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EFFECT OF REPEATED TREATMENT WITH REBOXETINE ON THE CENTRAL α_1-ADRENERGIC AND DOPAMINERGIC RECEPTORS

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Reboxetine (REB) is a member of a new class of antidepressant drugs, which selectively inhibit the neuronal reuptake of noradrenaline. It is devoid of any affinity for neurotransmitter receptors nor does it inhibit monoamine oxidases A or B. Since our earlier studies have shown that antidepressant drugs administered repeatedly increase the responsiveness of α_1-adrenergic receptors and induce the up-regulation of postsynaptic dopamine D_2/D_3 receptors in the rat brain, we designed the present experiments to determine whether repeated administration of REB evokes similar effects.

The experiments were carried out on male Wistar rats. REB was administered at a dose of 10 mg/kg (or 30 mg/kg in some cases) once or repeatedly (twice daily for 14 days). The obtained results show that REB administered repeatedly increased exploratory behavior induced by phenylephrine and potentiated the hyperlocomotion induced by D-amphetamine. These behavioral effects indicate the hyperresponsiveness of α_1-adrenergic receptors. Biochemical studies did not show any changes in the binding parameters of [^{3}H]prazosin (B_{max} or K_d), but the ability of the α_1-adrenergic receptor agonist, phenylephrine, to compete for these sites was significantly increased upon repeated administration of REB.

Locomotor activity induced by quinpirole was not changed, although there was a potentiation of 7-OH-DPAT-induced locomotor hyperactivity in rats receiving repeated administration of REB. At the same time no significant changes in the binding of [^{3}H]quinpirole and [^{3}H]7-OH-DPAT, or at the level of mRNA coding for dopamine D_2 receptors in the rat brain were observed. Enhanced responsiveness to 7-OH-DPAT observed in the behavioral studies might, therefore, result from alterations at the postreceptor level.

The above results indicate that repeated administration of REB induces the adaptive changes in the α_1-adrenergic receptors, especially it enhances their functional responsiveness. However, the question whether this functional responsiveness is important for the clinical antidepressant efficacy, remains to be elucidated.

Key words*: reboxetine, repeated treatment, adaptive changes, rats, noradrenergic and dopaminergic system*

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INTRODUCTION

Reboxetine, (PNU-0155950E), (RS)-2-[2(RS)-α-(2-ethoxyphenoxy)benzyl]morpholine methanesulfate (REB), is a member of a new class of antidepressant drugs (NARIs), which only inhibit the neuronal reuptake of noradrenaline [36], but do not inhibit serotonin or dopamine reuptake in the animal brain tissue [46]. In contrast to the most of typical tricyclic reuptake inhibitors, it is devoid of any affinity for neurotransmitter receptors nor does it inhibit the rat brain monoamine oxidase A or B [46].

The clinical efficacy of REB is comparable to that of typical tricyclics (like desipramine, imipramine or amitriptyline) [2, 3, 29].

Our earlier studies have shown that antidepressant drugs (ADs) administered repeatedly (but not at a single dose) increase, among others, responsiveness of the α_1-adrenergic system (sensitivity of postsynaptic α_1-adrenergic receptors). The measure of the above activity is potentiation of the behavioral hyperexploration evoked by α_1-adrenergic agonists (phenylephrine, methoxamine). Moreover, ADs administered repeatedly increase the binding to α_1-adrenergic receptors in different brain regions, in particular, they elevate the affinity of these receptors for their agonists (i.e. also to noradrenaline, the endogenous neuromediator) [26, 31]. The above effects have been described for various ADs (tricyclic noradrenaline and serotonin reuptake inhibitors, selective serotonin reuptake inhibitors (SSRIs), MAO inhibitors, atypicals) [17, 20, 28, 40].

Also ADs administered repeatedly, but not at a single dose, potentiate behavioral effects (locomotor hyperactivity) evoked by dopamine stimulants such as amphetamine (AMP) and quinpirole (QUI) or 7-OH-DPAT (a $D_2/D_{/3}$ agonist) [14, 21, 22]. These findings indicate that ADs given repeatedly activate the dopaminergic system by increasing responsiveness to stimulants, including dopamine. Further support for this concept comes from biochemical results that show that repeated administration of ADs increases the binding (density and affinity) of dopamine D_2 and D_3 receptors in certain brain structures [7, 14, 15, 24, 41], as well as raises the concentration of mRNA encoding dopamine D_2 receptors [8}.

