Pharmacological Reports
2011, 63, 1349–1358
ISSN 1734-1140

Effects of the noradrenergic neurotoxin DSP-4 on the expression of α_1-adrenoceptor subtypes after antidepressant treatment

Grzegorz Kreiner[1], Agnieszka Zelek-Molik[1], Marta Kowalska[1], Adam Bielawski[1], Lucyna Antkiewicz-Michaluk[2], Irena Nalepa[1]

[1]Department of Brain Biochemistry, [2]Department of Neurochemistry, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Irena Nalepa, e-mail: nfnalepa@cyf-kr.edu.pl

Abstract:
We have previously reported that chronic imipramine and electroconvulsive treatments increase the α_{1A}-adrenoceptor (but not the α_{1B} subtype) mRNA level and the receptor density in the rat cerebral cortex. Furthermore, we have also shown that chronic treatment with citalopram does not affect the expression of either the α_{1A}- or the α_{1B}-adrenoceptor, indicating that the previously observed up-regulation of α_{1A}-adrenoceptor may depend on the noradrenergic component of the pharmacological mechanism of action of these antidepressants. Here, we report that previous noradrenergic depletion with DSP-4 (50 mg/kg) (a neurotoxin selective for the noradrenergic nerve terminals) significantly attenuated the increase of α_{1A}-adrenoceptor mRNA induced by a 14-day treatment with imipramine (IMI, 20 mg/kg, *ip*) and abolished the effect of electroconvulsive shock (ECS, 150 mA, 0.5 s) in the prefrontal cortex of the rat brain. The changes in the receptor protein expression (as reflected by its density) that were induced by IMI and ECS treatments were differently modulated by DSP-4 lesioning, and only the ECS-induced increase in α_{1A}-adrenoceptor level was abolished. This study provides further evidence corroborating our initial hypothesis that the noradrenergic component of the action of antidepressant agents plays an essential role in the modulation of α_{1A}-adrenoceptor in the rat cerebral cortex.

Key words:
α_{1A}-adrenoceptor, DSP-4, electroconvulsive shock, HPLC, imipramine, mRNA, northern blot, [^{3}H]prazosin binding

Abbreviations: 5-HT – serotonin, α_1-AR – α_1-adrenoceptors, α_2-AR – α_2-adrenoceptors, DBH – dopamine β-hydroxylase, DSP-4 – *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine, ECS – electroconvulsive shock, EtBr – ethidium bromide, HPLC – high-performance liquid chromatography, IMI – imipramine, NA – noradrenergic/noradrenaline, SAL – saline, WB4101 – 2-([2,6-dimethoxyphenoxy-ethyl]aminomethyl)-1,4-benzodioxane

Introduction

Chronic antidepressant treatments cause adaptive receptor changes in the noradrenergic (NA) system. The NA system is regarded as one of the central systems in which pathologies may be involved in number of psychiatric disorders, including depression (reviewed by [35]). There is a considerable amount of clinical evidence indicating a close relationship between the NA system and depression [16]. Noradrenaline also plays an important role in mediating acute behavioral and neurochemical actions of many antidepressants in animal models. For example, Cryan et al. reported that genetically modified and noradrenaline-deficient mice, DBH(–/–), did not respond to various antidepressant drugs (including the NE reuptake inhibitors) in forced swimming and tail suspension tests [5].

The α_1-adrenergic receptors (α_1-adrenoceptors, α_1-AR) and their signaling systems are important tar-

gets of antidepressant drugs [24–26]. The α_1-ARs, a class of G protein-coupled receptors that act through the Gαq/11 signaling pathway, are key modulators of NA transmission. There are three cloned subtypes of α_1-AR: 1A (α_{1A}-AR), 1B (α_{1B}-AR), and 1D (α_{1D}-AR). Each subtype displays distinct pharmacological properties and tissue distribution [39]. The putative fourth subtype, α_{1L}-AR, which has been identified in vascular tissues, most likely represents a functional phenotype of the α_{1A}-AR in an alternative conformational state of this subtype [8]. The α_1-ARs are involved in physiological processes mediated by noradrenaline and adrenaline, and their regulatory role has been well studied in the cardiovascular system. However, despite more than a decade of intensive study, there is relatively little information about the distinctive physiological function of the α_1-AR subtypes in the central nervous system due to the lack of highly subtype-selective drugs (reviewed by [28]). Although initially, it was thought that calcium influx response was dependant mainly on α_{1A}-AR, recent data have shown that all three subtypes can activate calcium signaling and phosphoinositol turnover [28]. Heterodimerization of α_1-AR subtypes, which has been shown to be important for their cell surface expression, signaling and internalization, may help to elucidate the functional role of a particular subtype [3].

We have previously reported that chronic imipramine (IMI) and electroconvulsive (ECS) treatments increase α_{1A}-AR but not α_{1B}-AR mRNA levels in the rat cortex [22]. Furthermore, we have also shown that chronic treatment with citalopram, a selective serotonin reuptake inhibitor, does not affect the expression of either α_{1A}-AR or α_{1B}-AR [20]. These results suggest that the observed up-regulation of α_{1A}-AR after IMI and ECS might depend mainly on the NA component of pharmacological action of these antidepressants. Here, we report that NA depletion with neurotoxin DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine) abolished the ECS effect and diminished the IMI-induced increase in the α_{1A}-AR mRNA level in the rat prefrontal cortex. The effect of DSP-4 was more disputable in the case of the receptor protein.

