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Repeated imipramine and electroconvulsive shock increase α_{1A}-adrenoceptor mRNA level in rat prefrontal cortex

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Abstract

α_1-Adrenoceptors have been implicated in the mechanism of action of antidepressants, but their action on specific receptor subtypes was rarely reported. We compared now the action of two prototypic antidepressant treatments: repeated imipramine and electroconvulsive shock, on the expression of the α_{1A}- and α_{1B}-adrenoceptor mRNAs and on the receptor density in rats. The mRNA expression was assessed by Northern blot in the prefrontal cortex and the hippocampus, the receptor density was measured by [^{3}H]prazosin binding in the total cerebral cortex and hippocampus. In the cortex, both treatments elevated the α_{1A}-adrenoceptor mRNA and the expression of receptor protein. The expression of α_{1B}-adrenoceptor mRNA remained unaffected. In contrast, in the hippocampus, the antidepressant treatments augmented the density of α_{1A}-adrenoceptor protein without changing the level of its mRNA expression there. The results suggest that the α_{1A}-adrenoceptor subtype is specifically involved in the mechanism of action of classical antidepressant treatments.

Keywords: α_1-Adrenoceptor subtype; Brain, rat; [^{3}H]Prazosin binding; Imipramine; Electroconvulsive shock; α_1-Adrenoceptor mRNA

1. Introduction

Noradrenergic system in the brain is regarded as one of the central state systems (Shepherd, 1994) and its pathologies may be involved in several psychiatric conditions, including affective disorders. This possibility is implicated not only by the results of post mortem studies in the brain of depressed patients (Reiach et al., 1999; Dowlatshahi et al., 1999), but also by data coming from fibroblasts of patients with major depression (Shelton et al., 1999). While these results indicate the disturbances in the cyclic AMP signaling cascade in bipolar affective disorder, the reports on changes in the β-adrenoceptor density were controversial (e.g., Biegon and Israeli, 1988; Young et al., 1994). Apparent discrepancies were also reported concerning α_1-adrenoceptor system. While the density of α_1-adrenoceptor in the cortex of suicidal victims remained at control level (De Paermentier et al., 1997), the α_1-adrenoceptor-associated $G_{q/11}$ protein in bipolar disorder patients was elevated in some cortical regions (Mathews et al., 1997).

Chronic antidepressant treatments cause adaptive receptor changes in noradrenergic system (reviewed by Vetulani and Nalepa, 2000). While downregulation of β- and α_2-adrenoceptors was usually reported, at least for classical antidepressants and electroconvulsive shock, the results concerning α_1-adrenoceptors were less coherent and possibly dependent on the strain of rats (Vetulani et al., 1991).

Molecular pharmacology and receptor cloning studies have revealed that α_1-adrenoceptors, which are ubiquitous in both human and rat tissues, exist in at least three subtypes, namely α_{1A}, α_{1B} and α_{1D} that are encoded by separate genes (Michel et al., 1995), and have distinct pharmacological profiles (Morrow and Creese, 1986; Minneman et al., 1988; Schwinn and Lomasney, 1992; Faure et al., 1994; Yang et al., 1998). The α_1-adrenoceptor subtypes are differently distributed (Price et al., 1994a,b) and their mRNA expression is not always paralleled by protein expression (Yang et al., 1997). In some areas of rat's brain, mRNA for both α_{1A}- and α_{1B}-adrenoceptor have been found to be equally expressed, while in others, one subtype predominates (Pieribone et al., 1994; Domyancic and Morilak, 1997).

The functional differences between α_{1A}- and α_{1B}-adrenoceptor are not clear, but it had been postulated that both

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subtypes differ between themselves in their role in signal transduction (Minneman, 1988). Originally, Han et al. (1987), studying the receptors of smooth muscles, proposed that α_{1A} subtype is coupled selectively to dihydropiridine-sensitive voltage-gated calcium channel, while α_{1B} operates through phospholipase C system. In other peripheral tissues, the distinction between the action of those subtypes is less sharp (see Zhong and Minneman, 1999). The situation in the brain has not been resolved yet. As most tissues expressed a mixture of α_{1A}- and α_{1B}-adrenoceptors, the complex interaction between these receptor subtypes and their cooperative effects in signal transduction have been suggested (Wilson and Minneman, 1990; Piascik et al., 1991).

α_1-Adrenoceptors and their signaling system are important targets of many psychoactive substances including antidepressants (Nalepa, 1994; Nalepa and Vetulani, 1993, 1994). However, the early studies on the effect of antidepressant treatments on α_1-adrenoceptors could not take into account the current knowledge about the existence of their subtypes. Thus, repetitive treatment with antidepressant drug, imipramine, was shown to increase the density of the total α_1-adrenoceptor binding sites labeled by [^{3}H]prazosin in rat's cerebral cortex (Vetulani et al., 1984; Maj et al., 1985). Similar effect was induced by chronic administration of electroconvulsive shock that is known to possess antidepressant properties. That was reported in the cerebral cortex (Vetulani et al., 1983; Stockmeier et al., 1987; Nowak and Przegalinski, 1988) as well as in most cortical regions of rat's brain (Blendy et al., 1990, 1991). Later on, studies concerned with particular subtypes of α_1-adrenoceptor have also yielded discrepant results (see Discussion).

