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The Effect of Chronic Imipramine and Electroconvulsive Shock Treatment on [^{3}H]DADLE Binding to Cortical Membranes of Rats Pretreated With Chronic Reserpine or 6-Hydroxydopamine

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ANTKIEWICZ-MICHALUK, L., J. MICHALUK, A. ROKOSZ-PELC, D. MARONA-LEWICKA AND J. VETULANI. *The effect of chronic imipramine and electroconvulsive shock treatment on [^{3}H]DADLE binding to cortical membranes of rats pretreated with chronic reserpine or 6-hydroxydopamine.* PHARMACOL BIOCHEM BEHAV **26**(2) 203–206, 1987.— Repetitive electroconvulsive shock treatment (for 8 days) or chronic administration of imipramine (10 mg/kg/day IP for 14 or 21 days) elevated the density of opioid δ receptors in the cerebral cortex of the rat. Electroconvulsive shock treatment produced a similar effect in rats treated subchronically with reserpine or receiving intraventricularly 6-hydroxydopamine, but these manipulations of central catecholamines prevented the action of imipramine.

Antidepressant treatment Imipramine Electroconvulsive shock Cortical opioid δ receptor Reserpine 6-Hydroxydopamine

STUDIES of the action of antidepressant drugs and electroconvulsive shock treatment on normal animals yielded important information indicating that the treatments applied chronically lead to changes in the responsiveness of several neurotransmitter systems, reflected often by changes in the parameters of receptor populations (see [9] for review). However, the effects of antidepressant treatments in normal animals may not be necessarily relevant to their clinical action in depression, as in normal human volunteers the psychological effects of antidepressant drugs are negligible and uncharacteristic [11]. Therefore, several attempts were undertaken to find out a suitable "animal model of depression."

Among "models" for depressions in animals, reserpinization and central chemosympathectomy with 6-hydroxydopamine (6-OHDA) were often used: reserpine is known to precipitate depression in humans [3] and to produce 'despair-like' behavior in monkeys [9], and behavioral changes resembling depression were produced by intraventricular 6-OHDA in free-ranging macaques [12]. Those treatments result in an enhancement of the response of the cyclic AMP generating system in the slices from the limbic forebrain, a response opposite to that induced by chronic administration of antidepressant agents and electroconvulsive shock treatment [19–21].

We have previously found that in reserpinized and centrally chemosympathectomized rats electroconvulsive shock treatment produces downregulation of central β-adrenoceptor as reflected by diminished response of the cyclic AMP generating system to norepinephrine [19,21]. Moreover, we have found that another receptor change, which is constantly observed in our experiments after chronic administration of antidepressants or electroconvulsive shock treatment, namely an increase in the density of α_1-adrenoceptors [14,15] is also produced not only in normal, but also in reserpinized and 6-OHDA-treated rats [10,16].

In this experiment we investigated if another receptor change produced by chronic electroconvulsive shock treatment, an increase in the density of opiate δ receptors [1], will be observed in rats with central noradrenergic transmission impaired by reserpinization or 6-OHDA. We also investigated the effect of chronic administration of imipramine in

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TABLE 1

THE EFFECT OF MULTIPLE ELECTROCONVULSIVE SHOCK TREATMENT (ECT) ON [^{3}H]DADLE BINDING TO CEREBRAL CORTICAL MEMBRANES OF RESERPINIZED AND CENTRALLY CHEMOSYMPATHECTOMIZED RATS

	N	B_{max} fmol/mg prot	%	K_D nmole/l	%
Control	24	116 ± 3	100	1.4 ± 0.05	100
ECT	5	164 ± 15	141†	1.9 ± 0.14	136†
Reserpine	5	132 ± 14	114	1.6 ± 0.14	114
ECT+Reserpine	4	152 ± 9	131†	1.5 ± 0.12	107
F(3/34)		9.72‡		5.29†	
Control	24	116 ± 3	100	1.4 ± 0.05	100
ECT	4	138 ± 9	119*	1.1 ± 0.11	79
6-OHDA	4	122 ± 5	105	1.2 ± 0.07	86
ECT+6-OHDA	4	133 ± 7	115	1.3 ± 0.07	93
F(3/32)		3.65*		2.60	

Electroconvulsive shock treatment was given 8 times, at 48 hr intervals. Reserpine, 4 mg/kg IP, was given always 24 hr before each electroconvulsive shock treatment. 6-OHDA, 250 μg, was given unilaterally into the lateral cerebral ventricle in a volume of 20 μl, 7 days before the first electroconvulsive shock treatment. The animals were killed 24 hr after the last electroconvulsive shock treatment.

‡$p<0.001$; †$p<0.01$; *$p<0.05$ (significance of the F value or of the difference from the control group, Duncan test).

these animals; our preliminary experiments have revealed that imipramine treatment also results in an increase in the density of opiate δ receptors [2].

METHOD

Animals and Treatment

The experiment was carried out on male Wistar rats weighing approximately 220 g. They were kept under standard laboratory conditions, ten to a large home cage, with free access to food and water, at ambient temperature approx. 23°C, and in natural day-light cycle (spring).

Electroconvulsive shock treatment consisted of 8 electroshocks given at 48 hr intervals produced by passing a current (sinusoidal, 150 mA, 50 cps, 500 msec) through ear-clip electrodes. This invariably produced convulsions lasting over 15 sec. Imipramine treatment consisted of daily intraperitoneal (IP) injections of 10 mg/kg imipramine (hydrochloride; Polfa) for 14 or 21 days. The electroconvulsive shock treatment controls were handled identically as the electroconvulsive shock treatment group, but without passing current through the electrodes, and imipramine controls were injected daily with 0.9% NaCl solution in a volume of 4 ml/kg IP.

