50 mM dithiotreitol). After hybridization, each

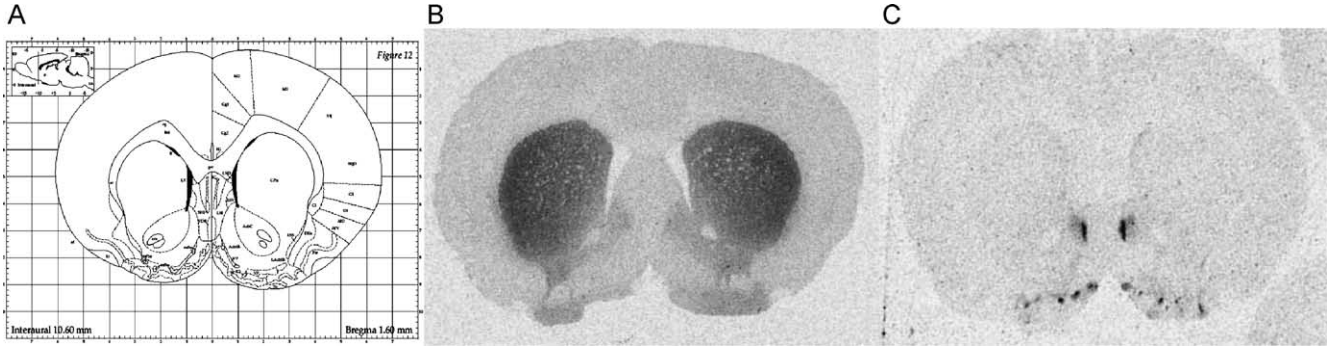

Fig. 1. Representative autoradiograms of [^{3}H]raclopride (B) and [^{3}H]7-OH-DPAT (C) binding in the rat brain. The brain regions used for quantitative analysis were chosen according to (A) Paxinos and Watson (1998).

slide was washed twice for 5 s in 1×SSC (room temperature), three times for 15 min in 1×SSC at 55 °C, then for 30 min in 1×SSC at room temperature and rinsed in deionized water. The sections were dehydrated in increasing concentration of ethanol (70–100%) and air dried.

The representative autoradiograms are presented in Fig. 2.

2.5. Image analysis

Radiolabeled, dried tissue sections were apposed to tritium-sensitive screens (FujiImaging plate) along with [^{3}H]microscales (Amersham) and the images were obtained using FujiFilm BAS 5000. The autoradiograms were analyzed using a computer imaging system and quantified with the use of computer-generated curves derived from the standards. In receptor autoradiography studies, the images of sections showing non-specific binding were subtracted from the images of adjacent sections with total binding, thus permitting direct observation of images representing specific binding on screen. The slides processed for in situ hybridization were exposed to Hyperfilm βmax (Amersham) for 3 weeks before being developed. Autoradiograms were analyzed using a MCID imaging analysis system (Imaging Research, Ontario, Canada). Data are expressed as a mean of the percentage of the control mRNA level±S.E.M. from six animals per group.

2.6. Statistical analysis

The effect of drug treatment on the measured parameters was compared with the appropriate control level using one-way analysis of variance (ANOVA), followed by Dunnett's test.

3. Results

3.1. Locomotor activity

Tramadol given acutely did not change the basal locomotor activity nor did it change the D-amphetamine-induced locomotor hyperactivity (data not shown). However, when given repeatedly, tramadol significantly enhanced the D-amphetamine-induced locomotor hyperactivity (Fig. 3).

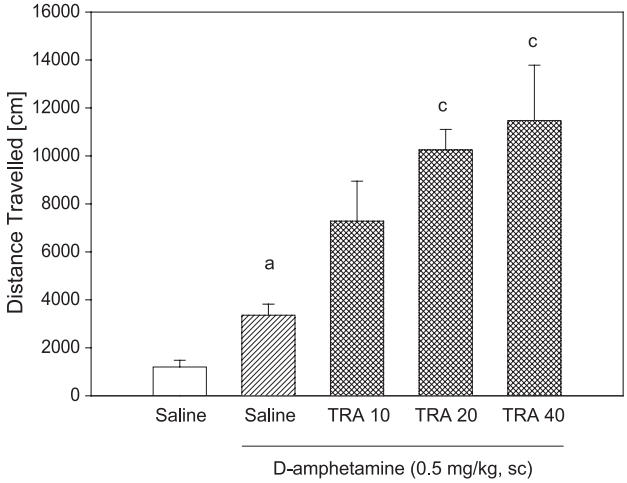

Fig. 3. Effect of repeated treatment with tramadol (TRA) on D-amphetamine-induced locomotor hyperactivity in rats. Tramadol was administered at the doses of 10, 20 and 40 mg/kg i.p. for 21 days. D-Amphetamine (0.5 mg/kg s.c.) was given 30 min before the test. The measurements lasted for 1 h. Data represent the mean±S.E.M., n=7. The statistical significance was assessed using one-way ANOVA, followed by the Dunnett's test for post-hoc comparison: $^c p<0.01$ vs. saline+D-amphetamine group; $^a p<0.05$ vs. saline-treated group (t-test).