Relatively little attention has been paid to the molecular basis of changes induced by antidepressant treatments in α_1-adrenoceptors. Therefore, in this study, we compared the effects of repeated treatments with electroconvulsive shock and imipramine on the expression of mRNAs encoding for α_{1A}- and α_{1B}-adrenoceptors in rats' cerebral cortex and hippocampus. In addition, we investigated whether changes in α_1-adrenoceptors mRNA level were reflected by alterations in expression of receptor protein, as assessed by receptor binding studies.

2. Materials and methods

Procedures involving animals and their care were conducted in conformity with the institutional guidelines, in compliance with national and international laws and policies.

2.1. Chemicals

Imipramine hydrochloride was obtained from Polfa, Cracow, Poland. [^{3}H]Prazosin (S.A. 24 Ci/mmol) was purchased from Radioactive Center, Amersham and 2-([2,6-dimetoxyphenoxy-ethyl]aminomethyl)-1,4-benzodioxane (WB4101) from Sigma, Saint Louis, MO. Aquascynt was from BioCare, Poland, and DNA molecular weight marker ϕX174 DNA/*Bsu*RI (*Hae*III) from MBI Fermentas, Lithuania. Formamide, ethidium bromide, morpholinopropane sulfonic acid (MOPS), agarose HRB, agarose I, agarose SFR, and glycerol loading dye 5 × were purchased from Amresco, Solon, OH.

2.2. Animal treatment, tissue and RNA isolation

Male Wistar rats, weighing approximately 200–250 g, were injected with imipramine (10 mg/kg, twice daily, i.p.) or saline for 3 days (subchronically) and for 14 days (chronically). Other two groups of animals were treated with electroconvulsive shock (150 mA, 0.5 s, once daily) or sham for 3 and 14 days. After 24 h from the last treatment, animals were decapitated, their brains excised and brain structures dissected, along the natural borders, on an ice-cold porcelain plate. The tissues designated for RNA isolation were frozen in liquid nitrogen, while these for receptors binding, on dry ice. All of them were stored at − 70 °C until extraction of total RNA and membrane preparation.

Total cellular RNA was isolated and purified from the prefrontal cortex and hippocampus by the method of Chomczynski (1993) utilizing TRIzol reagent (Gibco-BRL). RNA quantity and purity were determined by spectrophotometry (Pharmacia Ultrospec 2000 UV/Vis) and RNA gel electrophoresis.

The membrane preparation (P_2 fraction) was prepared from the entire cerebral cortex and hippocampus as described previously (Nalepa and Vetulani, 1991). Briefly, the tissue was homogenized in 20 volumes of 50 mM Tris–HCl buffer, pH 7.5, at 0–4 °C using Polytron homogenizer. The supernatant after centrifugation of homogenate (1000 × *g*, 10 min) was centrifuged at 25,000 × *g* for 30 min. The resulting pellet was resuspended and recentrifuged under the same conditions, and the preparation was stored at − 20 °C until incubation. Immediately before the assay, the pellet was reconstituted in 50 mM Tris–HCl buffer, pH 7.6, to obtain the final concentration of protein of approximately 0.6 mg/ml. Protein was determined by the method of Lowry et al. (1951).

2.3. Selection of primers for polymerase chain reaction (PCR)

Plasmids containing cDNAs for α_{1A}-adrenoceptor (a 2520 bp rat α_{1a}-adrenoceptor receptor encoding cDNA in *Eco*RI/*Pst*I site of pCMV5) and α_{1B}-adrenoceptor (~ 2000 bp rat homolog of the hamster α_{1b}-adrenoceptor encoding cDNA in SP65 at *Eco*RI site) (Lomasney et al., 1991), generously provided by Dr. J.W. Lomasney, were amplified in PCR with following sense and antisense primers: 5′-TggCTgACCTgCTTTTgAgT-3′ and 5′-CTCCTTCCAAC-

CTA-3′, for α_{1A}-adrenoceptor; 5′-gggTCgCAAggggACAA-ggg-3′ and 5′-CACCgAACAgACACACgCAC-3′, for α_{1B}-adrenoceptor. Primer sequences were selected in sequences of plasmid inserts (OLIGO PrimerAnalysis Software ver. 5.0, NBI) to generate segments of the 299 and 233 bp that were used for α_{1A}- and for α_{1B}-adrenoceptor cDNA probes preparation, respectively. The amplified regions, selected on the basis of a determined optimum ratio for total G/C content (60–65%) and minimal homology (not greater than 80%) with GenBank-entered eukaryotic sequences (as of November 1996), were neither homologous for other published sequences nor between α_{1A}- and α_{1B}-adrenoceptor (not greater than 65% homology).