The present study was aimed at determining whether REB, which inhibits noradrenaline reuptake and shows no receptor affinity, could evoke, when given repeatedly, adaptive changes in the α_1-adrenergic and dopamine D_2/D_3 system, similar to those produced by tricyclic drugs. To this end, we administered REB for two weeks (twice daily), i.e. with a period and dosage generally accepted in the treatment with tricyclics, and studied its effects on the response to the agonists of α_1-adrenergic (phenylephrine, PHEN) or dopamine D_2/D_3 (QUI, 7-OH-DPAT) receptors by examining its influence on the effects of PHEN (exploratory activity in the open field test) and dopaminergic agonists in the AMP-, QUI-, or 7-OH-DPAT-induced locomotor hyperactivity test.

We also studied the effect of repeated REB administration on the binding of [^{3}H]prazosin to α_1-adrenergic receptors in the rat cortex and on the binding at dopamine D_2 and D_3 receptor (autoradiography procedure) as well as on the concentration of mRNA coding for D_2 receptor in the nucleus accumbens, islands of Calleja and nucleus caudatus, i.e. brain regions considered to be abundant in D_2 and D_3 receptors.

MATERIALS and METHODS

The experiments were carried out on rats (male Wistar, healthy, ca. 80 days old). At the beginning of the experiment, rats weight was 220–230 g, increasing up to 270–300 g after 14 days of repeated drug administration. 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 12/12 h light/dark cycle (light on at 7 a.m.). The experiments were performed in accordance with the ethical requirements.

Substances

D-amphetamine sulfate (AMP; Sigma, St Louis, USA), (±)-7-hydroxy-dipropylaminotetralin hydrochloride (7-OH-DPAT; Research Biochemicals Int., USA), phenylephrine hydrochloride (PHEN; Research Biochemicals Int., USA), quinpirole hydrochloride (QUI; Research Biochemicals Int., USA), reboxetine hydrochloride (REB; PNU-0155950E, Pharmacia & Upjohn, Kalamazoo, MI, USA).

Drug administration

REB (10 or 30 mg/kg) was dissolved in distilled water and was administered perorally (*po*) with a stomach tube, once or repeatedly (twice daily for 14 days). Drug was administered at 8–9 a.m. and

8–9 p.m. All animals received treatment twice daily for 14 days. Control animals received vehicle for the whole period. Repeatedly treated animals received the appropriate drug, and animals treated acutely received vehicle for 13 days, and on the day 14, they received the appropriate drug, so all groups of animals were handled in the same manner. Using this experimental paradigm, we avoided the effect of a single intragastric intubation which inevitably, as a stressful event for an animal, might mask or change the actual effect of acute administration of the studied drug. All groups of animals, treated acutely or repeatedly, were subjected to the behavioral experiment at the same time.

Data analysis

The behavioral data were evaluated by one-way analysis of variance (ANOVA) followed, when appropriate, by individual comparisons with the control using Dunnett's test. The binding results were statistically assessed by ANOVA, intergroup differences were analyzed by Duncan's multiple range test.

Exploratory behavior induced by phenylephrine in rats

For experiments with PHEN, the rats were operated under pentobarbital anesthesia (30 mg/kg *ip*). They were implanted chronically and unilaterally with stainless steel guide cannulae 9.00 mm long (0.4 mm o.d.), according to the method described by Kolasiewicz and Maj [10]. After a 4-day postoperative period, the animals were administered REB (10 mg/kg *po*) twice daily for 14 days. Control animals were given vehicle.

PHEN was injected at a dose of 25 μg/5 μl into the lateral brain ventricle (at 24 h after the last dose of REB and 30 min before the test), using an inner injection cannula (11.6–14.6 mm long; 0.3 mm o.d.). The tip of injection cannula was aimed at the lateral ventricle (AP (–) 0.4–0.8, L 1.1–1.7) using stereotaxic coordinates [34]. Injection of the volume of 5 μl lasted 2 min. The inner cannula was withdrawn 1 min after the termination of the injection. Control animals (operated) were treated with appropriate volume of the solvent. Exploratory activity was assessed in the elevated open field test. The black circular elevated platform (without walls, 1 m in diameter, divided into six symmetrical sectors, elevated 50 cm above the floor) was used. During the experiment, the laboratory room was dark and only the centre of the open field was illuminated with 75 W bulb, hung 75 cm above it. The animals were placed in the open field and their exploratory behavior, i.e. the time of walking, number of crossings (ambulation), episodes of peeping outside the edge of the arena and rearing, was assessed for 5 min. After completion of the experiments, the rats were anesthetized with 45 mg/kg of pentobarbital, perfused through the heart with 4% paraformaldehyde, and decapitated. The brains were cut into 50 μm sections and the location of all the injection cannulae tips was determined histologically. Only those animals with histologically confirmed injection sites were used for the data analysis. Each group consisted of 8 rats.