Materials and Methods

Animal treatment

The experiments were performed on male Wistar rats (200–250 g). The experimental procedure consisted of two parts, and the analyses concerning IMI and ECS were carried out independently. In the first part of experiment, the animals were divided into four groups. The rats belonging to the SAL/SAL and SAL/IMI groups were injected with saline, while the two other groups, DSP-4/SAL and DSP-4/IMI, received the noradrenergic neurotoxin, DSP-4, (50 mg/kg, *ip*, one dose). To protect 5-HT neurons, rats receiving DSP-4 were additionally pretreated with citalopram (5 mg/kg, 60 min before DSP-4 injection) as previously described [32]. Four days after DSP-4 injection, the animals received either imipramine (10 mg/kg, twice daily, *ip*; groups SAL/IMI, DSP-4/IMI) or saline (groups SAL/SAL, DSP-4/SAL) for 14 days.

The second series of experiments was performed analogously, but IMI was substituted with ECS. ECS (150 mA, 0.5 s, once daily, 14 days) was generated with the GE-01 apparatus (COTM, Białystok, Poland) and was administered through ear clips. Control animals received a sham treatment (clips were placed on the ears without applying an electric current).

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval of the Bioethics Commission according to Polish law regulations.

Tissues

Animals were decapitated 24 h after the last injection of saline or IMI and ECS treatment; their brains were excised, and selected structures were dissected on ice. The tissues (cerebral cortex and its selected regions, the parietal and prefrontal cortices) were deep frozen until membrane preparation, HPLC assay and extraction of total RNA.

Evaluation of DSP-4 lesion efficacy

To assess the efficacy and selectivity of the DSP-4 action, the levels of noradrenaline (NA) and serotonin (5-HT) in the parietal cortex were assessed by high-performance liquid chromatography (HPLC) with electrochemical detection. The chromatograph HP 1050 (Hewlett-Packard, USA) was equipped with Hypersil columns BDS-C18 (4 × 100 mm, 3 μm). The tissue samples were weighed and homogenized in ice-cold 0.1 M perchloroacetic acid containing 0.05 mM ascorbic acid. After centrifugation (10,000 × g, 5 min), the supernatants were filtered through RC58 cellulose

membranes with a pore diameter of 0.2 μm (Bioanalytical Systems, West Lafayette, IN, USA). The mobile phase consisted of 0.05 M citrate-phosphate buffer, pH 3.5, 0.1 mM EDTA, 1 mM sodium octyl sulfonate and 3.5% methanol. The flow rate was maintained at 1 ml/min [1].

RNA isolation

Total cellular RNA was isolated from the prefrontal cortex and purified by the method described by Chomczynski [4] utilizing TRIzol reagent (Gibco). RNA quantity and purity were determined spectrophotometrically.

Analysis of mRNA expression by northern blot

Expression ofα_{1A}-AR mRNA was analyzed by northern blot hybridization as described previously by Nalepa et al. [22]. Briefly, samples of total RNA were denatured at 55°C for 15 min. Each sample of total RNA was loaded (20 μg/well) and separated by electrophoresis (1% agarose/2.2 M formaldehyde denaturing gel and 1x northern running buffer, 90 V for 3.5 h), transferred to the Hybond N^+ membrane (Amersham/Pharmacia) in 10x saline-sodium citrate buffer (SSC, Amresco). The blots were baked for 10 min at 80°C and cross-linked with UV light. Filters were prehybridized for 20 min at 65°C in Rapid-hyb buffer (Amersham/Pharmacia), hybridized with the α_{1A}-AR cDNA probe (6×10^6 cpm/ml of buffer, prepared as described by Nalepa et al. [22]) at 65°C for 2.5 h and washed as follows: 1 × 20 min (2 × SSC/0.1% sodium dodecyl sulfate, SDS) at room temperature and 2 × 15 min (0.1 × SSC/0.1% SDS) at 65°C. Blots were exposed to an autoradiographic screen and analyzed quantitatively on a phosphoimager (Fuji BAS 5000, Japan) using Science Lab 4.0 software. Ethidium bromide (EtBr) fluorescence of 28S ribosomal RNA was used as an internal standard as described by Duhl et al. [10]. The signal density was expressed as a percent of the mean signal density for all appropriate controls.

Analysis of mRNA expression by competitive PCR

Expression of α_{1B}-AR mRNA was analyzed by the competitive PCR method as described previously [20]. The primer sequences (sense primer: 5'-GTA GCC CAG CCA GAA CAC CA -3'; antisense primer: 5'-GGA AAA GAA AGC AGC CAA AAC CT-3') were selected from the sequence of rat mRNA for α_{1B}-AR to generate the 151-base pair (bp) segment of α_{1B}-AR cDNA. Briefly, isolated total RNA was reverse transcribed (RT) into cDNA in a 20 μl reaction mixture containing 1x AMV RT Buffer, 1 mM dNTP, 1 μM antisense primer, 2 μg RNA and 10 units of AMV reverse transcriptase (Amresco). Then, cDNA for the α_{1B}-AR was co-amplified with prepared 248 bp internal standard in a 25 μl PCR reaction containing 1x PCR buffer, 0.625 units of Taq polymerase (Finnzymes), 1.5 mM dNTP, 0.4 μM sense and antisense primers, 1 μl of RT mix (containing α_{1B}-AR cDNA) and 1 μl of a chosen concentration of internal standard. The PCR was performed as follows: one initial denaturation cycle (94°C/5 min); 34 cycles of (i) 94°C/1 min (denaturation), (ii) 64°C/1 min (annealing), and (iii) 72°C/1 min (extension); and a final elongation cycle (72°C/7 min). The relative fluorescence of cDNA PCR product *versus* internal standard fragment was measured in an EtBr-stained 3% agarose SFR (Amresco) gel using the FluoroImager system (Fuji LAS-1000). The signal density was expressed as a percent of the mean signal density for all appropriate controls.