2.4. PCR and probe preparations

The 299 and 233 bp cDNAs for the α_{1A}- and α_{1B}-adrenoceptor, respectively, were amplified by PCR in a 25-μl reaction mixture containing 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 1% Triton X-100 (i.e., 1 × thermophilic buffer, Promega, Madison, WI), 1.5 mM $MgCl_2$, 200 μM dNTP, 0.4 μM sense and antisense primers, 0.1 ng (α_{1A}) or 38.5 ng (α_{1B}) of template and 0.625 units Taq DNA polymerase (Promega). For the amplification of some probes, REDTaq DNA polymerase and 1 × REDTaq PCR reaction buffer [50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.1 mM $MgCl_2$, 0.1% gelatin] (Sigma) were used. The PCR was performed with a Biometra Personal Thermocycler with the following cycle parameters: one denaturation cycle for 5 min at 95 °C; 30 cycles of 45 s at 94 °C (denaturation), 45 s annealing at 58 °C, 45 s extension at 72 °C (for α_{1A}) or 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 61 °C, 1 min extension at 72 °C (for α_{1B}). The final extension cycle was run for each reaction for 7 min at 72 °C. The PCR products were separated by horizontal gel electrophoresis in a 2% agarose (Agarose I, Amresco) gel against a DNA molecular ladder, appropriate cDNAs were extracted and purified using QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA). Then, 10 μl of the cDNA probes were electrophoresed in a 2% of agarose gel (Agarose SFR, Amresco) and cDNA concentration was assessed by Kodak Electrophoresis Documentation and Analysis System (EDAS) with 1D Image Analysis Software.

Probes were radiolabelled with [α^{32}P]-dCTP by a random primer method (Rediprime DNA Labelling System, Amersham) to a specific activity of approximately 2.4×10^9 dpm/μg. The separation of unincorporated nucleotides from radiolabeled DNA probes were done on NucTrap Probe Purification Columns (Stratagene, La Jolla, CA).

2.5. Analysis of mRNA levels

mRNA was analyzed by Northern blot hybridization procedure. Samples of total RNAs were denatured at 55 °C for 15 min, in an RNA sample buffer containing: 50% formamide, 1 × Northern gel buffer (20 mM MOPS, 3 mM sodium acetate, 1 mM NaEDTA), 0.5 × glycerol loading dye (Amresco, Solon, OH) and 20 mM formaldehyde. Each sample of total RNA was loaded in duplicate (20 μg/slot) and electrophoresed in 1% agarose/2.2 M formaldehyde denaturing gel and 1 × Northern running buffer at 90 V for 3.5–4.5 h. Then, RNA was transferred to the Hybond N^+ membrane (Amersham) by capillary action for 18–24 h in 10 × saline–sodium citrate buffer (SSC), the blots were cross-linked on UV. Filters were prehybridized for 20 min at 65 °C in Rapid-hyb buffer (Amersham) and hybridized with the α_{1A}- or α_{1B}-adrenoceptor cDNA probe (6×10^6 cpm/ml of buffer) at 65 °C for 2.5 h. The blots that have been hybridized with α_{1A} cDNA probe were washed as follows: once for 20 min in 2 × SSC/0.1% sodium dodecyl sulfate (SDS) at room temperature and twice for 15 min in 0.1 × SSC/0.1%SDS at 65 °C and these with α_{1B}-probe once for 20 min in 2 × SSC/0.1% SDS at room temperature, once for 15 min in 0.5 × SSC/0.1% SDS at 65 °C and once for 15 min in 0.2 × SSC/0.1% SDS at 65 °C. Blots were exposed to autoradiographic film (BIOMAX MS, Kodak) at −70 °C for 36–48 h. The quantitative analysis of hybridization products was done using KODAK EDAS and 1D Image Analysis Software. Ethidium bromide fluorescence of 28S ribosomal RNA was used as an internal standard to control for loading errors as described by Duhl et al. (1992).

The signal density of each experimental sample was expressed as a percent of the mean signal density for all controls and significance was determined by Student's *t*-test.

2.6. Receptor binding study

To estimate α_{1A}- and α_{1B}-adrenoceptor densities, the procedure described by Hayakawa et al. (1992) was used. In this procedure, α_{1A}-adrenoceptor may be masked by low concentration of WB4101, and the relative densities of the subtypes assessed from the difference between [^{3}H]prazosin binding in the presence of low and high concentrations of WB4101.

Six concentrations of [^{3}H]prazosin, ranging from 0.09 to 3.63 nM, were used to characterize α_1-adrenoceptor binding sites. Total α_1 binding (including both α_{1A} and α_{1B} subtypes) was assessed by subtracting baseline binding, in the presence of 10 μM WB4101 as a displacer, from total binding in the absence of WB4101. The α_{1A} sites were determined by [^{3}H]prazosin binding in the presence of 2 nM WB4101 and the density of α_{1B}-adrenoceptor subtype was assessed by subtraction the α_{1A} binding from the total binding. The final incubation mixture in all cases contained 450 μl of membrane suspension, 50 μl of the radioligand solution, and 50 μl of the Tris–HCl buffer or of solution of displacers. The incubations were carried out at 25 °C for 30 min in a water bath shaker, and were terminated by vacuum-assisted filtration (Brandel Harvester, Labortechnik, Germany) through Whatman GF/C filters. The filters were then