STUDIES ON THE α_1-ADRENERGIC SYSTEM

α_1-Adrenergic receptor binding in the rat brain cortex

The experiment was carried out according to the method used previously [16, 23]. For [^{3}H]prazosin (specific activity: 19.5 Ci/nmol) binding studies, the tissue was homogenized for 15 s in 20 vol. (w/v) of an ice-cold Tris-HCl buffer (50 mM, pH 7.4) using Ultra Turrax homogenizer. The nonspecific binding was defined in the presence of 10 μM regitine. The homogenates were centrifuged at 25,000 × g for 10 min. That step was repeated twice. Final pellets were resuspended in 170 vol. (w/v) of a Tris-HCl buffer (50 mM, pH 7.4). Saturation isotherms were generated using eight concentrations (0.01–2 nM) of [^{3}H]prazosin. The bound ligand was separated by vacuum filtration through Whatman GF/C filters and was washed three times with 5 ml of ice-cold Tris-HCl buffer. Radioactivity was measured in 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 6–8 rats.

Phenylephrine competition for the [^{3}H]prazosin binding in the rat brain cortex

The experiment was carried out according to the method used previously [16, 23]. The affinity of α_1-adrenergic receptors for an agonist was estimated by studying the ability of various concentrations of PHEN (0.1 nM – 1 mM) to compete for

[^{3}H]prazosin binding sites. To a volume of 1.7 ml of tissue suspension, 200 µl of PHEN and 100 µl of [^{3}H]prazosin (at final concentration of 0.5 nM) were added. Afterwards, the samples were incubated at 25°C for 25 min, followed by a 10 min ice-cold bath. Finally, a total incubation volume of 2 ml was poured over glass filters (Whatman GF/C) and rinsed three times with 5 ml of an ice-cold Tris-HCl buffer. All assays were performed in duplicate. The data were analyzed using iterative fitting routines (Graph PAD Prism 2.0). Each group consisted of 6–8 rats.

STUDIES ON THE DOPAMINERGIC D_2/D_3 SYSTEM

D-amphetamine-, quinpirole- or 7-OH-DPAT-induced locomotor hyperactivity

Locomotor activity was measured in photoresistor actometers (two light beams, two photoresistors; L × W × H = 40 × 40 × 25 cm), starting at 24 h after single (acute experiment) or last (repeated experiment) administration of REB or saline. AMP (0.5 mg/kg *sc*) or QUI (0.3 mg/kg *sc*) was given at 23.5 h and 7-OH-DPAT (3 mg/kg *sc*) at 24 h after REB or vehicle administration. Locomotor activity measurement started 30 min after AMP and QUI or 5 min after 7-OH-DPAT administration, and lasted for 1 h (AMP) or 2 h (QUI or 7-OH-DPAT). Each experimental group consisted of 8 rats.

Dopamine D_2/D_3 receptor binding in the rat nucleus accumbens septi, nucleus caudatus and islands of Calleja – an autoradiographic procedure

After administration of REB or vehicle, the rat brains were carefully removed and rapidly frozen in dry ice liquid n-heptane. Consecutive coronal sections (12 µm) were cut at –19°C using a cryostat Jung CM 3000 (Leica). The effect of the drugs on dopamine D_2/D_3 receptor expression was evaluated in the coronal sections between the levels 1.0–1.7 mm from bregma and between 10.0–10.7 mm from interaural line including the nucleus caudatus, nucleus accumbens septi, olfactory tubercles and islands of Calleja, according to the Paxinos and Watson rat brain atlas [34].

Receptor binding with [^{3}H]QUI was visualized using the procedure described by Levant and de Souza [11] and Rogoż and Dziedzicka-Wasylewska [37]. Briefly, the sections were preincubated for 10 min at room temperature in 50 mM Tris-HCl buffer (pH 7.4) containing the following ions: 5 mM KCl, 2 mM $MgCl_2$ and 2 mM $CaCl_2$. The sections were then incubated at room temperature in the same buffer with 10 nM radioligand for 90 min. Non-specific binding was determined with 1 µM (+)-butaclamol. The experiment was terminated by dipping the sections in ice-cold buffer and rinsing them twice in distilled water. The sections were then dried as described above. Each experimental group consisted of 6–8 rats.

Dopamine D_3 receptor binding in the rat nucleus accumbens septi and islands of Calleja – an autoradiographic procedure

Dopamine D_3 receptors were labeled with [^{3}H]7-OH-DPAT, as described by Lévesque et al. [12] and Maj et al. [14, 16]. 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. 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 of 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.

