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Effects of Venlafaxine Given Repeatedly on α_1-Adrenergic, Dopaminergic and Serotonergic Receptors in Rat Brain

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Venlafaxine (VEN), a representative of a new class of antidepressants (serotonin and noradrenaline reuptake inhibitors, SNRI), administered repeatedly affects — as was demonstrated by us previously — the behavioural responsiveness of α_1-adrenergic, dopaminergic (D_2 and D_3) and serotonergic systems to their agonists. In the present study we aimed to find out whether parallel changes in the binding to the respective receptors also occurred. The experiment was carried out on male Wistar rats. VEN was administered in a dose of 10 mg/kg once or repeatedly (14 days, twice daily). The obtained results showed that VEN did not change the binding (B_{max} and K_D) of α_1-adrenergic receptors to [^{3}H]-prazosin in the cerebral cortex, having increased only its displacement by phenylephrine. The binding (B_{max} and K_D) to D_1 and D_2 receptors in the limbic forebrain and the striatum was not affected by repeated venlafaxine when [^{3}H]-SCH 23390 and [^{3}H]-spiperone, respectively, were used as ligands. When [^{3}H]-quinpirole was used as a ligand, the binding was enhanced in the striatum, the nucleus accumbens (shell and core) and islands of Calleja. VEN also increased the binding of [^{3}H]-7-OH-DPAT to D_3 receptors in islands of Calleja and the nucleus accumbens (shell). In the serotonergic system, a decrease in the density of 5-HT_{1A} receptors was observed in the hippocampus, whereas no changes occurred in the binding of 5-HT_2 receptors in the cortex. Thus VEN given repeatedly enhanced the binding (of the ligands that are agonists) to dopamine D_2 and D_3 receptors. Weaker effects were observed in the α_1-adrenergic and the serotonergic systems. Copyright © 1999 John Wiley & Sons, Ltd.

KEY WORDS — repeated venlafaxine; brain neurotransmitter receptors

INTRODUCTION

Venlafaxine [Wy-45,030; 1-[2-(dimethylamino)-1-(4-methyloxphenyl)ethyl]cyklohexanol, VEN] is a member of a new class of antidepressant drugs (SNRIs) which inhibit the neuronal reuptake of 5-hydroxytryptamine (5-HT) and noradrenaline (NA) (Muth *et al*., 1986). VEN also inhibits, to a lesser extent, though, the dopamine (DA) uptake (Muth *et al*., 1986). The drug differs chemically and pharmacologically from typical tricyclic reuptake inhibitors, as it is devoid of any affinity for α_1-, α_2-, β-adrenergic, serotonergic (5-HT_{1A}, 5-HT_{2A}), dopaminergic, muscarinic or histaminergic receptors (Cusack *et al*., 1994; Muth *et al*., 1986, 1991). In acute experiment, VEN antagonizes — among others — reserpine and apomorphine hypothermia, potentiates head twitches evoked by 5-hydroxy-L-tryptophan, and reduces the immobility time in the behavioural despair test (Lloyd and Mitchell, 1992; Moyer *et al*., 1984; Rogóż *et al*., 1998), i.e. it produces effects similar to those evoked by tricyclic 5-HT- and NA reuptake inhibitors. The latter drugs administered repeatedly, i.e. in such a way as to obtain an antidepressant effect in depressive patients, induce adaptative changes; among others, they enhance the responsiveness of α_1-adrenergic and dopaminergic receptors and also alter the responsiveness of 5-HT receptor subpopulations to the respective agonists (Maj, 1984, 1986; Maj *et al*., 1981, 1984, 1985b, 1989a,b,c, 1996a). Our behavioural results indicate that VEN administered repeatedly induces adaptive changes similar to those described by us earlier for tricyclic antidepressants (Maj and Rogóż, 1999). The drug

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potentiates the clonidine-induced aggressiveness in mice and the methoxamine-induced exploratory hyperactivity in rats, both of these effects being mediated by α_1-adrenoceptors (Maj *et al*., 1981, 1989b; Ozawa *et al*., 1975). It potentiates the locomotor hyperactivity induced by D-amphetamine and (±)7-OH-DPAT and increases dopaminergic (mainly D_2/D_3) systems. VEN decreases the head twitch reaction induced by 5-hydroxy-L-tryptophan or (±)-1-(2,5-dimethoxy-4-iodo-phenyl)-2-aminopropane and the hypothermia induced by trifluoromethylphenylpiperazine (Maj and Roǵoż, 1999), all those effects being mediated by 5-HT_2 receptors (Arnt *et al*., 1984; Green *et al*., 1983; Kodzińska and Chojnacka-Wójcik, 1992; Ortmann *et al*., 1982).

In the case of various antidepressants administered repeatedly, apart from the above-described behavioural effects, also biochemical changes were observed. The latter changes were measured by methods of binding of [^{3}H] labelled ligands to the respective receptors (α_1-adrenergic, dopaminergic and serotonergic) (Klimek and Nielsen, 1987; Klimek *et al*., 1994; Maj *et al*., 1990, 1996a).