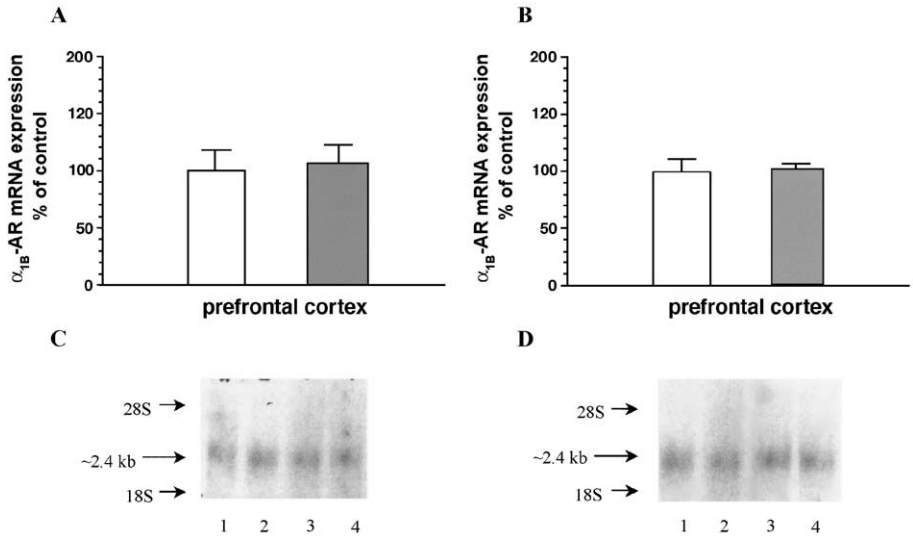

Fig. 1. Lack of effect of repeated treatment with imipramine and electroconvulsive shock on steady-state levels of α_{1B}-adrenoceptor (α_{1B}-AR) mRNA in the rat prefrontal cortex determined by Northern blot analysis. Animals were sacrificed after 24 h from the last treatment. Samples of 20 μg of total RNA were loaded in duplicates and mRNA levels were analyzed as described under Materials and methods. (A,C) Imipramine, 10 mg/kg, i.p., twice daily for 14 days. (B,D) Electroconvulsive shock, 150 mA, 0.5 s, once daily for 14 days. Results are expressed as a percent of control treated with saline or sham and are the means ± S.E.M. of values from five to seven individuals. White bars — control; shaded bars — treatment; (C,D) Representative Northern blot analyses (one of four) of α_{1B}-adrenoceptor mRNA expression in rats treated with imipramine and electroconvulsive shock, respectively. (C) Lanes 1 and 2 — saline; 3 and 4 — imipramine; (D) Lanes 1 and 2 — sham; 3 and 4 — electroconvulsive shock. Arrows show location of α_{1B}-adrenoceptor (~ 2.4 kb) and of residual 28S and 18S ribosomal RNA, visualized by ethidium bromide staining.

rinsed twice with 5-ml portions of ice-cold Tris–HCl buffer, placed in polyethylene minivials in 3 ml of Aquascynt scintillation liquid, and counted for radioactivity in a Beckman liquid scintillation counter.

The B_{max} and K_d values were calculated from binding isotherm using GraphPad Prism 2.01 program. Statistical analysis of the results were preformed with STATISTICA 4.0 software using one way analysis of variance followed by