3.2. α_1-Adrenoceptor binding parameters

Both acute and repeated administration of tramadol increased the density (B_{max}) of α_1-adrenoceptors in the rat brain cortex, while their affinity (K_d) was changed only in the group of animals treated repeatedly with this drug. Affinity of α_1-adrenoceptors for an agonist (K_i), as measured using phenylephrine competition for [^{3}H]prazosin binding sites, was not changed upon acute or repeated administration of tramadol (Table 1).

3.3. Dopamine D2/D3 receptors

Binding of [^{3}H]7-OH-DPAT to dopamine D3 receptors was measured in the islands of Calleja and shell region of the nucleus accumbens septi, i.e. in the rat brain regions

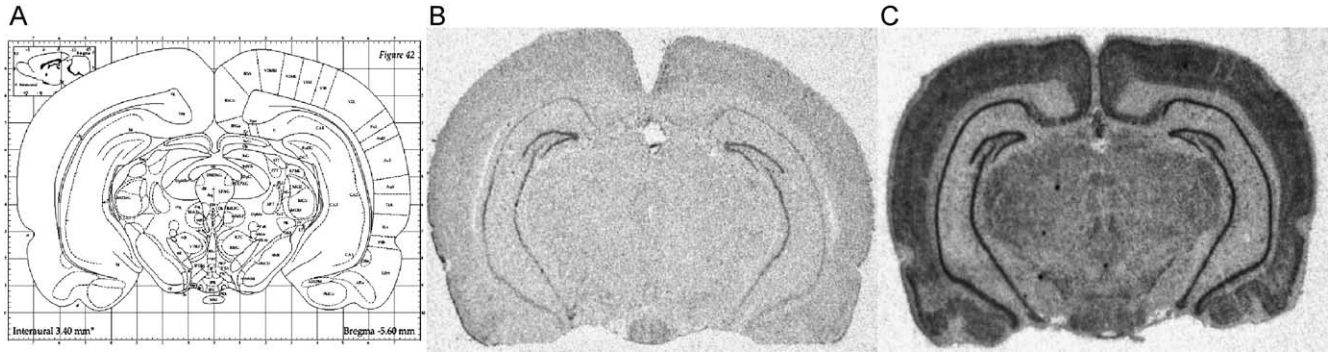

Fig. 2. Representative autoradiograms of mRNA encoding for BDNF (B) and TrkB (C) in the rat brain. The brain regions used for quantitative analysis were chosen according to (A) Paxinos and Watson (1998).

Table 1
Effects of acute (1×) and repeated (21×) treatment with tramadol on the binding of [^{3}H]prazosin to α_1-adrenoceptors in the rat brain cortex

Treatment	B_{max} (fmol/mg protein)	K_d (nM)	K_i (μM)
Control	22.135±0.964	0.249±0.022	3.367±0.473
Tramadol 1×	26.057±0.634[b]	0.289±0.011	3.226±0.466
Tramadol 21×	26.495±0.951[b]	0.343±0.022[b]	3.574±0.415

Data represent mean±S.E.M., n=8. The statistical significance was assessed using one-way ANOVA, followed by Dunnett's test for post-hoc comparison: [b]p<0.01 vs. control. B_{max} and K_d values have been obtained using saturation analysis of [^{3}H]prazosin binding; K_i value is a competition constant of phenylephrine.

having the highest expression of these receptors. In the nucleus accumbens, the increase in the [^{3}H]7-OH-DPAT binding was observed after repeated administration of tramadol (Fig. 4A), while in the island of Calleja Magna also a single dose of this drug induced the increase in the binding of [^{3}H]7-OH-DPAT (Fig. 4B). No effect of tramadol was observed in the islands of Calleja (data not shown).

Binding of [^{3}H]raclopride enabled us to measure the effects of tramadol on dopamine D2 receptors in the rat caudate putamen and nucleus accumbens. In the latter region, a significant increase in [^{3}H]raclopride binding was observed both in the core (Fig. 5A) and shell (Fig. 5B) upon repeated treatment with tramadol. However, in the caudate putamen, especially in the lateral part of this brain region, a significant decrease was observed upon both acute and repeated administration of tramadol (Fig. 5C), while no changes were observed in the medial part (Fig. 5D).