After the experiments, the sections together with tritiated standards (Amersham), were exposed to [^{3}H]-Hyperfilm (Amersham) at 4°C, for 6–8 weeks. Following this period, the films were developed, fixed and washed under running water. Each experimental group consisted of 6–8 rats.

The level of mRNA encoding dopamine D_2 receptors in the nucleus accumbens septi and and nucleus caudatus – *in situ* hybridization

The effect of REB on the level of mRNA encoding dopamine D_2 receptors was determined as described previously [8], using a commercially available mixture of 48-mer synthetic deoxyoligonucleotides complementary to bases 4–51, 766–813 and 901–948 of the rat D_2 dopamine receptor (NEN Du Pont), which was labelled using [^{35}S]dATP (1,200 Ci/mmol, NEN DuPont) with terminal transferase (Roche Molecular Biochemicals). Each experimental group consisted of 6–8 rats.

RESULTS

STUDIES ON THE α_1-ADRENERGIC SYSTEM

Exploratory behavior induced by phenylephrine in rats

PHEN (25 μg/5 μl) given intraventricularly increased exploratory behavior in the open field test (time of walking, ambulation and peeping and rearing). REB at a single dose (10 mg/kg) neither affected the exploratory activity in normal rats (Tab. 1) nor it did change the action of PHEN (Tab. 1).

Table 1. Effect of single and repeated treatment with reboxetine (REB) on the exploratory behavior induced by phenylephrine (PHEN) in rats

Compounds (mg/kg)	Mean ± SEM		
	Time of walking	Ambulation	Peeping and rearing
Vehicle	42.4 ± 1.0	14.0 ± 1.2	12.4 ± 0.8
PHEN	75.4 ± 4.5**	28.2 ± 2.0**	20.9 ± 0.9*
REB, single	41.9 ± 1.5	15.4 ± 1.2	14.0 ± 0.9
REB, repeated	40.0 ± 1.3	17.2 ± 1.5	14.9 ± 1.8
REB, single + PHEN	75.2 ± 2.2	27.4 ± 2.1	19.4 ± 1.1
REB, repeated + PHEN	120.6 ± 6.0$^{\#\#}$	46.5 ± 3.2$^{\#\#}$	31.5 ± 1.1$^{\#}$

PHEN, 25 μg/5 μl was injected into the lateral brain ventricle 30 min before the test. The test was carried out at 24 h after the last dose of REB (10 mg/kg *po*). Data represent means ± SEM, n = 8. The statistical significance was assessed using ANOVA, followed, when appropriate, by the Dunnett's test. * $p < 0.05$, ** $p < 0.001$ vs vehicle-receiving group, $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.001$ vs PHEN-receiving groups

REB (10 mg/kg) administered repeatedly did not affect the exploratory activity in the open field test (Tab. 1). Repeated treatment with REB at 10 mg/kg enhanced the effect of PHEN in the open field test. It prolonged the time of walking and increased the number of ambulations or peeping and rearing episodes measured 24 h after the last dose of the drug (Tab. 1).

α_1-Adrenergic receptor binding in the rat brain cortex

REB (10 mg/kg) given acutely or repeatedly, did not influence the density (B_{max}) of [^{3}H]prazosin binding sites and the binding affinity (K_d) in the cerebral cortex measured 24 h after the last dose of the drug (Tab. 2).

Table 2. Effect of single and repeated administration of reboxetine (REB) on the binding of [^{3}H]prazosin to α_1-adrenergic receptors in the rat brain cortex

Treatment	B_{max} (fmol/mg protein)	K_d (nM)	K_i (μM)
Vehicle	8.74 ± 0.42	0.80 ± 0.08	11.65 ± 2.1
REB 10, single	10.28 ± 0.94	0.81 ± 0.06	8.90 ± 1.3
REB 10, repeated	9.81 ± 0.46	0.82 ± 0.08	4.60 ± 0.8*

Reboxetine (REB, 10 mg/kg *po*) was administered at a single dose or repeatedly (twice daily for 14 days). The tissue for biochemical measurements was taken out at 24 h after single or last dose of the drug. [^{3}H]prazosin was used as a ligand. The following binding parameters were determined: density, B_{max}; dissociation constant, K_d and K_i, inhibition constant of [^{3}H]prazosin binding by phenylephrine. Data represent means ± SEM, n = 6–8. The statistical significance was assessed using ANOVA followed, when appropriate, by Dunnett's test, * $p < 0.001$ vs vehicle-receiving group

Phenylephrine competition for the [^{3}H]prazosin binding in the rat brain cortex

Competition studies showed that acute administration of REB (10 mg/kg) did not affect the ability of PHEN to displace [^{3}H]prazosin from cortical α_1-adrenoreceptors (Tab. 2). Repeated treatment with REB (10 mg/kg) significantly enhanced the ability of PHEN to displace [^{3}H]prazosin from cortical α_1-adrenoreceptors, since the K_i value was significantly decreased. That effect was observed 24 h after the last dose of the drug (Tab. 2).