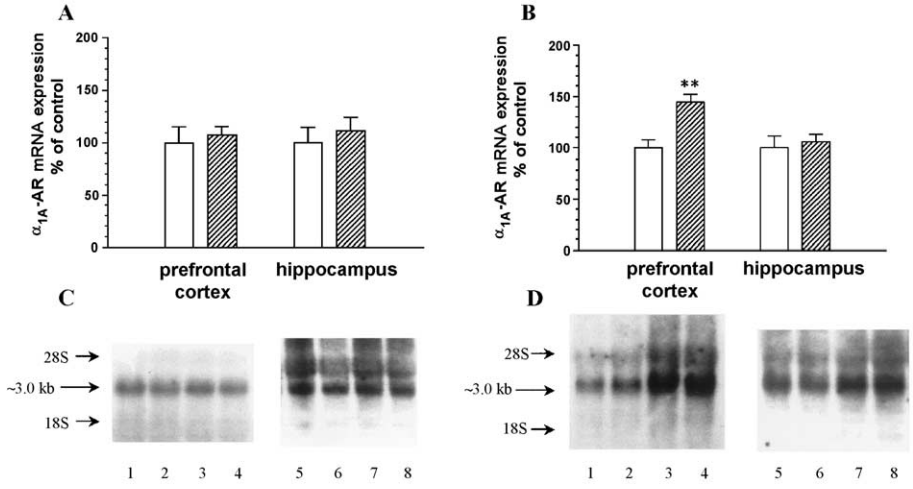

Fig. 2. Effect of imipramine administration on steady-state level of α_{1A}-adrenoceptor (α_{1A}-AR) mRNA in rat prefrontal cortex and hippocampus determined by Northern blot analysis. Imipramine was given twice daily (i.p., 10 mg/kg), for 3 days (A,C) and for 14 days (B,D). Animals were sacrificed after 24 h from the last injection. Samples of 20 μg of total cellular RNA were loaded in duplicates and mRNA levels were analyzed as described under Materials and methods. (A,B) Results are expressed as a percent of controls treated with saline and are the means ± S.E.M. of values from 6 to 10 individuals. Open bars — saline; crossed bars—imipramine. ** Significantly different ($P < 0.01$) from the group treated with saline. (C,D) Representative Northern blot analyses (one of five, for each schedule) of α_{1A}-adrenoceptor mRNA after 3 and 14 days treatment, respectively. Lanes: 1, 2 (prefrontal cortex) and 5, 6 (hippocampus) — saline; 3, 4 (prefrontal cortex) and 7, 8 (hippocampus) — imipramine. Arrows show location of α_{1A}-adrenoceptor (~ 3.0 kb) and of residual 28S and 18S ribosomal RNA, visualized by ethidium bromide staining.

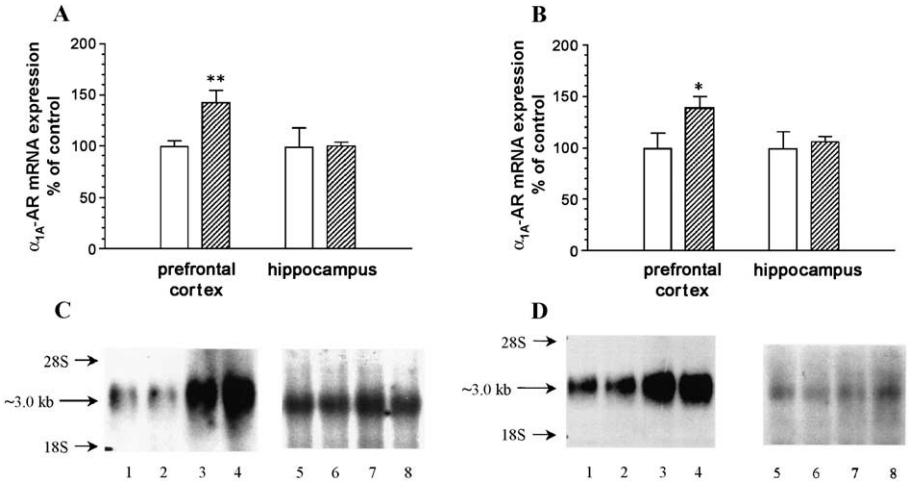

Fig. 3. Effects of treatment with electroconvulsive shock on steady-state levels of α_{1A}-adrenoceptor (α_{1A}-AR) mRNA in rat prefrontal cortex and hippocampus determined by Northern blot analysis. Electroconvulsive shock (150 mA, 0.5 s, once daily) or sham were delivered through ears' clips, for 3 days (A,C) and 14 days (B,D). Animals were sacrificed after 24 h from the last treatment. Samples of 20 μg of total cellular RNA were loaded in duplicates and mRNA levels were analyzed as described under Materials and methods. (A,B) Results are expressed as a percent of controls treated with sham and are the means ± S.E.M. of values from five to nine individuals. Open bars — sham; crossed bars — electroconvulsive shock. * $P<0.05$, ** $P<0.01$, compared with the sham-treated group. (C,D) Representative Northern blot analyses (one of four, for each schedule) of α_{1A}-adrenoceptor mRNA after treatment with electroconvulsive shock for 3 and 14 days, respectively. Lanes: 1, 2 (prefrontal cortex) and 5, 6 (hippocampus) — sham; 3, 4 (prefrontal cortex) and 7, 8 (hippocampus) — electroconvulsive shock. Arrows show location of α_{1A}-adrenoceptor (~ 3.0 kb) and of residual 28S and 18S ribosomal RNA, visualized by ethidium bromide staining.

the Fisher's LSD (least significant difference) test; $P<0.05$ was considered significant.

3. Results

3.1. Effects of imipramine and electroconvulsive shock on the expression of mRNAs encoding for α_{1A}- and α_{1B}-adrenoceptors

Northern blot hybridization revealed that the cDNA probes specific for α_{1A}- and α_{1B}-adrenoceptors detected a single 3.0-kb mRNA and 2.4-kb mRNA species, respectively (Figs. 1–3). Both α_{1A} and α_{1B} subtypes were represented in the prefrontal cortex. On the contrary, in the hippocampus, only the mRNA encoding for α_{1A}-adrenoceptor was strongly expressed and the expression of the α_{1B} mRNA was barely detectable. The latter was not changed by any treatments.