Analysis of receptor protein ([^{3}H]prazosin binding)

The membrane preparation (P_2 fraction) from the cerebral cortex of the rat was performed as previously described [22, 25]. To discriminate the α_{1A}-AR and α_{1B}-AR densities, the procedure described by Hayakawa et al. [13] was used. Following this concept, α_{1A}-AR can be masked by a low concentration of WB4101, and the relative densities of these subtypes can be assessed from the difference between [^{3}H]prazosin binding in the presence of low and high concentrations of WB4101. The procedure was performed as described by Nalepa et al. [22]. Briefly, six concentrations of [^{3}H]prazosin, ranging from 0.09–3.63 nM, were used to characterize α_1-AR binding sites. Total α_1-AR binding (including both α_{1A}-AR and α_{1B}-AR subtypes) was assessed by subtracting baseline binding, in the presence of 10 μM WB4101 as a displacer, from the total binding in the absence of WB4101. The α_{1A}-AR sites were determined by [^{3}H]prazosin binding in the presence of 2 nM WB4101, and the density of α_{1B}-AR was assessed by subtraction the α_{1A}-AR

binding from the total α_1-AR 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) through Whatman GF/C filters. Afterwards, the filters were rinsed twice with 5-ml portions of ice-cold Tris-HCl buffer, placed in polyethylene mini-vials in 3 ml of scintillation liquid, and counted for radioactivity in a liquid scintillation counter. The B_{max} and K_D values were calculated from binding isotherm using GraphPad Prism software.

Drugs and chemicals

Citalopram was a gift from H. Lundbeck A/S. Imipramine hydrochloride was obtained from Polfa, Kraków, Poland. [^{3}H]Prazosin (S.A. 24 Ci/mmol) was purchased from Radioactive Center, Amersham, WB4101 and DSP-4 from Sigma, St. Louis, MO. Formamide, ethidium bromide and agarose SFR were purchased from Amresco, Solon, OH.

Statistical analysis

Statistical analysis of the results were preformed with Statistica 6.0 software using one way analysis of variance followed by the Fisher's LSD (Least Significant Difference) test; p value less than 0.05 was considered to be statistically significant.

Results

The effect of noradrenergic lesion on neurotransmitter levels

Efficacy and selectivity of the NA depletion after DSP-4 lesioning were quantified by HPLC. Administration of DSP-4 produced a significant decrease of noradrenaline content in the samples of the parietal cortex to approximately 44%, 27%, 27% and 18% of appropriate control levels (SHAM or SAL treated) in the DSP-4/SHAM, DSP-4/ECS, DSP-4/SAL and DSP-4/IMI groups, respectively, while the 5-HT level was unchanged (Tab. 1)

Tab. 1. Levels of noradrenaline and serotonin in the parietal cortex after DSP-4 treatment

Group (n)	[ng/g tissue]	
	NA	5-HT
SAL/SHAM (14)	406 ± 49	325 ± 30
SAL/ECS (10)	418 ± 62	301 ± 25
DSP-4/SHAM (14)	179 ± 33**	342 ± 22
DSP-4/ECS (11)	108 ± 57**	304 ± 25
SAL/SAL (10)	213 ± 15	230 ± 36
SAL/IMI (8)	255 ± 42	210 ± 40
DSP-4/SAL (10)	58 ± 21**	245 ± 49
DSP-4/IMI (8)	39 ± 13**	192 ± 39

SAL – saline, ECS – electroconvulsive treatment, IMI – imipramine, NA – noradrenaline, 5-HT – serotonin; ** $p < 0.01$ *vs.* appropriate controls (SAL/SHAM or SAL/SAL)

Noradrenergic lesion and antidepressant-induced changes in the expression of α_1-adrenoceptor subtype mRNAs

DSP-4 neurotoxin did not change the mRNA expression of any of the investigated subtypes (α_{1A} and α_{1B}) of α_1-AR in the prefrontal cortex (Fig. 1 and 2). Northern blot hybridization revealed that the ECS-induced up-regulation of the mRNA encoding for α_{1A}-AR was completely abolished when ECS was applied to the rats that underwent NA lesion (Fig. 1A). The IMI induced enhancement of α_{1A}-AR expression was attenuated when IMI was administered after the DSP-4 injection. Though the up-regulation of α_{1A}-AR was still present compared to the saline-treated controls, there was no statistically significant difference between the animals receiving DSP-4/SAL and DSP-4/IMI (Fig. 1B).

The level of expression of α_{1B}-AR mRNA was not changed by any treatment, as assessed by competitive RT-PCR (Fig. 2A, B).