The present paper was aimed at determining whether VEN, which inhibits the reuptake of 5-HT and NA but is devoid of affinity for the above-mentioned receptors, evokes — when given repeatedly — biochemical changes similar to those produced by tricyclic drugs which show a high affinity for these receptors. To this end we administered VEN — like tricyclics — for two weeks (twice daily) and studied its effects on the binding to rat brain α_1-adrenergic, dopaminergic D_1, D_2 and D_3, serotonergic 5-HT_{1A} and 5-HT_{2A} receptors using [^{3}H]-prazosin, [^{3}H]-SCH 23390, [^{3}H]-spiperone, [^{3}H]-7-OH-DPAT, [^{3}H]-quinpirole, [^{3}H]-8-OH-DPAT and and [^{3}H]-ketanserin, respectively, as ligands.

Our preliminary results were presented at the XXIst CINP Congress in Glasgow (Maj *et al*., 1998b).

MATERIALS AND METHODS

Animals

The experiments were carried out on rats (male Wistar, 270–300 g). The animals had free access to food and water before the experiment and were kept at a constant room temperature (22 ± 1°C), on a 12 h light/dark cycle (the light on at 7 a.m.). The experiments were performed according to ethical requirements.

Substances used

Phenylephrine hydrochloride (Research Biochemical Int.), venlafaxine hydrochloride (VEN; Wyeth-Ayerst Research).

Drug administration

VEN in a dose of 10 mg/kg p.o. was administered twice a day for 14 days. Rats were killed 2 or 72 h after a single (acute treatment) or the last dose (repeated treatment) of VEN. The tissue [cortex, striatum, hippocampus and limbic forebrain (containing the olfactory tubercle, preoptic area, nucleus accumbens, septum, amygdala and ventral cortex)] was dissected and frozen.

Statistical analysis

The binding results were statistically assessed by a one-way analysis of variance (ANOVA), and inter-intergroup differences were analyzed by Duncan's multiple range test.

The autoradiograms were analyzed by using a computer imaging system MCID-M1 (Canada) and quantified with the use of computer-generated curves derived from the standards. Film images of sections with non-specific binding were subtracted from those of adjacent sections with total binding, thus permitting the direct observation of images representing specific binding on screen. The regional densities of labelled receptors were compared by using ANOVA, followed by Fisher's test to compare each treatment with the appropriate control level.

BINDING STUDIES

α_1-Adrenergic receptor binding in the rat brain cortex

The experiment was carried out according to the method used previously (Maj *et al*., 1990). For [^{3}H]-prazosin (specific activity: 25 Ci/mmol) binding studies, the tissue was homogenized for 15 s in 20 vol (w/v) of an ice-old Tris-HCl buffer (50 mM, pH 7·4) using a Polytron homogenizer. The non-specific binding was defined in the presence of 5 μM phentolamine. 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 over Whatman GF/C filters and was washed three times with 5 ml of an ice-cold Tris-HCl buffer. Radioactivity was measured in a Backman LS 3801 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 eight rats.

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

The experiment was carried out according to the method used previously (Maj *et al*., 1990). The agonist affinity was estimated by studying the ability of various concentrations of phenylephrine (0·1 nM–1 mM) to compete for [^{3}H]-prazosin binding sites. To a volume of 1·7 ml tissue suspension there were added: 200 µl of phenylephrine and 100 µl of [^{3}H]-prazosin (final concentration: 0·5 nM). 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. Each group consisted of eight rats.

Dopamine D_1 receptor binding in the rat limbic forebrain and striatum

According to the method used previously (Klimek and Nielsen, 1987), the tissue suspension (250 µl) was incubated together with 150 µl of phosphate buffer (50 mM, pH 7·4), 50 µl of 1 µM cis-flupentixol (displacer) or H_2O, and 50 µl of [^{3}H]-SCH 23390 (specific activity 85 Ci/mmol) were incubated at 30°C for 60 min, followed by rapid vacuum filtration through Whatman GF/C glass filters using a Brandell cell harvester. The filters were washed three times with 5 ml portions of an ice-cold phosphate buffer, and tritium was estimated by the conventional liquid scintillation counting. Saturation isotherms were generated using 6–8 concentrations (0·1–2 nM) of [^{3}H]-SCH 23390. The assays were performed in duplicate. Each group consisted of eight rats. The data were analyzed using iterative curve fitting routines (Graph PAD Prism 2·0).

Dopamine D_2 receptor binding in the rat limbic forebrain and striatum

According to the method used previously (Klimek and Nielsen, 1987) the membrane suspension, 250 µl, was incubated with 150 µl of a phosphate buffer, 50 µl of 5 µM (±) butaclamol (displacer) or H_2O, and 50 µl of [^{3}H]-spiperone (specific activity of 16·7 Ci/mmol, N.E.N. Chemicals) at 37°C for 30 min. The total incubation volume (0·5 ml) was filtered through Whatman GF/C glass filters using a Brandell cell harvester. The filters were rinsed three times with 5 ml of an ice-cold phosphate buffer. Saturation isotherms were generated using 6–8 concentrations (0·1–2 nM) of [^{3}H]-spiperone. Each group consisted of eight rats. The data were analyzed using iterative curve fitting routines (Graph PAD Prism 2·0).