STUDIES ON THE DOPAMINERGIC D_2/D_3 SYSTEM

D-Amphetamine-induced locomotor hyperactivity

AMP at a dose of 0.5 mg/kg increased the locomotor activity of the rats in comparison with the control (vehicle-injected) group.

REB (10 mg/kg), given at a single dose, affected neither the locomotor activity of naive rats (data not shown) nor the locomotor hyperactivity induced by AMP (0.5 mg/kg), as shown in Table 3.

Table 3. Effect of reboxetine (REB) administered on the AMP-induced hyperactivity in rats

Compounds	Activity counts (mean ± SEM)
Vehicle + vehicle	135.0 ± 7.0
Vehicle + AMP	391.8 ± 40.3*
REB, single + AMP	392.0 ± 41.8
REB, repeated + AMP	669.8 ± 81.8#

REB (10 mg/kg *po*) was given at a single dose or repeatedly (twice daily, 14 days). AMP (0.5 mg/kg *sc*) was injected at 23.5 h after the single dose or after the last administration of REB. The measurement of the locomotor activity began 30 min after the AMP injection and lasted for 1 h. Data represent means ± SEM, n = 8. Statistical evaluation was carried out by ANOVA followed by Dunnett's test, * $p < 0.001$ vs vehicle-treated group; # $p < 0.05$ vs AMP-treated group

Repeated treatment with REB (10 mg/kg) increased the locomotor hyperactivity induced by AMP in rats (measured at 24 h after the last administration of this antidepressant) (Tab. 3).

Quinpirole-induced locomotor hyperactivity

QUI (0.3 mg/kg) increased the locomotor activity in rats (Tab.4). REB (10 mg/kg) given acutely or repeatedly did not change the locomotor hyperactivity evoked by QUI (0.3 mg/kg) in rats (Tab. 4).

Table 4. Effect of reboxetine (REB) administered on the QUI-induced hyperactivity in rats

Compounds	Activity counts (mean ± SEM)
Vehicle + vehicle	144.7 ± 12.4
Vehicle + QUI	684.2 ± 40.5*
REB, single + QUI	598.5 ± 60.2
REB, repeated + QUI	745.8 ± 94.2

REB (10 mg/kg *po*) was given at a single dose or repeatedly (twice daily, 14 days). QUI (0.3 mg/kg *sc*) was injected at 23.5 h after the single dose or after the last administration of REB. The measurement of the locomotor activity began 30 min after the QUI injection and lasted for 2 h. Data represent means ± SEM, n = 8. Statistical evaluation was carried out by ANOVA followed by Dunnett's test, * $p < 0.001$ vs vehicle-treated group

7-OH-DPAT-induced locomotor hyperactivity

7-OH-DPAT (3 mg/kg) increased the locomotor hyperactivity in rats (Tab. 5). Repeated (but not acute) treatment with REB (10 mg/kg) increased the locomotor hyperactivity induced by 7-OH-DPAT (Tab. 5).

Table 5. Effect of reboxetine (REB) administered on the 7-OH-DPAT-induced hyperactivity in rats

Compounds	Activity counts (mean ± SEM)
Vehicle + vehicle	144.8 ± 13.8
Vehicle + 7-OH-DPAT	496.0 ± 63.9*
REB, single + 7-OH-DPAT	483.1 ± 53.1
REB, repeated + 7-OH-DPAT	927.1 ± 146.1#

REB (10 mg/kg *po*) was given at a single dose or repeatedly (twice daily, 14 days). 7-OH-DPAT (3 mg/kg *sc*) was injected at 24 h after the single dose or after the last administration of REB. The measurement of the locomotor activity began 5 min after the 7-OH-DPAT injection and lasted for 2 h. Data represent means ± SEM, n = 8. Statistical evaluation was carried out by ANOVA followed by Dunnett's test, * $p < 0.05$ vs vehicle-treated group, # $p < 0.001$ vs 7-OH-DPAT-treated group

