Pharmacology Biochemistry & Behavior, Vol. 25, pp. 743–746, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Increase in β- and α_1-Adrenoceptor Binding Sites in the Rat Brain and in the α_1-Adrenoceptor Functional Sensitivity After the DSP-4-Induced Noradrenergic Denervation

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Received 27 March 1986

MOGILNICKA, E. *Increase in β- and α_1-adrenoceptor binding sites in the rat brain and in the α_1-adrenoceptor functional sensitivity after the DSP-4-induced noradrenergic denervation.* PHARMACOL BIOCHEM BEHAV **25**(4) 743–746, 1986.—Changes in the density of β- and α_1-adrenoceptors were studied following denervation of the rat cerebral cortex and hippocampus, caused by systemic administration of DSP-4. The noradrenergic denervation increased both β- and α_1-adrenoceptor density by about 30 and 17%, respectively in the cortex, and by about 30% in the hippocampus. In order to estimate the behavioral response of normal and DSP-4-treated rats to α_1-agonist, the influence of phenylephrine (25 μg ICV) on the exploratory activity of rats in the open field was measured. Phenylephrine failed to change the exploratory activity of normal rats, but significantly increased it in DSP-4 animals. The results indicate that noradrenergic denervation produces an increase in number of both β- and α_1-adrenoceptors and the functional supersensitivity to the α_1-adrenergic agonists.

DSP-4 Noradrenergic denervation β-Receptor α_1-Receptor Exploratory behavior Phenylephrine Rat

WE have recently described the functional supersensitivity to β- and α_2-adrenergic agonists in the rat after DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine), a selective noradrenergic neurotoxin. It was just demonstrated that the exploratory activity of DSP-4-treated animals was disrupted by clonidine or clenbuterol to a greater degree than in control animals [3]. This finding correlates with earlier neurochemical data stating that DSP-4-treated rats have supersensitive α_2- and β-adrenoceptors in some regions of the central nervous system [2].

Following an intracerebral ventral or intraperitoneal administration of the catecholaminergic neurotoxins 6-hydroxydopamine (6-OHDA) or DSP-4, respectively, the number of β-adrenoceptor binding sites in the rat cerebral cortex increases [5, 9–11] however, there is some controversy whether the α_1-adrenergic receptor density increases after a noradrenergic denervation [2, 9, 11, 12, 15]. Only recently Sutin and Minneman [11] found that the α_1-adrenergic density increases following 6-OHDA treatment, yet the degree of changes is smaller than the increase in β-adrenoceptor density. Considering the fact that a percent change in the density of one receptor type is well correlated with that of the other, the authors suggest that these two types of receptors are co-regulated in response to the noradrenergic denervation. Our own behavioral observations permit us to think that the stimulation of β-system is accompanied with facilitation of α_1-system [8]; therefore, we decided to use the model of noradrenergic denervated rats with a supersensitive β-system for the investigation of possible changes in the α_1-adrenergic system. In the present study we examined β- and α_1-adrenoceptor densities in the cerebral cortex and hippocampus of DSP-4-lesioned rats. DSP-4 markedly depleted norepinephrine (NE) in the central nervous system, especially in the regions innervated by noradrenergic neurons of the locus coeruleus (LC), the greatest reduction of the NE content occurring in the cerebral cortex, hippocampus and spinal cord [2, 6, 7].

Functional consequences of DSP-4 treatment were measured in a behavioral model involving the exploratory activity of rats in response to administration of the α_1-agonist phenylephrine.

METHOD

Subjects

The experiments were carried out on male Wistar rats weighing 200–220 g. The animals were kept under standard laboratory conditions (a continuous 12 hr light-dark cycle,

TABLE 1

EFFECT OF DSP-4-TREATMENT ON THE DENSITY OF α_1- AND β-ADRENOCEPTORS IN CEREBRAL CORTEX AND HIPPOCAMPUS

	^{3}H-DHA			^{3}H-Prazosin		
	B_{max} (pmol/g weight)	% of control	K_D (nM)	B_{max} (pmol/g weight)	% of control	K_D (nM)
Neocortex						
Control	4.72 ± 0.20	100	0.73 ± 0.12	8.27 ± 0.35	100	0.32 ± 0.09
DSP-4	6.25 ± 0.25	132.4‡	0.64 ± 0.12	9.70 ± 0.50	117.3*	0.35 ± 0.09
Hippocampus						
Control	3.34 ± 0.15	100	0.91 ± 0.08	3.52 ± 0.19	100	0.19 ± 0.05
DSP-4	4.2 ± 0.04	125.7‡	0.80 ± 0.04	4.74 ± 0.20	134.6†	0.21 ± 0.05

The dissociation affinity constants K_D (nM) and the maximal binding B_{max} were obtained by Scatchard analysis. Scatchard plots, each of 6 points, were determined in duplicate using concentrations of ^{3}H-DHA and ^{3}H-prazosin ranging from 0.1 to 2.0 nM and from 0.05 to 1.5 nM, respectively.