In the prefrontal cortex, both imipramine and electroconvulsive shock induced changes in the expression of mRNA encoding for α_{1A}, but they did not affect the level of expression of mRNA encoding for α_{1B}-adrenoceptor (Fig. 1A,B). The maximum effects of both treatments were

Table 1
Lack of effect of a 3-day treatment with imipramine (IMI) and electroconvulsive shock (ECS) on binding sites for [^{3}H]prazosin in the cerebral cortex (A) and hippocampus (B) of the rat

Treatment	Total α_1		α_{1A}		α_{1B}	
	B_{max} (fmol/mg protein)	K_d (nM)	B_{maxH}	K_{dH}	B_{maxL}	K_{dL}
(A)						
SAL (6)	137.3 ± 8.1	0.23 ± 0.05	113.6 ± 7.0	0.11 ± 0.02	64.1 ± 5.6	1.42 ± 0.11
IMI (6)	118.8 ± 7.9	0.18 ± 0.03	111.4 ± 12.7	0.12 ± 0.01	78.3 ± 5.0	1.09 ± 0.27
Sham (6)	133.6 ± 6.8	0.23 ± 0.04	107.1 ± 4.4	0.20 ± 0.03	75.1 ± 5.3	0.97 ± 0.23
ECS (6)	128.5 ± 3.1	0.20 ± 0.09	104.0 ± 2.4	0.16 ± 0.05	89.5 ± 7.3	0.99 ± 0.21
(B)						
SAL (4)	101.1 ± 5.0	0.24 ± 0.02	83.2 ± 5.9	0.16 ± 0.02	53.9 ± 4.4	0.80 ± 0.10
IMI (4)	95.6 ± 7.8	0.20 ± 0.02	82.8 ± 9.1	0.17 ± 0.01	34.6 ± 10.7	0.57 ± 0.07
Sham (4)	98.6 ± 10.2	0.24 ± 0.03	75.8 ± 5.2	0.16 ± 0.02	27.2 ± 6.4	0.46 ± 0.05
ECS (4)	104.4 ± 5.2	0.27 ± 0.03	77.6 ± 5.7	0.15 ± 0.03	30.6 ± 5.2	0.68 ± 0.15

The concentration of [^{3}H]prazosin ranged from 0.09 to 3.61 nM. Total binding (including both α_{1A} and α_{1B} subtypes) was assessed by the difference in binding in the absence and presence of 10 μM WB4101. The α_{1A} sites were assessed from the difference in [^{3}H]prazosin binding in the absence and presence of 2 nM WB4101. The difference between total and α_{1A}-adrenoceptor binding was assumed to be α_{1B}-adrenoceptor binding. The data are expressed as the mean ± S.E.M. from four (hippocampus) or six (cerebral cortex) independent experiments. SAL—saline.

similar. After 14 days of administration, the increase in the level of mRNA encoding for α_{1A}-adrenoceptor reached 39–45% over the control level ($P<0.01$; Figs. 2B,D and 3B,D). Electroconvulsive shock acted more rapidly, as the full effect of this treatment was observed as early as after 3 days, which was too short a time to produce any change by imipramine (Figs. 2A,C and 3A,C).

The action of both antidepressant treatments on mRNA encoding for α_{1A}-adrenoceptor was structure dependent, as they did not affect it in the hippocampus (Figs. 2A–D and 3A–D).

3.2. Effect of imipramine and electroconvulsive shock on α_1-adrenoceptor density

Short-term treatment (3 days) with imipramine or electroconvulsive shock did not affect the binding parameters of α_1-adrenoceptor subtypes (Table 1). However, a more protracted treatment (for 14 days) resulted in a significant increase in the density (B_{max}) of α_1-adrenoceptors (Table 2). In the cerebral cortex, the total binding sites for [^{3}H]prazosin were increased by 14% ($P<0.05$) in animals treated with electroconvulsive shock and a similar effect was induced by imipramine (by 16%, $P<0.05$). In both cases, the analysis of subtypes indicated that the increase was due to the augmentation of the high-affinity binding site, supposedly the α_{1A}-adrenoceptor subtype (by 25%) (Table 2A). The same direction of changes was observed in the rat hippocampus (Table 2B), in which the effects induced by imipramine were more pronounced than those of electroconvulsive shock. Imipramine increased the total α_1-adrenoceptors and the α_{1A}-adrenoceptor densities by 27% ($P<0.01$) and by 35% ($P<0.01$), while the treatment with electroconvulsive shock enhanced only the α_{1A}-subtype density by 19% ($P<0.05$), having insignificant effect on the total binding of α_1-adrenoceptors (an increase by 10%). The K_d values for either total α_1-adrenoceptor or each subtype was not changed after these treatments.

4. Discussion

The adaptive changes in noradrenergic system were considered as an important part of action of antidepressant treatment (see, for review, Vetulani and Nalepa, 2000), but the consistent results were obtained mainly with β-adrenoceptor, while the results concerning changes in α_1-adrenoceptors were often contradictory. The analysis of the literature results and our own experiments on radioligand binding suggested that antidepressant treatments produce upregulation of total population of α_1-adrenoceptor in the rats of Wistar strain (Nowak and Przegalinski, 1988; Vetulani et al., 1983, 1984), while significant changes were usually not observed in Sprague–Dawley rats (Stockmeier et al., 1987; Vetulani et al., 1991). The binding results in Wistar rats were consistent with behavioral effects of antidepressant treatments (Maj et al., 1983). The discovery of at least three subtypes of α_1-adrenoceptor: α_{1A}-, α_{1B}-, α_{1D}-adrenoceptor (see Zhong and Minneman, 1999; Michel et al., 1995), playing probably different roles in signal transduction in some tissues, prompted studies on the antidepressant-induced changes in separate subtypes.