Dopamine D_3 receptor binding in rat islands of Calleja and nucleus accumbens — an autoradiographic procedure

The method has been used previously (Maj *et al*., 1998a). Rat brains were carefully removed and rapidly frozen in a dry ice liquid *n*-heptane. Consecutive coronal sections (12 µm) were cut out at −19°C, using a cryostat Jung CM 3000 (Leica). The effect of drug treatment on dopamine D_3 receptors was evaluated in brain sections between the levels 1·0–1·7 mm from the Bregma and 10·0–10·7 mm from the Interaural including the shell part of the nucleus accumbens and islands of Calleja, according to rat brain atlas (Paxinos and Watson, 1986).

The slices were sectioned and thaw-mounted on pre-cleaned and gelatin-coated glass microscope slides. They were stored at −70°C. Immediately prior to use, the slide-mounted sections were dried at room temperature. To label dopamine D_3 receptors with [^{3}H]-7-OH-DPAT, the binding was carried out as described by Lévesque *et al*. (1992). Briefly, tissue sections were first preincubated for 10 min at room temperature in 50 mM of the HEPES/NaOH buffer (pH 7·5) containing 1 mM EDTA and 0·1 per cent bovine serum albumin. The sections were then incubated for 60 min at room temperature in the buffer described above with 0·5–1 nM of [^{3}H]-7-OH-DPAT. To determine the non-specific binding, parallel sections were incubated in the presence of 10 µM of dopamine. Following incubation, the tissue sections were washed four times in ice-cold HEPES/NaOH

buffer (50 mM pH 7·5) containing 100 NaCl, twice in distilled water, and then dried under cool air.

After the experiment, the sections were exposed, together with tritiated standards (Amersham), to a ^{3}H-Hyperfilm (Amersham) for 6–8 weeks at 4°C. Then the films were developed, fixed, and washed under running water.

Dopamine D_2 and D_3 receptor binding in the striatum, islands of Calleja and nucleus accumbens (core and shell) — an autoradiographic procedure

Rat brains were carefully removed and rapidly frozen in a dry ice liquid *n*-heptane. Consecutive coronal sections (12 μm) were cut out at −19°C using a cryostat Jung CM 3000 (Leica). The effect of the treatment on the D_2/D_3 receptors expression was evaluated in coronal sections between the levels 1–1·7 mm from the Bregma and 10–10·7 mm from the Interaural including the nucleus caudatus, nucleus accumbens, olfactory tubercles and islands of Calleja, according to the Paxinos and Watson (1986) rat brain atlas. The slices were sectioned and thaw-mounted on pre-cleaned and gelatin-coated glass microscope slides. They were stored at −70°C. Immediately prior to use, the slide-mounted sections were dried at a room temperature.

The receptor binding with [^{3}H]-quinpirole was carried out under conditions described by Levant *et al.* (1993). Briefly sections were preincubated for 10 min at room temperature in 50 mM Tris-HCl buffer (pH 7·4) containing the ion solutions: 5 mM KCl, 2 mM $MgCl_2$ and 2 mM $CaCl_2$. The sections were then incubated for 120 min at room temperature in the same buffer with 10 nM of a radioligand. Non-specific binding was determined with 1 μM of (+)-butaclamol. The experiment was terminated by dipping the sections in an ice-cold buffer and rinsing them twice with distilled water. The sections were then dried under cool air.

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

5-HT_{1A} binding in the rat hippocampus

[^{3}H]-8-OH-DPAT (specific activity 221 Ci/mmol; NEN DuPont) was used for labelling the 5-HT_{1A} receptor. Membrane preparation and assay procedures, were carried out according to Schlegel and Peroutka (1986) with slight modifications. Briefly, the hippocampal tissue was homogenized in 20 vol. of 50 mM Tris-HCl buffer (pH 7·7 at 25°C) using an Ultra-Turrax T25, and was then centrifuged at 32 000 *g* for 10 min. The supernatant was discarded, and then pellets were resuspended in the same volume of Tris-HCl buffer and then recentrifuged. Before the third centrifugation, the samples were preincubated at 37°C for 10 min. The final pellets were resuspended in the Tris-HCl buffer containing 10 μM of pargyline, 4 mM of calcium chloride and 0·1 per cent ascorbic acid. One millilitre of the tissue suspension (5 mg of wet weight), 100 μl of 10 μM 5-HT (for nonspecific binding) and 100 μl of [^{3}H]-8-OH-DPAT were incubated at 37°C for 15 min. Saturation isotherms were generated using six concentrations (0·1–2 nM) of [^{3}H]-8-OH-DPAT. The incubation was followed by rapid vacuum filtration through Whatman GF/B glass filters, which were then washed three times with 5 ml of a cold buffer (50 mM Tris-HCl, pH 7·7) using a Brandel cell harvester. Radioactivity was measured in a Beckman L53801 scintillation counter. All assays were performed in duplicate. Each group consisted of 6–8 animals. The data were analyzed using iterative curve fitting routines (Graph PAD Prism 2·0).