Mean ± S.E.M. values are given. *$p<0.05$; †$p<0.01$; ‡$p<0.001$ (Student's t-test).

constant humidity and temperature) with free access to food and water.

Subject Preparation

The preparation of DSP-4 animals was as previously described [2]. Briefly, they were pretreated with CGP 6085 A (2.7 mg/kg IP), an inhibitor of 5-hydroxytryptamine uptake [14], 30 min before the injection of DSP-4 (60 mg/kg IP) or a control solution. The control and DSP-4-treated rats were then tested or killed 10 days later between 09.00 and 11.00 hr. These results of previous neurochemical experiments [2] indicated, 10 days after DSP-4 injection, an almost total depletion of NE in the neocortex, hippocampus, cerebellum and spinal cord, and a smaller depletion in the hypothalamus and other brain regions.

Binding Assay

The brains were removed and the neocortex and hippocampus were dissected and rapidly frozen. For ^{3}H-dihydroalprenolol (^{3}H-DHA) and ^{3}H-prazosin binding studies the rat brain neocortex and hippocampus were homogenized in 20 vol (w/v) of ice-cold Tric-HCl buffer (50 nM, pH 7.4) using a Polytron homogenizer. The homogenates were centrifuged at 25.000 × g for 10 min. The pellets were rehomogenized in another portion of buffer, then centrifuged. The final pellets were resuspended in 140 vol (w/v) of Tric-HCl buffer (50 nM, pH 7.4). To 1.4 ml of membrane suspensions were added ^{3}H-DHA (NEN, sp. act. 52.1 Ci/mmol) or ^{3}H-prazosin (NEN, sp. act. 19.8 Ci/mmol) in 100 μl and the samples were incubated at 25°C for 25 min. The total incubation volume of 2 ml was then poured over glass filters (Whatman GF/C) and rinsed 3 times with 5 ml of ice-cold buffer (Tris-HCl, pH 7.4). Tritium was estimated by a conventional liquid scintillation counting. Non-specific binding of ^{3}H-prazosin or ^{3}H-DHA were defined in the presence of 1 μM phentolamine; or in the presence of 1 μM (−)alprenolol, respectively.

The specific bindings of ^{3}H-DHA and ^{3}H-prazosin were about 75 and 70%, respectively of the total binding; ^{3}H-DHA and ^{3}H-prazosin concentrations ranging from 0.1 nM to 2.0 nM and from 0.05 nM to 1.5 nM, respectively, were used for Scatchard plots. B_{max} and K_D values were calculated individually for each rat by Scatchard analysis, using 6 concentrations of ^{3}H-DHA or ^{3}H-prazosin and performing the assay in duplicate.

Behavioral Testing

Exploratory activity was investigated in the open field by a slightly modified method of Janssen *et al.* [4], using an open arena without walls. The center of the open field was illuminated with a 75 W bulb suspended directly above it. During all the experiments the laboratory room was dark. Individual control or drug-injected animals were placed gently in the center of the arena and were allowed to explore freely. Ambulation (the number of crossings of the diameters of the arena by an animal) and the total time of walking were recorded for 3 min. Saline or phenylephrine (25 μg/10 μl) were given into cerebral ventricles (ICV) 30 min before the beginning of the test.

The following drugs were used: CGP 6085 A (4-(5,6)-dimethyl-2-benzofuranyl/piperidine hydrochloride) (CIBA-GEIGY), DSP-4 (Astra), phenylephrine HCl (Sigma).

Statistical Analyses

The results were statistically analysed by Student's t-test (binding data) and according to the Dunnet's procedure, which was posterior to ANOVA (behavioral data).

RESULTS

Table 1 presents the effect of DSP-4 treatment on the density of β- and α_1-adrenergic receptor binding sites in the rat brain cortex and hippocampus. In the samples of the cortical tissue from DSP-4-treated animals the concentration of β- and α_1-adrenergic receptors was by about 32 and 17%, respectively higher in comparison with the membranes prepared from control rats. In the hippocampus of DSP-4-treated rats the density of both the β- and α_1-type of adrenoceptors was increased by about 26 and 35%, respectively.