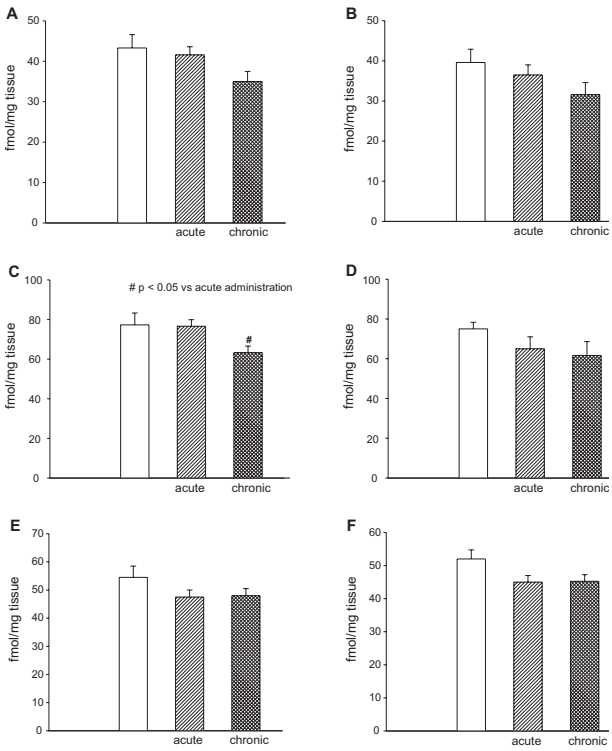

Fig. 1. Effect of acute and chronic treatment with REB (30 mg/kg *po*) on the binding [^{3}H]quinpirole in the rat brain regions: A – shell of nucleus accumbens septi, B – core of nucleus accumbens septi, C – islands of Calleja, D – islands of Calleja Magna, E – rostral striatum (bregma 1.6 mm), F – caudal striatum (bregma 1.0 mm). The tissue for biochemical analysis was taken 24 h after acute or last administration of REB. Data represent density of radioligand binding (fmol/mg of tissue ± SEM), n = 6–8. The statistical significance was assessed using ANOVA followed by Dunnett's test, # $p < 0.05$ vs acute administration

Dopamine D_2/D_3 receptor binding in the rat nucleus accumbens septi, nucleus caudatus and islands of Calleja – an autoradiographic study

REB given acutely or repeatedly at a dose of 10 mg/kg did not induce any statistically significant changes in the binding of [^{3}H]quinpirole to the dopamine D_2/D_3 receptors (data not shown), therefore, we used the higher dose of the drug, i.e. 30 mg/kg.

REB given acutely or repeatedly at a dose of 30 mg/kg did not induce any changes in the binding of [^{3}H]quinpirole in the rat nucleus caudatus and nucleus accumbens septi, however, in the islands of Calleja we observed the statistically significant decrease in the binding of this radioligand (Fig. 1).

Dopamine D_3 receptor binding in the rat nucleus accumbens septi and islands of Calleja – an autoradiographic study

REB given acutely or repeatedly at a dose of 10 mg/kg did not induce any statistically significant changes in the binding of [^{3}H]7-OH-DPAT to the dopamine D_3 receptors (data not shown), therefore, we used the higher dose of the drug, i.e. 30 mg/kg.

After the repeated administration of REB, the binding of [^{3}H]7-OH-DPAT was decreased in the islands of Calleja but not in the shell region of the nucleus accumbens (Fig. 2).

The level of mRNA encoding dopamine D_2 receptors in the nucleus accumbens septi and nucleus caudatus – *in situ* hybridization

REB given acutely or repeatedly at a dose of 10 mg/kg did not induce any statistically significant changes in the levels of mRNA encoding dopamine D_2 receptors (data not shown), therefore, we used the higher dose of the drug, i.e. 30 mg/kg. However, even this higher dose of REB, administered acutely or repeatedly, did not influence the levels of mRNA encoding dopamine D_2 receptors in any of the rat brain regions studied (Fig. 3).

DISCUSSION

The aim of the present study was to investigate the effect of the new antidepressant REB, administered repeatedly (14 days), on the α_1-adrenergic and dopaminergic receptors in the rat brain.