Noradrenergic lesion and antidepressant-induced changes in the density of [^{3}H]prazosin binding sites of α_1-adrenoceptor subtypes

DSP-4 increased the density (B_{max}, by approximately 17% and 28% *vs.* appropriate controls receiving sham or saline injections, respectively) and reduced the affinity (approximately 2-fold increase of K_D value) of

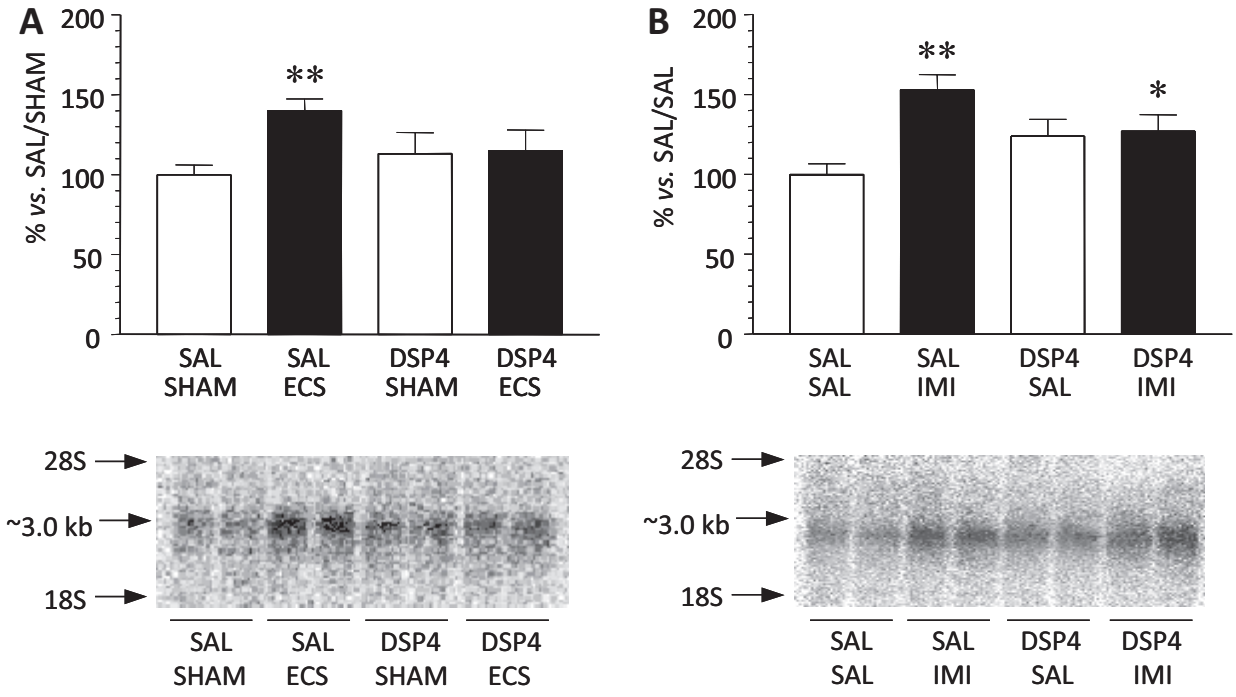

Fig. 1. The effect chronic treatment with electroconvulsive shock (**A**) or imipramine (**B**) administered after DSP-4 on steady-state level of α_{1A}-AR mRNA in the rat prefrontal cortex determined by northern blot analysis. Samples of 20 μg of total RNA were loaded in duplicates, and mRNA levels were analyzed as described under Materials and Methods. The results are expressed as a percent of controls treated with saline and are the means ± SEM of values from at least five individuals. Bottom panels show the representative northern blot analyses of α_{1A}-AR mRNA. Arrows show location of α_{1A}-AR (~3000 base pairs) and of residual 28S and 18S ribosomal RNA; SAL – saline, ECS – electroconvulsive shock, IMI – imipramine; ** $p < 0.01$, * $p < 0.05$ *vs.* appropriate controls (SAL/SHAM or SAL/SAL)

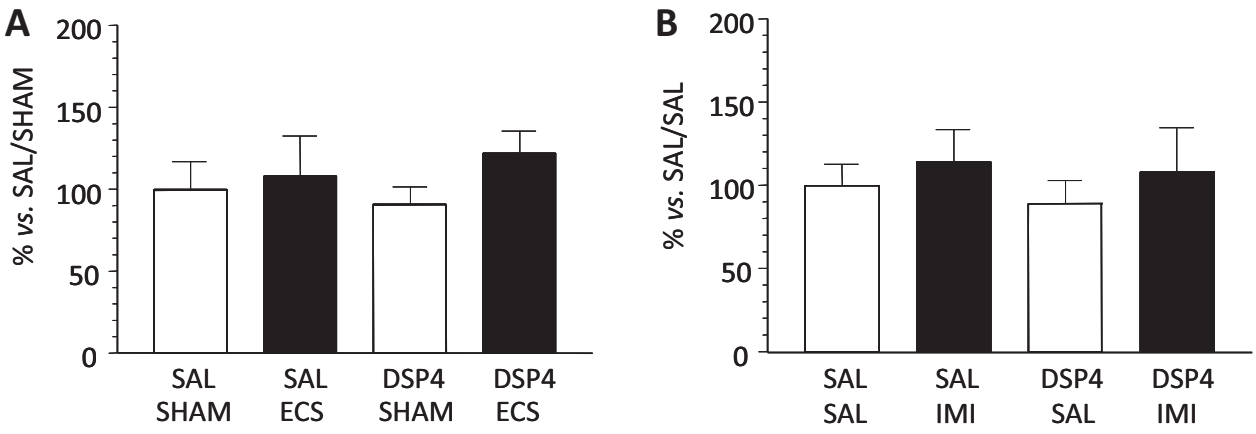

Fig. 2. The lack of effects of DSP-4 and chronic treatment with electroconvulsive shock (**A**) or imipramine (**B**) on steady-state level of α_{1B}-AR mRNA in the rat prefrontal cortex determined by competitive RT-PCR. Competitive RT-PCR analysis was conducted as described in the Materials and Methods. The results are expressed as a percent of controls treated with saline and are the means ± SEM of values from at least six individuals. SAL – saline, ECS – electroconvulsive shock, IMI – imipramine