5-HT_{2A} binding in the rat brain cortex

[^{3}H]-ketanserin (specific activity 77 Ci/mmol; NEN DuPont) was used for labelling 5-HT_{2A} receptors. The assay was carried out according to the method of Leysen *et al.* (1982) with slight modifications. The cerebral cortex tissue was homogenized in 20 vol. of 50 mM Tris-HCl buffer (pH 7·7 at 25°C), and was centrifuged at 32 000 *g* for 20 min. The resulting pellets were resuspended in the same volume of the buffer, preincubated at 37°C for 15 min, and centrifuged for 20 min. The final pellets were resuspended in 50 vol. of the same buffer. One millilitre of the membrane suspension, 100 μl of 1 μM methysergide (displacer) and 100 μl of [^{3}H]-ketanserin were incubated at 37°C for 20 min, followed by a rapid vacuum filtration through Whatman GF/B glass filters, and were then washed three times with 5 ml of a cold Tris-HCl buffer. Saturation isotherms were generated using eight concentrations (0·001–4 nM) of [^{3}H]-ketanserin. Radioactivity was measured in a Beckman L53801 scintillation counter. All essays were performed in duplicate. Each group consisted of

6–8 animals. The data were analyzed using iterative curve fitting routines (Graph PAD Prism 2·0).

RESULTS

α_1-Adrenergic receptor binding in the rat brain cortex

VEN administered in a single dose or repeatedly (14 days, twice daily) did not change the density (B_{max}) of [^{3}H]-prazosin binding sites, or the binding affinity (K_D) in the rat cerebral cortex (Table 1), measured 2 or 72 h after a single dose or the last dose (repeated treatment) of the drug.

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

Competition studies showed that repeated administration of VEN significantly enhanced the ability of phenylephrine to displace [^{3}H]-prazosin from cortical α_1-adrenoceptors, since the K_i value was significantly decreased (Table 2). That effect was observed 72 h after the last dose of VEN, but not 2 h after it and not after single-dose treatment.

Dopamine D_1 receptor binding in rat limbic forebrain and striatum

The binding of [^{3}H]-SCH23390 to dopamine D_1 receptors was carried out in the rat limbic forebrain and striatum, following single-dose or repeated administration of VEN (Table 3). No significant changes in the density of dopamine D_1 receptors (B_{max}) were observed in those brain regions. The affinity for [^{3}H]-SCH 23390 was increased in the striatum only at 72 h after single dose of VEN.

Table 1. Effect of single and repeated administration of venlafaxine on the binding of [^{3}H]-prazosin to α_1-adrenergic receptors in the rat brain cortex

Treatment	Cortex	
	B_{max}(fmol/mg protein)	K_D (nM)
Vehicle	9·80 ± 0·90	0·13 ± 0·013
VEN, single, 2 h	9·30 ± 1·10	0·11 ± 0·015
VEN, single, 72 h	9·95 ± 0·86	0·11 ± 0·019
VEN, repeated, 2 h	9·60 ± 0·90	0·11 ± 0·020
VEN, repeated, 72 h	10·80 ± 1·30	0·12 ± 0·020

VEN (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily, 14 days). The tissue for biochemical measurements was taken out at 2 or 72 h after single or last dose of the drug. Data represents means ± SEM, $n = 8$. Statistical evaluation was carried out by the Duncan's test.

Table 2. Effect of single and repeated administration of venlafaxine on the phenylephrine competition for [^{3}H]-prazosin binding sites in the rat cortex

Treatment	Cortex K_i (μM)
Vehicle	18·90 ± 1·8
VEN, single, 2 h	15·96 ± 1·9
VEN, single, 72 h	16·30 ± 1·9
VEN, repeated, 2 h	17·70 ± 2·9
VEN, repeated, 72 h	14·30 ± 1·3*

VEN (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily, 14 days). The tissue for biochemical measurements was taken out at 2 or 72 h after single or last dose of the drug. Data represents means ± SEM, $n = 8$. Statistical evaluation was carried out by the Duncan's test, $*p < 0.05$ vs vehicle-treated group.

Table 3. Effect of single dose and repeated administration of venlafaxine on the binding of [^{3}H]-SCH 23390 to D_1 receptors in the rat limbic forebrain and striatum

Treatment	Limbic forebrain		Striatum	
	B_{max} (fmol/mg protein)	K_D (nM)	B_{max} (fmol/mg protein)	K_D (nM)
Vehicle	6·38 ± 1·10	1·44 ± 0·17	19·11 ± 0·45	1·60 ± 0·27
VEN, single, 2 h	6·90 ± 0·34	1·33 ± 0·15	14·62 ± 3·20	1·25 ± 0·08
VEN, single, 72 h	5·54 ± 0·57	1·05 ± 0·18	15·24 ± 3·00	0·83 ± 0·11*
VEN, repeated, 2 h	6·56 ± 1·00	1·40 ± 0·35	17·92 ± 0·90	1·27 ± 0·10
VEN, repeated, 72 h	6·76 ± 1·00	1·45 ± 0·27	19·80 ± 3·20	1·58 ± 0·17

VEN (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily, 14 days). The tissue for biochemical measurements was taken 2 or 72 h after the last dose of the drug. Data represents means ± SEM, $n = 8$. Statistical evaluation was carried out by the Duncan's test, $*p < 0.05$ vs vehicle-treated group.