In this study, we investigated whether the changes in α_1-adrenoceptor subtypes induced by repeated antidepressant treatments: electroconvulsive shock and imipramine, do appear at the level of gene expression. We compared the expression of α_{1A}- and α_{1B}-adrenoceptor mRNAs in the hippocampus and prefrontal cortex of the rat and investigated whether antidepressant treatments affected both α_1-adrenoceptor subtypes.

In both structures, the α_{1A}-adrenoceptor cDNA probe detected a single 3.0-kb mRNA while the α_{1B} was repre-

Table 2
The effect of a 14-day treatment with imipramine (IMI) and electroconvulsive shock (ECS) on binding sites for [^{3}H]prazosin in the cerebral cortex (A) and hippocampus (B) of the rat

Treatment	Total α_1		α_{1A}		α_{1B}	
	B_{max} (fmol/mg protein)	K_d (nM)	B_{maxH}	K_{dH}	B_{maxL}	K_{dL}
(A)						
SAL (11)	108.0 ± 4.7	0.18 ± 0.02	75.4 ± 3.7	0.07 ± 0.01	76.6 ± 4.1	1.58 ± 0.24
IMI (11)	125.3 ± 4.7[a]	0.19 ± 0.02	93.1 ± 5.9[a]	0.09 ± 0.01	71.1 ± 5.5	1.59 ± 0.16
Sham (11)	116.1 ± 4.5	0.21 ± 0.02	88.2 ± 5.4	0.13 ± 0.02	61.2 ± 3.2	0.92 ± 0.20
ECS (9)	132.8 ± 3.3[a]	0.19 ± 0.02	110.6 ± 6.4[a]	0.18 ± 0.03	66.5 ± 4.9	1.05 ± 0.20
(B)						
SAL (4)	106.6 ± 2.1	0.13 ± 0.02	75.3 ± 3.5	0.10 ± 0.02	49.3 ± 6.1	1.27 ± 0.20
IMI (4)	135.4 ± 4.1[b]	0.17 ± 0.02	102.0 ± 3.5[b]	0.08 ± 0.02	51.9 ± 4.2	1.03 ± 0.08
Sham (4)	93.9 ± 2.9	0.20 ± 0.02	73.3 ± 2.6	0.11 ± 0.02	46.8 ± 2.9	0.91 ± 0.05
ECS (4)	103.3 ± 2.8[c]	0.21 ± 0.05	86.9 ± 4.0[a]	0.10 ± 0.01	52.7 ± 3.6	0.89 ± 0.05

The concentration of [^{3}H]prazosin used ranged from 0.085 to 3.55 nM. The parameters of binding to α_1-adrenoceptor subtypes were calculated as described in Table 1. The data are expressed as the mean ± S.E.M. from 4 (hippocampus) or 9–11 (cerebral cortex) independent experiments.

[a] $P<0.05$ versus saline (SAL) control.

[b] $P<0.01$ versus SAL control.

[c] $P<0.1$ versus SAL control.

sented by a 2.4-kb mRNA species, what was in agreement with the data reported by Lomasney et al. (1991). Both subtypes were similarly represented in the prefrontal cortex, but in the hippocampus, only the α_{1A}-adrenoceptor was well expressed while the α_{1B}-subtype was on the border of detection limit. This agrees with the in situ hybridization data (Pieribone et al., 1994; Domyancic and Morilak, 1997).

The present results suggest that at least one subtype of α_1-adrenoceptors, namely α_{1A}-adrenoceptor, undergoes upregulation upon chronic antidepressant treatment. However, when the expression of mRNA encoding for α_1-adrenoceptor subtypes is concerned, the changes were present only in the frontal cortex, but not in the hippocampus. To ascertain that the negative result was not due to the limitations of Northern blot methodology, we carried out an additional experiment, employing the competitive PCR (cPCR) analysis of α_1-adrenoceptor subtypes mRNA, which also did not detect changes in the hippocampus. The time course of the action of electroconvulsive shock and imipramine on α_{1A}-adrenoceptor mRNA expression paralleled the time course of their clinical effects.

The correlation between the expression of mRNA coding for various receptor subtypes and the expression of receptor protein in various structures is loose. This is due to the fact that the receptor protein is synthesized in the nuclei, but the protein finally is imbedded in the membrane of often-distant nerve terminals. This may explain the presence of α_{1B}-adrenoceptor protein in the hippocampus in the virtual absence of appropriate mRNA, similarly as described in other brain structures (Nicholas et al., 1996).

The fact that only α_{1A}-adrenoceptor is affected by antidepressant treatment, while α_{1B}-adrenoceptor is not, may be explained by the fact that the subtypes of α_1-adrenoceptors may differ in their pattern of regulation by noradrenaline. Thus, α_{1A}-adrenoceptor is positively regulated by adrenergic agonists that regulate negatively α_{1B}-adrenoceptor (Rokosh et al., 1996; Yang et al., 1999).