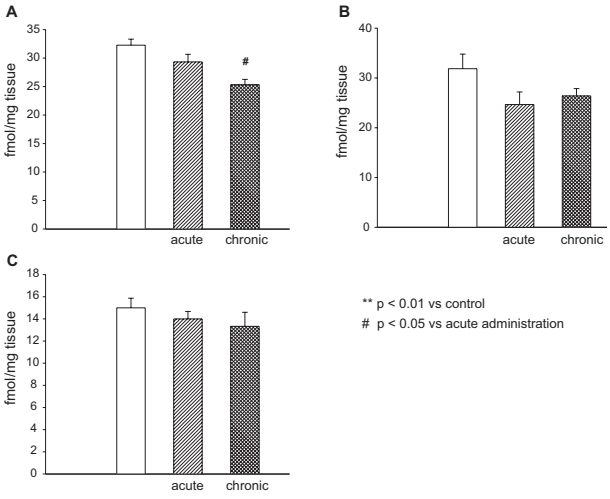

Fig. 2. Effect of acute and chronic treatment with REB (30 mg/kg *po*) on the binding of [^{3}H]7-OH-DPAT in the rat brain regions: A – islands of Calleja, B – islands of Calleja Magna, C – shell of nucleus accumbens septi. The tissue for biochemical analysis was taken 24 h after acute or last administration of REB. Data represent density of radioligand binding (fmol/mg of tissue ± SEM), n = 6–8. The statistical significance was assessed using ANOVA followed by Dunnett's test, ** $p < 0.01$ vs control, # $p < 0.05$ vs acute administration

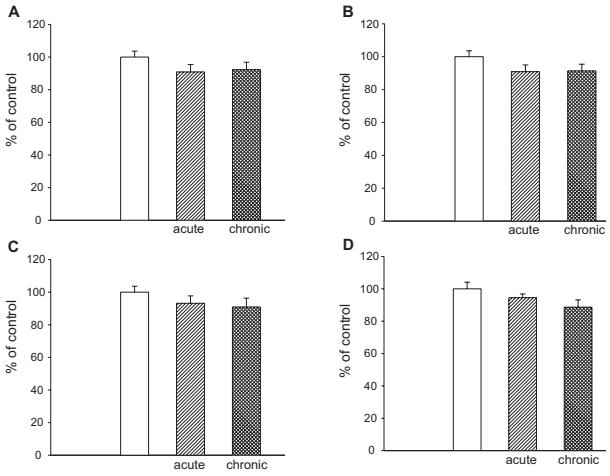

Fig. 3. Effect of acute and chronic treatment with REB (30 mg/kg *po*) on the level of mRNA for D_2 dopamine receptor in the rat brain regions: A – shell of nucleus accumbens septi, B – core of nucleus accumbens septi, C – rostral striatum (bregma 1.6 mm), D – caudal striatum (bregma 1.0 mm). The tissue for biochemical analysis was taken 24 h after acute or last administration of REB. Data represent percent of control level (arbitrary optical density units) ± SEM, n = 6–8. The statistical significance was assessed using ANOVA

The obtained results indicate that REB, given repeatedly (but not acutely), potentiated the PHEN-induced exploratory hyperactivity in the open field test in rats, the effect connected with α_1-adrenergic receptor stimulation [5]. This finding is in agreement with previous reports, which demonstrated that the methoxamine-induced exploratory hyperactivity in the open field test in rats and the clonidine-induced aggression in mice were enhanced by repeated treatment with REB [38]. This aggressiveness results from the stimulation of α_1-adrenergic receptors [17, 32]. Therefere, it may be concluded that REB given repeatedly evokes hyperresponsiveness of α_1-adrenoceptors. Similar results were observed in earlier studies after repeated administration of tricyclic antidepressants [17, 18, 20, 28].

However, the results obtained in the biochemical part of the present studies show that REB (10 mg/kg), administered neither acutely nor repeatedly changed the binding parameters of [^{3}H]prazosin to α_1-adrenergic receptors in the rat brain cortex. On the other hand, repeated treatment with REB (10 mg/kg) increased the affinity of α_1-adrenergic receptors for their agonist (PHEN), when measured at 24 h after the last dose.

These results indicate that the increased responssiveness of α_1-adrenergic receptors observed in the behavioral experiments (i.e. increased PHEN-induced exploratory hyperactivity) might result from the increased affinity of α_1-adrenergic receptors for their agonist. Our earlier investigations showed that ADs, given repeatedly, increased the density (B_{max}) of α_1-adrenoceptors in the cerebral cortex of the rat when [^{3}H]prazosin was used as a ligand. The increased density of α_1-adrenergic receptors in the cortex and other brain structures after repeated administration of ADs was confirmed by several authors [31, 45]. However, the lack of an increase in density of α_1-adrenergic receptors after repeated administration of ADs was also demonstrated [28, 42]. On the other hand, using a different approach, i.e. the method of [^{3}H]prazosin displacement by PHEN, Menkes et al. [26] showed that amitriptyline, desipramine and iprindole increased the affinity of α_1-adrenergic receptors for their agonists. Similar effect was demonstrated for imipramine, mianserin, citalopram [9] as well as milnacipran, venlafaxine or tianeptine [16, 19, 39].