Tab. 2. The effect of a 14-day treatment with electroconvulsive shock or imipramine administered after DSP-4 lesioning on [^{3}H]prazosin binding sites in the cerebral cortex

Group (n)	total α_1-AR		α_{1A}-AR		α_{1B}-AR	
	B_{max} [fm/mg protein]	K_D [nM]	B_{max} [fm/mg protein]	K_D [nM]	B_{max} [fm/mg protein]	K_D [nM]
SAL/SHAM (6)	114.0 ± 6.5	0.19 ± 0.02	89.2 ± 8.9	0.10 ± 0.02	64.0 ± 5.0	1.13 ± 0.36
SAL/ECS (6)	134.9 ± 2.8*	0.19 ± 0.02	110.9 ± 6.4*	0.17 ± 0.04	63.4 ± 6.1	1.17 ± 0.29
DSP-4/SHAM (7)	133.1 ± 3.8*	0.39 ± 0.07**	87.2 ± 3.1	0.21 ± 0.03**	59.7 ± 4.3	0.91 ± 0.12
DSP-4/ECS (6)	109.9 ± 8.3	0.28 ± 0.02	64.9 ± 10.7*	0.11 ± 0.03	50.0 ± 1.6*	0.73 ± 0.05
SAL/SAL (6)	108.1 ± 2.4	0.23 ± 0.02	79.8 ± 4.3	0.13 ± 0.03	61.4 ± 7.5	0.85 ± 0.18
SAL/IMI (5)	131.1 ± 9.1**	0.23 ± 0.02	101.7 ± 8.9**	0.11 ± 0.01	61.6 ± 4.7	1.01 ± 0.15
DSP-4/SAL (4)	138.9 ± 4.0**	0.46 ± 0.12*	90.2 ± 2.5	0.23 ± 0.05*	66.6 ± 4.3	1.00 ± 0.19
DSP-4/IMI (4)	146.2 ± 0.4**	0.41 ± 0.04**	99.0 ± 0.2*	0.25 ± 0.02**	62.4 ± 2.6	1.03 ± 0.05

Six concentrations of [^{3}H]prazosin (range: 0.086–3.46 nM) were used. Total binding (including both α_{1A}-AR and α_{1B}-AR) was assessed by the binding difference in the absence and presence of 10 μM WB4101. The α_{1A}-AR sites were assessed from the [^{3}H]prazosin binding difference in the absence and presence of 2 nM WB4101. The difference between total and α_{1A}-AR binding was assumed to be α_{1B}-AR binding. The data are expressed as the mean ± SEM, the number of animals within each group is indicated in parentheses; SAL – saline, ECS – electroconvulsive shock, IMI – imipramine. ** $p < 0.01$, * $p < 0.05$ *vs.* appropriate controls (SAL/SHAM or SAL/SAL)

total α_1-AR considerably in the rat cerebral cortex (Tab. 2).

NA lesion counteracted the ECS-induced elevation of the total α_1-AR density and the α_{1A} receptor subtype as well. Moreover, in the DSP-4/ECS group, the B_{max} value of the α_{1A}-AR dropped below that of the control level (by 28% *vs*. SAL/SHAM), and a reduction in the α_{1B}-AR (by 22% *vs*. SAL/SHAM) appeared in the cerebral cortex of these animals.

The effect induced by chronic treatment with IMI was not influenced by NA lesion; the increase in the density of total α_1-AR and α_{1A}-AR was still observed in the DSP-4/IMI group. Moreover, the affinity of the receptors (the total α_1-AR and its α_{1A} subtype) in this group was diminished as indicated by K_D values that were similar to those of the DSP-4/SAL-treated animals (Tab. 2).

Discussion

Research into the mechanisms of action of antidepressant therapies suggests that as a result of long-term use, a wide selection of adaptive changes at various membrane receptors and intracellular signal transduction occur, including modulation of transcriptional gene activity [23, 35]. The noradrenergic system is one target for antidepressant action, and the present study extends our previous research on the modulation of the subtypes of α_1-AR by antidepressant treatments. We have already shown that among the α_1-AR subtypes, the α_{1A}-AR underwent adaptive changes after repeated administration of ECS and IMI, while the α_{1B} receptor was unchanged [22]. This observation was also confirmed by the current study. The fact that only α_{1A}-AR is affected by antidepressant treatment, while α_{1B}-AR is not, may be explained by a difference in susceptibility of the α_1-AR subtypes to their regulation by noradrenaline. Thus, α_{1A}-AR is positively regulated by adrenergic agonists that regulate negatively α_{1B}-AR [31, 38].

Our previous findings show that both ECS and IMI antidepressant therapies increased the density of α_{1A}-AR and enhanced its mRNA expression in the rat prefrontal cortex, which was not caused by chronic administration of a selective 5-HT reuptake inhibitor, citalopram [20]. These results prompted us to hypothesize that the phenomenon is associated specifi-

cally with the stimulation of the NA transmission. To investigate this hypothesis, we examined whether NA depletion with the noradrenergic neurotoxin DSP-4 affects the up-regulation of the α_{1A} subtype of the α_1-AR observed after chronic ECS and IMI treatment. Administration of DSP-4 led to a reduction (approximately 70%) of NA tissue concentration in the parietal cortex, without altering the level of 5-HT, and corroborating the data shown by other authors [11, 17, 30]. The influence of NA lesion on the expression of α_1-ARs and their changes brought about by antidepressant treatment was studied in the cerebral cortex, which is the brain structure that receives considerable NA input from the locus coeruleus (LC) and where both α_{1A} and α_{1B} receptors are the most abundant among all three α_1-AR subtypes with equal distribution [21].