Table 4. Effect of single dose and repeated administration of venlafaxine on the binding of [^{3}H]-spiperone to D_2 receptors in the rat limbic forebrain and striatum

Treatment	Limbic forebrain		Striatum	
	B_{max} (fmol/mg protein)	K_D (nM)	B_{max} (fmol/mg protein)	K_D (nM)
Vehicle	8·90 ± 1·10	1·38 ± 0·17	15·80 ± 1·16	1·30 ± 0·15
VEN, single, 2 h	8·30 ± 1·60	1·40 ± 0·21	16·25 ± 1·3	1·30 ± 0·08
VEN, single, 72 h	7·60 ± 0·80	1·53 ± 0·21	15·46 ± 1·17	1·30 ± 0·21
VEN, repeated, 2 h	7·63 ± 1·20	1·48 ± 0·20	15·50 ± 1·4	1·25 ± 0·20
VEN, repeated, 72 h	7·72 ± 0·70	1·36 ± 0·25	14·15 ± 1·2	1·20 ± 0·22

VEN (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily, 14 days). The tissue for biochemical measurements was taken 2 or 72 h after the last dose of the drug. Data represents means ± SEM, $n = 8$. Statistical evaluation was carried out by the Duncan's test.

Dopamine D_2 receptor binding in rat limbic forebrain and striatum

The effects of VEN administration on the binding parameters of [^{3}H]-spiperone to dopamine D_2 receptors in the limbic forebrain and striatum are presented in the Table 4. In both those brain regions no significant changes were observed in the receptor density (B_{max}) or affinity for dopamine D_2 receptors (K_D).

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

The effects of VEN, administered acutely or repeatedly, on the binding of [^{3}H]-7-OH-DPAT to D_3 receptors in the islands of Calleja and the shell part of the nucleus accumbens septi are presented in Figure 1. In the islands of Calleja, VEN given acutely or repeatedly induced a statistically

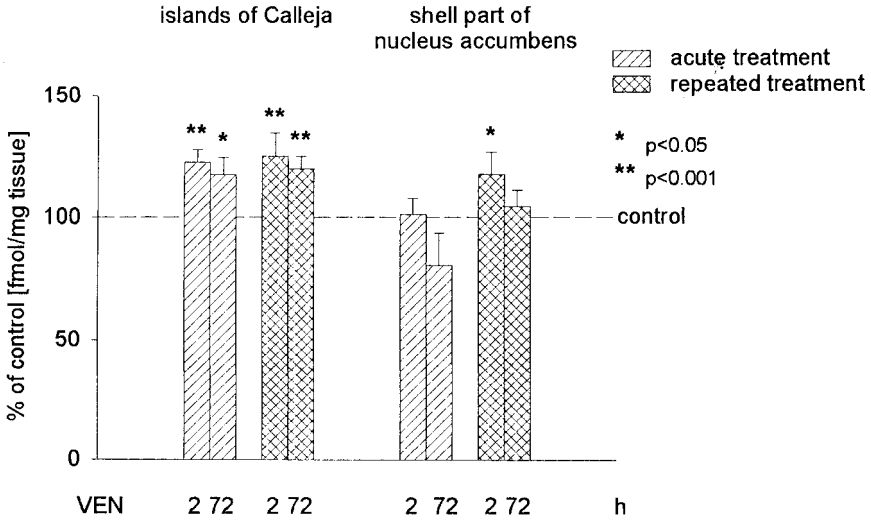

Figure 1. Effect of venlafaxine (10 mg/kg p.o.) administered acutely (single dose) or repeatedly (twice a day, for 14 days) on the binding of [^{3}H]-7-OH-DPAT in the islands of Calleja and shell part of nucleus accumbens of the rat. Rat brains were taken for autoradiographic analysis at 2 or 72 h after the last administration of VEN. Data represents means ± SEM, $n = 6$–8 animals per group. Results are expressed as percentage of the control value ($B_{max} = 4·21 \pm 0·3$ fmol/mg protein — islands of Calleja and $1·53 \pm 0·3$ fmol/mg protein — shell part of nucleus accumbens). Statistical analysis was performed on raw data, statistical significance (ANOVA followed by Fischer's test) is referred to control