The above-described results suggest a mechanism whereby α_1-adrenoceptors become functionally supersensitive after antidepressant treatment (increase in either their number or affinity for an agonist). This finding may have relevance for the noradrenergic hypothesis of depression which posits a functional deficit of noradrenaline in this disease. On the other hand, clinical neuroendocrinological studies have demonstrated α_1-adrenergic subsensitivity in certain depressive states [25], which may indicate that the regulation of α_1-adrenergic receptors might be related also to the therapeutic action of REB in man, besides other possible mechanisms.

On the other hand, our studies show that REB, administered repeatedly, did not induce any significant alterations at the level of central dopamine D_2/D_3 receptors, as has been shown previously for other ADs, displaying different pharmacological profile [14, 15, 37]. Only higher dose of REB (30 mg/kg), administered repeatedly, evoked the decrease in the dopamine D_3 receptor expresion in the islands of Calleja, however, the functional role of these receptors in the islands of Calleja is rather poorly understood at present. Studies conducted by Barik and De Beaurepaire [1] with the local administration of dopamine D_3 receptor agonists indicated that these receptors mediate a decrease in body temperature, but have no effect on the locomotor activity.

Repeated administration of REB did not change the locomotor activity stimulated by QUI but significantly increased the effect of AMP or 7-OH-DPAT. However, in the biochemical studies we did not observe any changes at the level of dopamine D_2/D_3 receptors (autoradiographic analysis using [^{3}H]quinpirole or [^{3}H]7-OH-DPAT) nor at the level of mRNA coding for dopamine D_2 receptors in various regions of the rat brain. As has been shown by Darracq et al. [6], locomotor activating effects of AMP are caused by the stimulation of cortical α_1-adrenergic receptors by noradrenaline, which increases the release of a functionally activity subcortical dopamine. Since in animals receiving REB the affinity of α_1-adrenergic receptors for the agonist is increased, as has been shown in the present study, this is the most probable explanation why we observed an enhancement of AMP-stimulated locomotor activity following repeated administration of REB, even without any changes at the level of dopamine D_2/D_3 receptors.

However, it is difficult to interpret the results obtained in the behavioral studies with the use of 7-OH-DPAT, an agonist of dopamine D_3 receptors,

which show that repeated administration of REB strongly enhances the locomotor activity induced by 7-OH-DPAT, while the binding of [^{3}H]7-OH-DPAT was not significantly changed. Such results suggest that it may well be that even in case of the lack of changes in the density of dopamine D_3 receptors, the enhancement of functional coupling takes place between these receptors and their effector system *via* appropriate G protein. Such results have been already reported by Dziedzicka-Wasylewska and Rogoż [7], who have shown that, following repeated administration of imipramine, despite no changes in the density of dopamine D_2 receptors in the striatum, the significantly stronger inhibitory effect of QUI on the activity of adenylate cyclase was observed as compared to the effect of QUI in the striatal slices obtained from control animals. The use in this experiment of the nonhydrolyzable analog of GTP, GppNHp, which acts at the level of G_i protein, permitted to conclude that upon repeated imipramine administration, the enhancement of functional coupling between the receptor and adenylate cyclase takes place. Similar changes have been reported by other authors [27, 33]. In the recent paper, it has also been shown that transgenic mice lacking the noradrenaline transporter are supersensitive to psychostimulants [47], however, ligand binding studies failed to demonstrate any genotypic differences in D_1 ([^{125}I]iodoSCH23982) or D_2 ([^{3}H]spiperone) binding. However, analysis of striatal D_2/D_3 receptor function using [^{35}S]GTPγS binding assay revealed that these receptors were more efficiently coupled to their G proteins in the mutants that those in the wild-type animals.

Other studies also show that the effects of REB on the dopaminergic system are complex. Systemic administration of REB increased extracellular dopamine concentrations in the prefrontal cortex, but not in the nucleus acumbens [13]. These results are supported by several previous studies showing a substantial effect of systemic administration of noradrenaline reuptake inhibitors on central dopamine output, particularly in the medial prefrontal cortex [4, 43] with small or no effects in the nucleus accumbens septi [30, 35, 43]. The elevated extracellular levels of dopamine in the medial prefrontal cortex may be due to the inhibition of dopamine reuptake by the noradrenaline transporter in the areas where noradrenergic terminals are in abundance, such as medial prefrontal cortex [44, 48]. Consequently, regional variations in the density of the noradrenergic innervation may largely explain the differential effects of systemically administered noradrenaline reuptake inhibitors on extracellular dopamine concentrations in various brain regions.

Concluding the analysis of the results obtained in the present study we may state that repeated REB administration induces the adaptive changes in the α_1-adrenergic system, especially it enhances the functional responsiveness of α_1-adrenergic receptors. However, the question whether this increased functional responsiveness is important for the clinical antidepressant efficacy, remains open.

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