The reason why mRNA encoding α_{1A}-adrenoceptor is not increasing in the hippocampus while it is increasing in the frontal cortex after chronic treatment both with imipramine and electroconvulsive shock is not clear. One explanation may be that in the hippocampus the α_{2C}-adrenoceptors are much more abundant than other subtypes (Nicholas et al., 1996). These receptors, in contrast to other α_2-adrenoceptor subtypes, do not undergo desensitization (Eason and Liggett, 1992), and thus may exert their full inhibitory activity in spite of prolonged exposure to increased concentrations of noradrenaline. Thus, during an antidepressant treatment, when the noradrenaline availability, important for upregulation of α_{1A}-adrenoceptor mRNA, is increased, it is increased more in the cortex than in the hippocampus. Therefore, in the latter structure, noradrenaline is less capable of upregulation of α_{1A}-adrenoceptor.

While the results concerning changes in mRNAs encoding for α_{1A}-adrenoceptor and α_{1B}-adrenoceptor seem to provide a firm data, due to the specificity of the probes, it should be noted that they do not take into account the possible changes in the third α_1-adrenoceptor subtype, the α_{1D}-adrenoceptor. Along with other subtypes, mRNA encoding for α_{1D}-adrenoceptor has been found to be expressed in the brain and peripheral tissues of humans and rats (see Zhong and Minneman, 1999). However, using receptor-binding techniques, Yang et al. (1997, 1998) could not detect α_{1D}-adrenoceptor protein in a variety of tissues of mice and Wistar rats in which the expression of its mRNA has been described.

In addition to investigation on mRNA expression, the binding studies were carried out. While owing to the specificity of probes, the discrimination between mRNAs encoding for α_1-adrenoceptor subtypes poses no difficulties, the discrimination between receptor subtypes is difficult because of the lack of sufficiently specific ligands. The mostly used of available methods was that of Hayakawa et al. (1992), who employed the difference in affinity of WB4101 to receptor subtypes. Using a low concentration of the antagonists (2 nM), one can semiquantitatively block the population of α_{1A}-adrenoceptor subtype, and may use the data from binding in the presence of low and high WB4101 concentrations to calculate the relative proportion of the α_1-adrenoceptor subtypes, of different affinity to [^{3}H]prazosin. As the blockade of α_{1A}-adrenoceptor subtypes was probably not complete, the calculated sums of the densities of separate subtypes is always higher than the total value. Our results, obtained with this method, demonstrated an increase in the specific binding to α_{1A}-adrenoceptor in the cerebral cortex and the hippocampus after prolonged repeated treatment with imipramine and electroconvulsive shock.

The possibility of using a higher WB4101 concentration to mask specifically α_{1A}-adrenoceptors may pose a similar problems, though in opposite direction. Such concentrations were used for autoradiographic studies by Blendy et al. (1990), but although this methodology yields very important information concerning localization of receptors, it must be regarded as semiquantitative (Nicholas et al., 1996).

The results of Hayakawa et al. (1992) differ from those presented here, as they did not observe any changes induced by electroconvulsive shock or imipramine in α_{1A}-adrenoceptors, while reported that α_{1B} adrenoceptor was regulated by electroconvulsive shock and imipramine in opposite manner. The exact reasons for the discrepancies between their results and ours are not clear, but there are some differences that might be responsible for that. While Hayakawa et al. (1992) were using the frontal cortex for receptor binding studies, we tested the effects of imipramine and electroconvulsive shock in the total cortex. As shown by Blendy et al. (1990), there are important differences in the regulation of α_{1A}-adrenoceptors in various areas of the cerebral cortex, at least after electroconvulsive shock. Thus, the antidepressant treatment did not produce any change in the frontal cortex of the rat, while it produced an important increase in the receptor density in the entorhinal, pyriform

and insular cortex, as well as in the amygdala. The reasons for differences in the regulation of α_{1B}-adrenoceptor in the experiments of Hayakawa et al. (1992) and us are not clear. It might only be noticed that while in our study, we observed no effect, in the study of Hayakawa, imipramine and electroconvulsive shock acted inconsistently—imipramine decreasing, while electroconvulsive shock increasing the α_{1B}-adrenoceptor density. Moreover, Hayakawa et al. (1992) used different parameters of electroconvulsive shock and had not specified the intensity of the current.

A similar pattern of changes to that reported by us was found by Hanft and Gross (1990). In their study, chronic desipramine treatment caused an increase in the ratio of α_{1A}-adrenoceptor/α_{1B}-adrenoceptor density without change in the total binding. Thus, the discrepancies described above can be due to the different strains of animals, to the different and structure-dependent expression of α_{1A}- and α_{1B}-adrenoceptor as well as to the method used for the receptor subtypes discrimination. While the reasons for differences in results of binding studies reported by several groups are difficult to resolve, the present study yields a consistent picture of changes obtained under our experimental conditions.

In summary, we have found that chronic administration of electroconvulsive shock and imipramine, typical representatives of antidepressant treatment, increases specifically the expression of mRNA encoding for cortical α_{1A}-adrenoceptor, without affecting α_{1B}-adrenoceptor and hippocampal α_{1A}-adrenoceptor expression. Although the correlation of the functional role with the anatomical distribution of a particular receptor is still unknown, it is tempting to speculate that the α_{1A}-adrenoceptor subtype in the prefrontal cortex is the receptor species specifically involved in the action of antidepressant treatments.

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