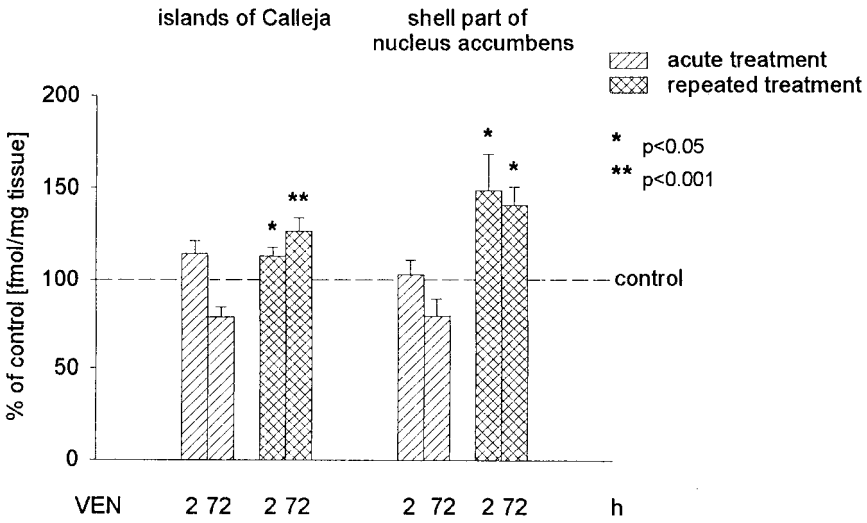

Figure 2. Effect of venlafaxine (10 mg/kg p.o.) administered acutely (single dose) or repeatedly (twice a day, for 14 days) on the binding of [^{3}H]-quinpirole in the islands of Calleja and shell part of nucleus accumbens of the rat. Rat brains were taken for autoradiographic analysis at 2 or 72 h after the last administration of VEN. Data represents means $\pm$ SEM, $n = 6$–8 animals per group. Results are expressed as percentage of the control value ($B_{max} = 1{\cdot}4 \pm 0{\cdot}09$ fmol/mg protein — islands of Calleja and $1{\cdot}01 \pm 0{\cdot}09$ fmol/mg protein — shell part of nucleus accumbens). Statistical analysis was performed on raw data, statistical significance (ANOVA followed by Fischer's test) is referred to control

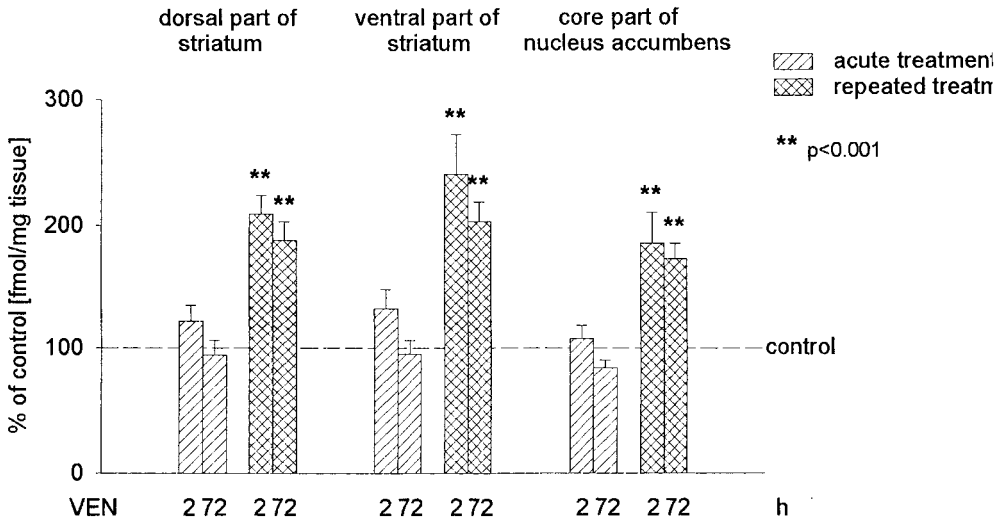

Figure 3. Effect of venlafaxine (10 mg/kg p.o.) administered acutely (single dose) or repeatedly (twice a day, for 14 days) on the binding of [^{3}H]-quinpirole in the striatum (dorsal and ventral part) and core part of nucleus accumbens of the rat. Rat brains were taken for autoradiographic analysis at 2 or 72 h after the last administration of VEN. Data represents means $\pm$ SEM, $n = 6$–8 animals per group. Results are expressed as percentage of the control value ($B_{max} = 0{\cdot}67 \pm 0{\cdot}06$ fmol/mg protein — dorsal part of striatum, $0{\cdot}64 \pm 0{\cdot}05$ fmol/mg protein — core part of nucleus accumbens). Statistical analysis was performed on raw data, statistical significance (ANOVA followed by Fischer's test) is referred to control

significant increase in the binding of [^{3}H]-7-OH-DPAT at 2 and 72 h after administration. In the shell part of the nucleus accumbens septi, only repeated but not acute administration of VEN induced a significant increase in the binding of [^{3}H]-7-OH-DPAT at 2 h (but not at 72 h) after administration.

Dopamine D_2 and D_3 receptor binding in the striatum, islands of Calleja and nucleus accumbens (core and shell)

The effects of VEN on the binding of [^{3}H]-quinpirole to D_2 and D_3 receptors in the striatum, nucleus accumbens and islands of Calleja are illustrated in Figures 2 and 3. VEN given repeatedly (but not acutely) increased the binding of [^{3}H]-quinpirole in the striatum (dorsal and ventral parts), the nucleus accumbens (core and shell) and in the islands of Calleja.

5-HT_{1A} and 5-HT_{2A} receptor binding in rat hippocampus and brain cortex

The effect of VEN administration on the binding to serotonergic 5-HT_{1A} and 5-HT_{2A} receptors was studied in rat hippocampus and brain cortex using [^{3}H]-8-OH-DPAT and [^{3}H]-ketanserin, as radioligands, respectively. The obtained results are presented in Table 5. A decrease in the density of binding sites of [^{3}H]-8-OH-DPAT in rat hippocampus was observed only at 2 h following repeated administration of VEN. No significant changes in the binding parameters of [^{3}H]-ketanserin were observed in rat brain cortex following single-dose or repeated administration of VEN.

DISCUSSION

The obtained results show that VEN administered in a single dose and repeatedly does not change the binding parameters (B_{max} and K_D) of [^{3}H]-prazosin to α_1-adrenergic receptors in rat cerebral cortex. Using the method of [^{3}H]-prazosin displacement by phenylephrine it was observed that repeated VEN (but not its single dose) diminished the K_i constant, i.e. increased the affinity of α_1-adrenoceptors to their agonist.