We found that DSP-4 lesion by itself did not influence the mRNA expression of either α_{1A}-AR or α_{1B}-AR in the prefrontal cortex, but it did affect the ECS and IMI-induced increase in α_{1A}-AR mRNA levels in this brain structure. While the ECS effect was abolished by previous noradrenergic depletion with DSP-4, the IMI-induced increase in the α_{1A}-AR was significantly attenuated. As ECS and IMI administration were shown to increase the NA release in the rat frontal cortex [34, 36], our results indicate an involvement of NA signaling in the modulation of transcriptional activity of the α_{1A}-AR gene that occurred as a result of these antidepressant treatments and was disturbed with NA depletion. However, the data also suggest that ECS and IMI efficacies may differ in this respect.

In addition to investigation of the mRNA expression, binding studies were carried out. The DSP-4-evoked NA lesion led to the increase in [^{3}H]prazosin binding sites, which corresponds to the total α_1-AR density. However, neither the α_{1A}-AR nor α_{1B}-AR subtype densities were affected. The latter finding remains difficult to explain. However, the increase in the density was mostly reversely correlated with the receptor affinity; thus, the eventual differences at the level of a particular subtype may be masked. Moreover, it should be emphasized that the discrimination between receptor subtypes is difficult due to the lack of sufficiently specific ligands. The method used by us (currently and previously [22]) and other authors [13] employed the difference in affinity of WB4101 to receptors subtypes. Using this method, one can semi-quantitatively block the population of the α_{1A}-AR subtype and can use the data from binding in the presence of low and high WB4101 concentrations to calculate the relative proportion of α_1-AR subtypes (of different affinity to [^{3}H]prazosin). However, this method does not allow the α_{1D}-AR (not considered in this study) to be discriminated, which may interfere with the binding. The affinity of WB4101 to the α_{1D}-AR was shown to be similar to that of the α_{1A}-AR [18]. Although the modulation of the α_{1D} receptor density by DSP-4 cannot be excluded and needs clarification, it should be emphasized that this receptor subtype is thought to be localized mainly intracellularly [2, 29]. The lack of correlation between the effect of DSP-4 lesion on receptor density and mRNA expression observed in this study may be explained by enhanced turnover of receptor protein, which most likely occurs in NA-depleted animals.

The results obtained after ECS administration to the animals depleted of noradrenaline were consistent on both mRNA and receptor protein levels. Similarly, as for mRNA, DSP-4 pretreatment abolished the ECS-induced enhancement of the total cortical population density of α_1-AR and the α_{1A}-AR subtype. On the other hand, one cannot exclude that ECS treatment normalized the receptor changes resulting from the DSP-4 action. The interaction between DSP-4 and ECS was especially marked at α_{1A}-AR, whose density dropped below the saline control level, but an unexpected similar change appeared at the α_{1B}-AR level, which is the receptor subtype that was not affected by ECS alone.

A different outcome was observed in the binding study concerning IMI effects. The densities and affinity of both the total α_1-AR and the α_{1A}-AR remained at a similar level when IMI was administered after DSP-4. However, there was no significant difference between the groups receiving DSP-4 and those treated with IMI after DSP-4, which may suggest that this effect is connected to the up-regulation of the α_1-AR observed after the neurotoxin. Here, it should be mentioned that in this case, there was no significant difference between the DSP-4/SAL and DSP-4/IMI groups.

Overall, the results indicate that the NA component of ECS and IMI pharmacological action are important in modulating the expression of α_1-AR and especially the α_{1A} subtype, though the pattern of changes induced by these two agents differed in the condition of the NA lesion. The data also raise questions about the mechanisms leading to an increase in α_1-AR that was

similarly expressed in the case of DSP-4 action and the two antidepressant treatments applied in our study.

DSP-4 has been considered an LC-selective noradrenergic neurotoxin that causes NA terminal loss (reviewed by [19]), but data exist suggesting that these terminals are not reduced (for review, see [33]) and that the amount of released NA into the synapse is not altered [15, 17]. Although the DSP-4-evoked increase in α_1-AR density was previously described by other authors who believed that it occurred due to a reduction in synaptic NA levels and postulated a compensatory response to enervated NA neurotransmission [27, 37], it remains unclear why α_1-AR binding sites would be elevated in the brain after treatment with DSP-4. The availability of synaptic NA, an important agent for the regulation of postsynaptic α_1-AR, will depend on noradrenergic transporter (NET) activity and on the amount of α_2-ARs, which inhibit NA release. Recently, DSP-4 was shown to induce a rapid, though transient, reduction in NET binding sites in many brain regions receiving innervations from the LC [33]. Thus, it is plausible that shortly after DSP-4 lesion, the availability of synaptic NA may be not diminished but instead increased enough to induce α_1-AR up-regulation (observed in our study), and paradoxically, the effect of up-regulation of α_1-AR density might be based on a similar mechanism as that employed by ECS and IMI treatments.

Interestingly, the paradoxical antidepressant-like effect of DSP-4 treatment observed in behavioral paradigms was reported by some authors who also postulated the role of α_1-AR supersensitivity in this phenomenon [7, 12]. Moreover, Cryan et al. [6] have shown that antidepressant-like effect of reboxetine (a selective noradrenaline reuptake inhibitor) was potentiated by pretreatment with DSP-4. In our study, DSP-4 only slightly (by 10%) enhanced the IMI-induced increase in the total α_1-AR binding.