3.4. mRNA encoding BDNF and TrkB

In the cortical areas, a decrease in the level of mRNA encoding BDNF was observed upon repeated tramadol administration, and the effect was most pronounced in the frontal cortex (Fig. 6B). No changes in the levels of mRNA encoding for BDNF were observed in all regions of the hippocampus (i.e. dorsal and ventral CA1, CA3 and dentate gyrus) following acute or repeated treatment with tramadol (data not shown).

No significant changes in the levels of mRNA encoding for TrkB were observed upon acute or repeated treatment with tramadol in all studied regions of brain cortex and hippocampus (data not shown).

4. Discussion

It has been shown that tramadol inhibits serotonin and noradrenaline uptake in much the same manner as the tricyclic antidepressant drugs (Bamigbade et al., 1997; Halfpenny et al., 1999), and reduces immobility in the forced swim test (Rojas-Corrales et al., 1998), which is a test commonly used to screen an antidepressant efficacy. Therefore, we decided to check whether tramadol induces other neurochemical alterations similar to the changes characteristic of conventional antidepressant drugs.

The results obtained in the present study indicate that tramadol administered repeatedly, but not in a single dose, enhanced the D-amphetamine-induced locomotor hyperactivity, as most of antidepressant drugs do (Maj, 1990). Considerable evidence suggests that D-amphetamine-induced psychomotor activation mainly results from an increased dopamine transmission in the nucleus accumbens, since it is antagonized by the intra-accumabal application of neuroleptics (Pijnenburg et al., 1975) and is disrupted after bilateral 6-hydroxydopamine lesions of the dopaminergic neurons projecting into this brain region (Kelly et al., 1975). Therefore, the up-regulation of dopamine D2/D3 receptors, observed in the rat brain upon repeated treatment with antidepressant drugs has often been regarded as biochemical correlate of the enhancement of D-amphetamine-induced locomotor hyperactivity. Indeed, in the present study, we have shown that in the nucleus accumbens septi the binding of both D2 and D3 receptors was increased by repeated treatment with tramadol, the effect that resembles most antidepressants studied so far (Lammers et al., 2000; Maj et

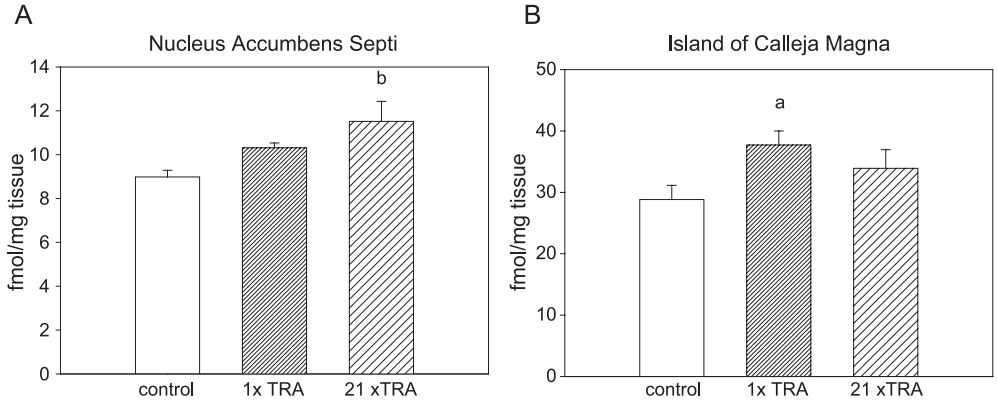

Fig. 4. Effects of acute (1×) and repeated (21×) treatment with tramadol (TRA, 20 mg/kg i.p.) on the binding of [^{3}H]7-OH-DPAT to dopamine D3 receptors in the rat brain: (A) nucleus accumbens and (B) islands of Calleja. Data represent the mean±S.E.M., n=7. The statistical significance was assessed using one-way ANOVA, followed by the Dunnett's test: [a]p<0.05, [b]p<0.01 vs. control.