Our earlier investigations showed that imipramine and amitryptyline, given repeatedly, increased the binding (B_{max}) to α_1-adrenoceptors in the cerebral cortex and the spinal cord of the rat when [^{3}H]-prazosin was used as a ligand (Maj *et al*., 1985a). The increased binding to α_1-adrenergic receptors in the cortex and the other brain structures after repeated administration of antidepressants was confirmed by some authors (Campbell and McKernan, 1982; Vetulani *et al*., 1984). Nowak and Przegaliński (1988) reported an increased binding to α_1-adrenoceptors in the cerebral cortex, but not in the hippocampus or thalamus. The lack of an increase in the density of α_1-adrenergic receptors after repeated antidepressants was also demonstrated (Hyttel *et al*., 1984; Mogilnicka *et al*., 1987; Stockmeier *et al*., 1987). Using the method of [^{3}H]-prazosin displacement by phenylephrine, Menkes *et al*. (1983) observed that amitryptyline, desipramine and iprindole increased the affinity of α_1-adrenergic receptors to their agonists. A similar effect was described for imipramine, mianserin and citalopram (Klimek *et al*., 1991).

Thus the data concerning the effect of repeated antidepressants on the binding to α_1-adrenoceptors are not unequivocal. In the light of the present

Table 5. Effect of single dose and repeated administration of venlafaxine on the binding of [^{3}H]-8-OH-DPAT to 5-HT_{1A} receptors in the rat hippocampus and [^{3}H]ketanserin to 5-HT_{2A} in the rat brain cortex

Treatment	Hippocampus		Cortex	
	B_{max} (fmol/mg protein)	K_D (nM)	B_{max} (fmol/mg protein)	K_D (nM)
Vehicle	4·62 ± 0·39	1·60 ± 0·15	6·78 ± 1·00	0·65 ± 0·1
VEN, single, 2 h	4·23 ± 0·48	1·77 ± 0·57	6·57 ± 1·50	0·71 ± 0·1
VEN, single, 72 h	4·80 ± 1·10	1·80 ± 0·40	6·25 ± 0·24	0·70 ± 0·1
VEN, repeated, 2 h	3·12 ± 0·41*	1·08 ± 0·25	8·20 ± 0·80	0·86 ± 0·2
VEN, repeated, 72 h	4·57 ± 0·97	1·30 ± 0·20	7·83 ± 0·90	0·72 ± 0·1

VEN (10 mg/kg p.o.) was given in a single dose or repeatedly (twice daily, 14 days). The tissue for biochemical measurements was taken out at 2 or 72 h after the last dose of the drug. Data represents means ± SEM, $n = 8$. Statistical evaluation was carried out by the Duncan's test, $*p < 0.05$ vs vehicle-treated group.

results, the effect of VEN on these receptors seems to be weak. Our behavioural studies showed the increase responsiveness of the α_1-adrenergic system to its agonists after repeated VEN (aggressiveness induced by clonidine in mice, or methoxamine-induced hyperexploration in rats) (Maj and Rogóż, 1999). We also recently observed the enhancement of repeated VEN of the locomotor hyperactivity induced by (±)-threo-3-(3,4-dihydroxy-phenyl)serine, a noradrenaline precursor (unpublished data).

VEN administered acutely and repeatedly does not change either the density (B_{max}) of or the affinity (K_D) for dopamine D_1, receptors. It differs in this respect from other antidepressants which, when given repeatedly, decrease the density of D_1 receptors (B_{max}), not affecting their affinity (K_D) in the limbic forebrain and the striatum (Klimek and Nielsen, 1987).

VEN given acutely and repeatedly has no effect on the binding parameters of [^{3}H]-spiperone (B_{max} and K_D) to dopamine D_2 receptors in the limbic forebrain and the striatum. Various antidepressants, including amine uptake inhibitors, do not affect the binding parameters of [^{3}H]-spiperone (Klimek and Maj, 1989; Klimek and Nielsen, 1987; Martin-Iverson *et al*., 1983; Peroutka and Snyder, 1980). On the other hand, in the case of repeated imipramine and mianserin, we observed an enhanced affinity of D_2 receptors for their agonist (i.e. a decreased value of K_i), measured by a method of displacement of [^{3}H]-spiperone, by quinpirole, a dopamine D_2 receptor agonist (Klimek and Maj, 1989). Furthermore, we found recently that repeated antidepressants increase the affinity of dopamine D_2 receptors for their selective agonist [^{3}H]-N-0437, as well as the density of these receptors (Maj *et al*., 1996b). The increased binding of [^{3}H]-quinpirole in the nucleus accumbens and the striatum after repeated VEN, found in the present study by an autoradiographic method, is in accordance with these findings.