IMI and ECS treatments both induce an increase in synaptic NA availability but with different mechanisms: IMI inhibits NET activity and NA reuptake from a synapse, while ECS will release neurotransmitters by depolarizing the nerve terminals. If synaptic NA is considered the main factor for regulation of α_{1A}-AR, then the question arises about the different effects of ECS and IMI in DSP-4 pretreated animals (this study). One possible explanation is an interaction of DSP-4 and ECS at the level of α_2-ARs. The critical role, at least in the frontal cortex, of the down-regulation of presynaptic α_2-AR in a mechanism leading to the increase in NA release that occurred after chronic ECS was postulated [34]. However, a comprehensive analysis of the DSP-4 effects in rats performed by Szot and colleagues [33] revealed that despite a decrease in α_2-AR reported by some authors shortly after DSP-4 treatment [14], the gradual up-regulation of these receptors in various brain structures was observed later on. Thus, the DSP-4 counteractive influence on ECS effects (this study) may be partially related to changes in presynaptic α_2-AR**.**

The importance of the α_{1A}-AR role in the mechanism of action of antidepressant therapy has been recently suggested by a study on transgenic animals showing that α_{1A}-AR and α_{1B}-AR differently modulate antidepressant-like behavior in mice [9]. The authors reported that only α_{1A}-AR signaling promotes antidepressant-like behavior in tail suspension and forced swimming tests, indicating that these two subtypes of α_1-AR may have separate and distinct functions in the central nervous system, even though overlapping of their distribution within many of brain regions was observed.

In conclusion, the present results confirm our hypothesis that the NA component of the action of antidepressants plays an important role in the modulation of α_{1A}-AR in the cerebral cortex. However, the final outcome of the modulation as reflected by α_{1A}-AR changes will depend on various factors involved in a complicated mechanism of regulation of the synaptic NA level.

Acknowledgments:
This work was supported by grant No. 4 P05A 044 11 from the State Committee for Scientific Research (KBN) and by the statutory funds of Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland

References:

1. Antkiewicz-Michaluk L, Romanska I, Vetulani J: Ca^{2+} channel blockade prevents lysergic acid diethylamide-induced changes in dopamine and serotonin metabolism. Eur J Pharmacol, 1997, 332, 9–14.
2. Chalothorn D, McCune DF, Edelmann SE, Garcia-Cazarin ML, Tsujimoto G, Piascik MT: Differences in the cellular localization and agonist-mediated internalization properties of the α_1-adrenoceptor subtypes. Mol Pharmacol, 2002, 61, 1008–1016.