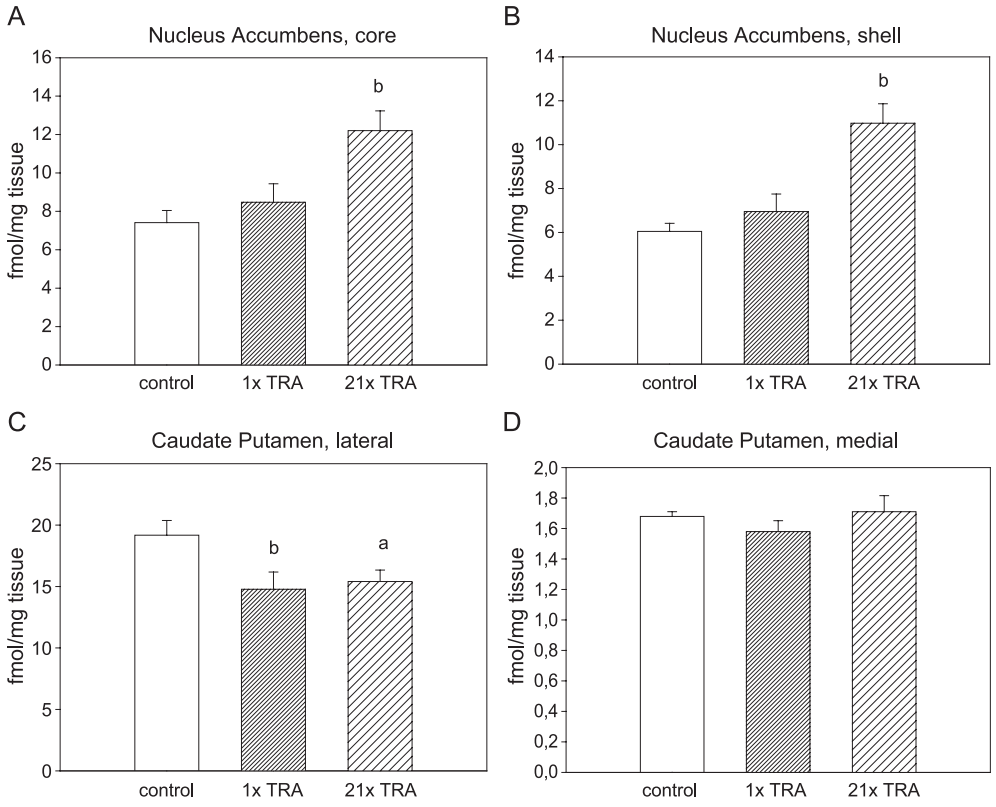

Fig. 5. Effects of acute (1×) and repeated (21×) treatment with tramadol (TRA, 20 mg/kg i.p.) on the binding of [^{3}H]raclopride to dopamine D2 receptors in the rat brain: (A) nucleus accumbens and (B) caudate putamen. (C) lateral part of caudate putamen, (D) medial part of caudate putamen. Data represent the mean±S.E.M., n=7. The statistical significance was assessed using one-way ANOVA, followed by the Dunnett's test: $^a p<0.05$, $^b p<0.01$ vs. control.

al., 1998; Rogoż and Dziedzicka-Wasylewska, 1999). In the rat caudate putamen, no changes or a slight increase in the density of dopamine D2 receptors upon treatment with antidepressant drugs have been usually reported, so the decrease in the binding of [^{3}H]raclopride observed in the present study following treatment with tramadol somehow differs this drug from conventional antidepressants. However, this is the nucleus accumbens, which, being a part of mesolimbic system, is often considered as one of the loci important for the action of antidepressant drugs (Willner, 1997).

On the other hand, Blanc et al. (1994) and Darracq et al. (1998) have demonstrated that locomotor activating effects of D-amphetamine are caused by the stimulation of cortical α_1-adrenoceptors by noradrenaline, and thereby the increased release of a functional pool of subcortical dopamine. In the present study, we have shown that, upon treatment with tramadol, the density of α_1-adrenoceptors in the rat brain cortex (B_{max}) was increased (however, also an increase in the value of K_d was observed after repeated, but not acute treatment with tramadol). Many authors have reported that repeated treatment with