Hence it may be assumed that repeated VEN enhances the binding to dopamine D_2 receptors. The potentiating effect of VEN on the D-amphetamine-induced locomotor hyperactivity, described by us previously (Maj and Rogóż, 1999), may stem from the above enhancement. On the other hand, repeated VEN does not increase, but inhibits the quinpirole-induced hyperactivity (Maj and Rogóż, 1999). The reason for such discrepancy is unclear. It may be due to the fact that the serotonergic component (uptake inhibition) in the action of VEN is stronger than the noradrenergic component, the former having presumably an antagonistic effect on the locomotor activity increased by quinpirole via dopaminergic stimulation. This supposition is supported by our latest observation that repeated VEN potentiates the quinpirole-induced hyperactivity in rats pretreated with ritanserin (a selective antagonist of 5-HT_2 receptors). The quinpirole hyperactivity in rats not treated with VEN is not modified by ritanserin (unpublished data).

Quinpirole shows affinity for both dopamine D_2 and D_3 receptors (Sautel *et al*., 1995; Sokoloff *et al*., 1990); therefore it may be assumed that the increased binding of [^{3}H]-quinpirole after repeated VEN, observed in our experiment, concerns dopamine D_3 receptors. However, the fact that an increase in the [^{3}H]-quinpirole binding was also observed in the striatum, which is practically devoid of D_3 receptors (Fallon *et al*., 1983; Sokoloff *et al*., 1992; Wallace and Booze, 1995) speaks against such a supposition. Besides, the binding increase of [^{3}H]-quinpirole is distinctly bigger in the striatum than in the islands of Calleja which have no D_2 receptors (Hillefors-Berglund and Von Euler, 1994).

Using a method of receptor autoradiography, we also studied the effect of VEN on the binding of [^{3}H]-7-OH-DPAT, a selective agonist of D_3 receptors, in islands of Callejas and in the shell of the nucleus accumbens, where the highest density of these receptors is observed (Bouthenet *et al*., 1991; Camacho-Ochoa *et al*., 1995; Hillefors-Berglund and Von Euler, 1994; Lévesque *et al*., 1992). The obtained results indicate that repeated VEN enhances the binding of [^{3}H]-7-OH-DPAT in both these brain regions, which explains the previously described increase in the 7-OH-DPAT-induced locomotor hyperactivity, produced by repeated VEN (Maj and Rogóż, 1999).

In islands of Calleja, an increase in the [^{3}H]-7-OH-DPAT binding after a single dose of VEN has also been found. This effect may result from the influence of acute VEN on the fluidity of the neuronal membrane. It has already been shown that, in contrast to repeated administration, some antidepressants given to rats in a single dose increase the fluidity of cortical membranes, as a result of non-specific interaction of these drugs with biological membranes, which leads to augmentation of a phospholipid content (Nocoń and Melzacka, 1991; Wesemann *et al*., 1988). In turn, changes in the membrane fluidity influence the binding of radioligands to their receptors, as has

been reported for serotonergic receptors, which may lead to misinterpretation of the binding data (Heron *et al*., 1980). In our studies, no enhancement of the (±)7-OH-DPAT-induced locomotor hyperactivity was observed after acute administration of VEN (Maj and Rogóż, 1999). Therefore it is justifiable to conclude that the increase in the binding of [^{3}H]-7-OH-DPAT, observed shortly after acute administration of VEN, may stem from a physical process which unmasks receptors hidden in the cell membrane; however, these receptors are not fully mature, nor do they transmit properly the functional signals. Similar effects were reported for other antidepressants (Maj *et al*., 1998a).

VEN administered repeatedly, but not acutely, lowers the density of 5-HT_{1A} receptors in the hippocampus (only 2 h after the last dose), but has no effect on the binding to 5-HT_{2A} receptors in the cerebral cortex. Our previous behavioural experiments demonstrated the lack of effect of repeated VEN on the action of 8-OH-DPAT, a 5-HT_{1A} agonist, and a decreased activity of 5-HT_2 agonists (5-hydroxy-L-tryptophan, (±)-1-(2,5-dimethyoxy-4-iodo-phenyl)-2-aminopropane, trifluoromethylphenylpiperazine) (Maj and Rogóż, 1999). Earlier experiments showed that repeated administration of tricyclic antidepressants, as well as mianserin and citalopram, increased the density (B_{max}) of 5-HT_{1A} receptors in the hippocampus, whereas fluoxetine and paroxetine decreased it (Klimek *et al*., 1994; Maj *et al*., 1996a). After administration of tricyclic antidepressants, mianserin and paroxetine, the affinity for 5-HT_{1A} receptors was enhanced. In the cerebral cortex, the density (B_{max}) of 5-HT_{2A} receptors was decreased after imipramine, mianserin, citalopram and paroxetine, but increased after fluoxetine. None of the antidepressants affected the affinity for 5-HT_{2A} receptors in the cortex (Klimek *et al*., 1994; Maj *et al*., 1996a).

Summing up, like other 5-HT and NA reuptake inhibitors, VEN administered repeatedly evokes adaptive biochemical (binding) changes in the dopaminergic system (an increase in the binding to D_2 and D_3 receptors). Changes in α_1-adrenergic receptors (an increased affinity for agonists) and in 5-HT ones (a transient decrease in the density of 5-HT_{1A} receptors; the lack of changes in the density of and affinity for 5-HT_2 receptors) are less distinct. Therefore the lack of affinity for neurotransmitter receptors (a property which differentiates VEN from tricyclic inhibitors of 5-HT and NA uptake) seem to be of no significance for the development of adaptive changes observed after repeated treatment with antidepressants, at least in the dopaminergic system.

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