3. Chen ZJ, Minneman KP: Recent progress in α_1-adrenergic receptor research. Acta Pharmacol Sin, 2005, 26, 1281–1287.
4. Chomczynski P: A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques, 1993, 15, 532–534, 536–537.
5. Cryan JF, O'Leary OF, Jin SH, Friedland JC, Ouyang M, Hirsch BR, Page ME et al.: Norepinephrine-deficient mice lack responses to antidepressant drugs, including selective serotonin reuptake inhibitors. Proc Natl Acad Sci USA, 2004, 101, 8186–8191.
6. Cryan JF, Page ME, Lucki I: Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swim test. Eur J Pharmacol, 2002, 436, 197–205.
7. Dabrowska J, Nowak P, Brus R: Reactivity of 5-HT_{1A} receptor in adult rats after neonatal noradrenergic neurons' lesion – Implications for antidepressant-like action. Brain Res, 2008, 1239, 66–76.
8. Daniels DV, Gever JR, Jasper JR, Kava MS, Lesnick JD, Meloy TD, Stepan G et al.: Human cloned α_{1A}-adrenoceptor isoforms display α_{1L}-adrenoceptor pharmacology in functional studies. Eur J Pharmacol, 1999, 370, 337–343.
9. Doze VA, Handel EM, Jensen KA, Darsie B, Luger EJ, Haselton JR, Talbot JN et al.: α_{1A}- and α_{1B}-adrenergic receptors differentially modulate antidepressant-like behavior in the mouse. Brain Res, 2009, 1285, 148–157.
10. Duhl DM, Gillespie DD, Sulser F: Ethidium bromide fluorescence of 28S ribosomal RNA can be used to normalize samples in northern or dot blots when analyzing small drug-induced changes in specific mRNA. J Neurosci Methods, 1992, 42, 211–218.
11. Ferdyn-Drosik M, Nowak P, Bojanek K, Balasz M, Kasperski J, Skaba D, Muchacki R et al.: Neonatal DSP-4 treatment impairs 5-HT_{1B} receptor reactivity in adult rats. Behavioral and biochemical studies. Pharmacol Rep, 2010, 62, 608–620.
12. Harro J, Pahkla R, Modiri AR, Harro M, Kask A, Oreland L: Dose-dependent effects of noradrenergic denervation by DSP-4 treatment on forced swimming and α-adrenoceptor binding in the rat. J Neural Transm, 1999, 106, 619–629.
13. Hayakawa H, Shimizu M, Yamawaki S: The effects of electroconvulsive shock or imipramine on subtypes of α_1-adrenoceptors in the frontal cortex of the rat. Neuropharmacology, 1992, 31, 955–960.
14. Heal DJ, Butler SA, Prow MR, Buckett WR: Quantification of presynaptic alpha 2-adrenoceptors in rat brain after short-term DSP-4 lesioning. Eur J Pharmacol, 1993, 249, 37–41.
15. Hughes ZA, Stanford SC: A partial noradrenergic lesion induced by DSP-4 increases extracellular noradrenaline concentration in rat frontal cortex: a microdialysis study in vivo. Psychopharmacology (Berl), 1998, 136, 299–303.
16. Itoi K, Sugimoto N: The brainstem noradrenergic systems in stress, anxiety and depression. J Neuroendocrinol, 2010, 22, 355–361.
17. Kask A, Harro J, Tuomaine P, Rago L, Mannisto PT: Overflow of noradrenaline and dopamine in frontal cortex after [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] (DSP-4) treatment: in vivo microdialysis study in anaesthetized rats. Naunyn Schmiedebergs Arch Pharmacol, 1997, 355, 267–272.
18. Kenny BA, Chalmers DH, Philpott PC, Naylor AM: Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br J Pharmacol, 1995, 115, 981–986.
19. Kostrzewa RM: Evolution of neurotoxins: from research modalities to clinical realities. Curr Protoc Neurosci, 2009, Chapter 1, Unit 1. 18.
20. Kreiner G, Bielawski A, Zelek-Molik A, Kowalska M, Nalepa I: Chronic treatment with citalopram does not affect the expression of α_1-adrenergic receptor (α_1-AR) subtypes. Pol J Pharmacol, 2004, 56, 831–836.
21. Kreiner G, Sanak M, Zelek-Molik A, Nalepa I: Using reverse transcription and a competitive polymerase chain reaction for quantification of α_{1B}-adrenoceptor mRNA. Pol J Pharmacol, 2002, 54, 401–405.
22. Nalepa I, Kreiner G, Kowalska M, Sanak M, Zelek-Molik A, Vetulani J: Repeated imipramine and electroconvulsive shock increase α_{1A}-adrenoceptor mRNA level in rat prefrontal cortex. Eur J Pharmacol, 2002, 444, 151–159.
23. Nalepa I, Sulser F: New hypothesis to guide future antidepressant drug development. Antidepressants: past, present and future. Handbook of Experimental Pharmacology, 2004, 519–563.
24. Nalepa I, Vetulani J: Enhancement of the responsiveness of cortical adrenergic receptors by chronic administration of the 5-hydroxytryptamine uptake inhibitor citalopram. J Neurochem, 1993, 60, 2029–2035.
25. Nalepa I, Vetulani J: Involvement of protein kinase C in the mechanism of in vitro effects of imipramine on generation of second messengers by noradrenaline in cerebral cortical slices of the rat. Neuroscience, 1991, 44, 585–590.
26. Nalepa I, Vetulani J: The responsiveness of cerebral cortical adrenergic receptors after chronic administration of atypical antidepressant mianserin. J Psychiatry Neurosci, 1994, 19, 120–128.
27. Nowak G, Zak J, Superata J: Role of dopaminergic neurons in denervation-induced alpha 1-adrenergic upregulation in the rat cerebral cortex. J Neurochem, 1991, 56, 914–916.
28. Perez DM: The adrenergic receptors in the 21 century. Humana Press, Totowa, New Jersey, 2006.
29. Piascik MT, Perez DM: α_1-Adrenergic receptors: new insights and directions. J Pharmacol Exp Ther, 2001, 298, 403–410.
30. Prieto M, Giralt MT: Effects of N-(2-chloroethyl)-N--ethyl-2-bromobenzylamine (DSP4) on α_2-adrenoceptors which regulate the synthesis and release of noradrenaline in the rat brain. Pharmacol Toxicol, 2001, 88, 152–158.
31. Rokosh DG, Stewart AF, Chang KC, Bailey BA, Karliner JS, Camacho SA, Long CS et al.: α_1-Adrenergic receptor subtype mRNAs are differentially regulated by α_1-adrenergic and other hypertrophic stimuli in cardiac myocytes in culture and in vivo. Repression of α_{1B} and α_{1D} but induction of α_{1C}. J Biol Chem, 1996, 271, 5839–5843.
32. Rossby SP, Nalepa I, Huang M, Perrin C, Burt AM, Schmidt DE, Gillespie DD et al.: Norepinephrine-

independent regulation of GRII mRNA in vivo by a tricyclic antidepressant. Brain Res, 1995, 687, 79–82.
33. Szot P, Miguelez C, White SS, Franklin A, Sikkema C, Wilkinson CW, Ugedo L et al.: A comprehensive analysis of the effect of DSP4 on the locus coeruleus noradrenergic system in the rat. Neuroscience, 2010, 166, 279–291.
34. Thomas DN, Nutt DJ, Holman RB: Effects of acute and chronic electroconvulsive shock on noradrenaline release in the rat hippocampus and frontal cortex. Br J Pharmacol, 1992, 106, 430–434.
35. Vetulani J, Nalepa I: Antidepressants: past, present and future. Eur J Pharmacol, 2000, 405, 351–363.
36. Wesołowska A, Kowalska M: Influence of serotonin 5-HT_7 receptor blockade on the behavioral and neurochemical effects of imipramine in rats. Pharmacol Rep, 2008, 60, 464–474.
37. Wolfman C, Abo V, Calvo D, Medina J, Dajas F, Silveira R: Recovery of central noradrenergic neurons one year after the administration of the neurotoxin DSP4. Neurochem Int, 1994, 25, 395–400.
38. Yang M, Ruan J, Voller M, Schalken J, Michel MC: Differential regulation of human α_1-adrenoceptor subtypes. Naunyn Schmiedebergs Arch Pharmacol, 1999, 359, 439–446.
39. Zhong H, Minneman KP: α_1-Adrenoceptor subtypes. Eur J Pharmacol, 1999, 375, 261–276.

Received: August 11, 2011; **in the revised form:** October 28, 2011; **accepted:** November 14, 2011.