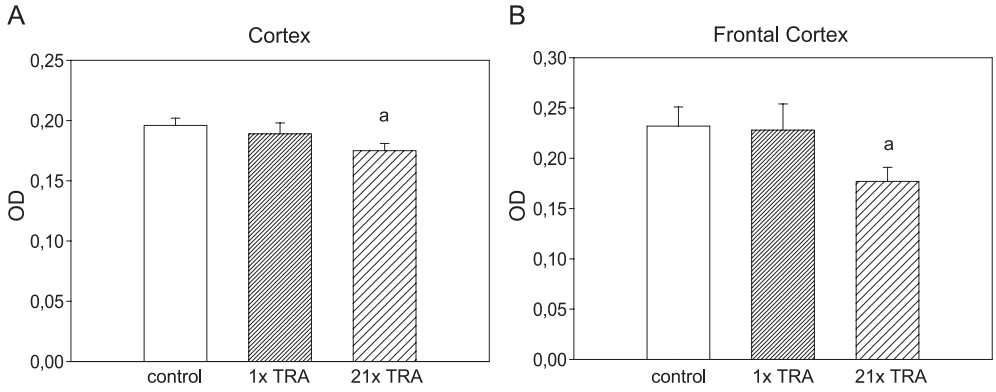

Fig. 6. Effects of acute (1×) and repeated (21×) treatment with tramadol (TRA, 20 mg/kg i.p.) on the level of mRNA encoding for BDNF in the rat brain. Data represent the mean±S.E.M., n=9. The statistical significance was assessed using one-way ANOVA, followed by the Dunnett's test: $^a p<0.05$ vs. control.

tricyclic antidepressants or electroconvulsive shock increases either the density or affinity of the brain α_1-adrenoceptors for agonists. However, not all investigators have been able to confirm the above findings (Heal, 1984; Stockmeier et al., 1987; Hayakawa et al., 1992) indicating that the variables involved have not yet been completely isolated. Nevertheless, the enhanced sensitivity to D-amphetamine observed in the rats treated repeatedly with tramadol correlates well with the increased density of the α_1-adrenoceptors.

The data obtained in the present study, together with the data obtained by others, show that tramadol manifests many of the actions previously observed for the recognized antidepressants. The decreased frontocortical β-adrenoceptor and 5-HT_{2A} receptor binding, as observed by Hopewood et al. (2001) or increased α_1-adrenoceptor and dopamine D2/D3 receptor binding shown in the present study are common findings with most classic antidepressants. These effects typically required days or even weeks to develop and thus may underlie the onset of clinical effectiveness of such compounds. Many antidepressant drugs, and tramadol as well, acutely increase levels of monoamines, but the requirement for repeated, chronic administration has led to the hypothesis that long-term adaptations are necessary for the therapeutic actions of these treatments. Indeed, in many experimental studies with antidepressant drugs, no significant alterations in the density or affinity of neurotransmitter receptors are typically observed; therefore, the hypothesis concerning the development of neuroadaptation upon repeated treatment with antidepressant drugs has been formulated. More recently, the up-regulation of BDNF and its receptor, TrkB, induced by antidepressant drugs has been considered as an important neuroadaptation, playing a role in synaptic plasticity and neuronal survival (Duman et al., 2001). However, rather a decrease or no change were observed in the present study in the levels of mRNA coding for BDNF and TrkB, which differs tramadol from conventional antidepressants, since possible neurotrophic effects of these drugs have been postulated.

In the present study, we observed the changes in the binding of the studied radioligands also after acute tramadol administration, however without any effect on the behavioral activity, as measured by D-amphetamine-induced locomotor hyperactivity. In binding experiments performed in vitro, tramadol did not show any affinity for [^{3}H]prazosin binding sites in the rat brain cortex (data not shown).

Since our experiments were performed at 24 h after tramadol administration, one can exclude direct influence of the compound on, e.g. the fluidity of neuronal membranes which might physically influence the radioligand binding parameters. Hopewood et al. (2001) studied the effects of tramadol on pre- and post-synaptic measures of monoamine function but they have not checked the effects of a single administration of tramadol, so we cannot discuss as yet, whether the effects observed in our study after acute administration of tramadol concern only its effects on dopamine D2/D3 receptors and α_1-adrenoceptors, or whether it is a more general feature of that drug. As has been shown by Matthiesen et al. (1998), the terminal elimination half-life of tramadol in the rat is about 3 h. After repeated administration, there is no evidence of accumulation of the compound or increased metabolism. Therefore, we interpret the effects observed in the present study as adaptive changes induced by tramadol—similar to those observed in the case of antidepressant drugs.

No human study of tramadol antidepressant activity has been published so far, so it may well be that the onset of its therapeutic efficacy is faster. Further preclinical studies are needed to explore the efficacy of tramadol in other types of tests used to screen its antidepressant activity, since the neurochemical data obtained so far indicate such a potential.

Recently, a case report has been published (Houlihan, 2004), which described the development of a significant serotonin syndrom resulting from addition of tramadol (for the treatment of pain) to an on-going combined therapy with mirtazapine and venlafaxine for major depression. Therefore, it may be suggested that while tramadol given alone can be beneficial, as far as antidepressant efficacy is concerned, when combined with already on-going antidepressant therapy with drugs, which themselves inhibit serotonin reuptake (e.g. venlafaxine), or are antagonists of α_2-adrenoceptors (e.g. mirtazapine), it could cause serious complications.

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