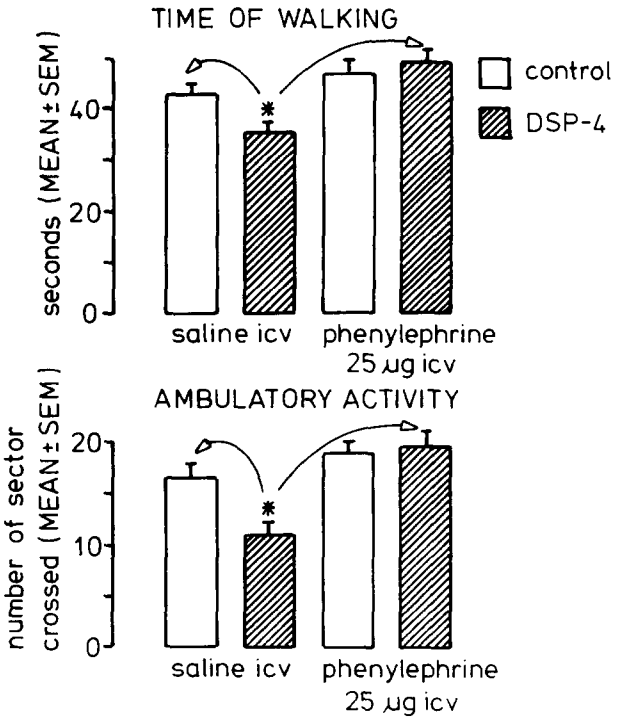

FIG. 1. Exploratory behavior of control and DSP-4 rats after administration of phenylephrine. All values were obtained during 3 min period of observation. Phenylephrine (25 µg ICV) was given 30 min before the beginning of the test. Open column: Control; striped column: DSP-4. Each group consisted of 8 rats. $^*p<0.05$. Significance according to Dunnet's procedure applied following ANOVA.

The effect of the α_1-agonist phenylephrine on the open field exploratory behavior of normal and DSP-4-treated rats is shown in Fig. 1. The exploratory activity (time of walking, ambulation) of DSP-4-treated rats was significantly reduced in comparison with normal controls. Phenylephrine, 25 µg ICV, did not affect the behavior of normal rats, but significantly stimulated the exploratory activity of DSP-4-treated animals.

DISCUSSION

The main findings of this study are: (1) the density of ^{3}H-prazosin and ^{3}H-DHA binding sites in the rat cerebral cortex and hippocampus was significantly higher in DSP-4-treated groups than in controls; (2) the behavioral response to α_1-agonist phenylephrine was found in DSP-4-treated rats.

As already mentioned elsewhere, the results concerning the α_1-adrenoceptor density in the central nervous system after NE-denervation are controversial. Some authors found a significant increase [11, 12, 15], while others observed no changes in the number of α_1-adrenoceptors after denervation with 6-OHDA [2,9].

After DSP-4-treatment Dooley *et al.* [2] found only slight (6%), non-significant increase in α_1-density in the rat neocortex. The hippocampal region of the ^{3}H-prazosin binding was not estimated.

In the present experiment the density of cortical ^{3}H-prazosin binding sites in DSP-4-treated rats was significantly higher—by about 17%—than in control animals. The NE-denervation also induced enhancement of the hippocampal ^{3}H-prazosin binding (by about 35%). In both structures, i.e., in the regions showing the greatest after DSP-4 depletion of NA [2, 6, 7], the number of β-adrenoceptors was also increased. The percent of changes in β-densities was similar (about 30%) to that reported by Dooley *et al.* [2].

Behavioral data show that the α_1-agonist phenylephrine used in a dose which did not affect the exploratory activity of control rats, stimulated that behavior in DSP-4-treated animals. Since the enhancement of exploration appears to be mediated by central α_1-adrenoceptor [1], it is likely that the increased behavioral response resulted from the changing sensitivity of α_1-adrenoceptors.

It is known that in DSP-4-treated rats there occurs supersensitivity of the β-adrenergic system, which consists of an increased cortical β-adrenoceptor density ([2]; present data), and a functional supersensitivity to the β-adrenergic agonist [3]. Recently it has been found that in DSP-4-treated rats also the α_1-adrenergic system is supersensitive. Therefore under conditions of the noradrenergic denervation there occur changes within both β- and α_1-adrenoceptor types, which follow the same direction. The increased β-adrenoceptor density is accompanied by an increase in the α_1-adrenoceptor concentration. However, β- and α_1-adrenoceptors do not always undergo similar changes; e.g., the α_1-up-regulation is observed after chronic treatment with some anti-depressants, yet it accompanies the β-down-regulation [13]. Obviously, receptor changes depend upon many different factors; therefore they may be diverse, dependent upon the pharmacological procedure used.

The results obtained here point to two substantial phenomena: (1) in response to the noradrenergic denervation the number of both types of receptors increases, which may confirm the results of Sutin and Minneman [11]; (2) facilitation of the responsiveness to α_1-stimulation in animals with an increased α_1-adrenoceptor density may testify to the relationship between the α_1-adrenoceptor density and functional response.

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