In the electroconvulsive shock treatment group reserpine (Sigma), 4 mg/kg IP, was given always 24 hr before each electroconvulsive shock, and 6-hydroxydopamine (hydrochloride; Sigma; 6-OHDA), dissolved in ice-cold aqueous solution of 0.9% NaCl and 0.1% ascorbic acid, was administered unilaterally into the lateral cerebral ventricle through a direct puncture technique to non-anesthetized animals [18] in a dose of 250 μg and a volume of 20 μl, 7 days before the first electroconvulsive shock. The animals were decapitated 24 hr after the last electroconvulsive shock.

In the imipramine-treated group reserpine, 4 mg/kg, was given every 72 hr, beginning on the first day of imipramine injection, and 6-OHDA was given as in the electroconvulsive shock treatment group, 7 days before the first imipramine injection. The animals were decapitated 24 hr after the last dose of imipramine. The presence or absence of postdecapitation convulsions was noted: the absence of convulsions indicates a deep depletion of cerebral noradrenaline [4,13].

Membrane Preparation

The brain was rapidly removed, placed on an ice-cold porcelain plate, and the cerebral cortex was dissected and stored under solid carbon dioxide until processed further. The tissues from individual animals were processed separately. They were homogenized (Polytron disintegrator, setting 4, 10 sec) at 0°C in 20 vol. of 50 mmol/l Tris-HCl buffer, pH (at 30°C) 7.6, and the homogenate was centrifuged at 0°C and 25,000 g for 30 min. The pellet was reconstituted in the original volume of the buffer, incubated in a shaking water bath at 37°C for 45 min, and then recentrifuged at 0°C and 25,000 g for 30 min. After decanting of the supernatant the pellet was stored at −18°C for not longer than 48 hr. For the incubation it was reconstituted in the incubation buffer (50 mmol/l Tris-HCl buffer, pH (at 30°C) 7.6, containing 3 mmol/l $CaCl_2$) to a final protein concentration (measured according to Lowry *et al.* [7]) of approximately 3.0 mg/ml.

[^{3}H]DADLE Binding

The radioligand [^{3}H]-D-Ala^2, D-Leu^5-enkephalin (Amersham, spec. act. 39.5 Ci/mmol; [^{3}H]DADLE) was prepared in 8 concentrations (0.05–4 nmol/l) in the incubation buffer. The incubation mixture (final volume 550 μl consisted of 450 μl of membrane suspension, 50 μl of a [^{3}H]DADLE solution

TABLE 2

THE EFFECT OF MULTIPLE IMIPRAMINE INJECTION (IMI) ON [³H]DADLE BINDING TO CEREBRAL CORTICAL MEMBRANES OF RESERPINIZED AND CENTRALLY CHEMOSYMPATHECTOMIZED RATS

	N	B_{max} fmol/mg prot	%	K_D nmole/l	%
Control	6	81 ± 5	100	1.0 ± 0.06	100
IMI[a]	5	101 ± 3	125†	1.0 ± 0.05	100
Reserpine	5	92 ± 6	114	1.0 ± 0.07	100
IMI[a]+Reserpine	6	75 ± 4	93	1.0 ± 0.07	100
F(3/18)		6.13†		0	
Control	7	97 ± 4	100	1.5 ± 0.09	100
IMI[b]	13	128 ± 8	132*	1.5 ± 0.06	100
6-OHDA	6	121 ± 5	125	1.6 ± 0.09	107
IMI[b]+6-OHDA	5	103 ± 4	106	1.4 ± 0.09	93
F(3/27)		4.14*		0.76	

Imipramine was given in a dose of 10 mg/kg IP daily for 21 (IMI[a]) or 14 (IMI[b]) days. Reserpine, 4 mg/kg IP, was given every third day from the first day of imipramine treatment. 6-OHDA, 250 μg in 20 μl unilaterally into the lateral brain ventricle, was given 7 days before the first imipramine injection. The animals were killed 24 hr after the last dose of imipramine.

†$p<0.01$; *$p<0.05$ (significance of the F value or of the difference from the control group, Duncan test).

and 50 μl of the buffer without (total binding) or with (unspecific binding) naloxone (hydrochloride, Endo; final conc. 10 μmol/l).

The incubation was carried out in duplicate, in a shaking water bath, at 30°C for 30 min. Addition of the radioligand initiated the incubation which was terminated by vacuum-assisted filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 ml portions of ice-cold incubation buffer, placed in plastic scintillation minivials, covered with 3 ml of Bray's fluid, and counted for radioactivity in a Packard Model B 3255 scintillation counter at approximately 36% yield.

The specific binding was defined as the difference between total and unspecific binding. The Scatchard analysis, which yielded always a rectilinear plot, was used for assessment of B_{max} and K_D values. The between-group comparisons were carried out with the one-way ANOVA followed by the Duncan's test (using harmonic mean of N).

RESULTS

Both reserpinized and centrally chemosympathectomized rats displayed no postdecapitation convulsions, regardless of electroconvulsive shock treatment or imipramine treatment. No apparent differences in the convulsions between saline, imipramine or electroconvulsive shock treated animals were noted. In the first series of experiments neither chronic administration of reserpine, nor central chemosympathectomy affected the density of [³H]DADLE binding sites. Electroconvulsive shock treatment in the control rats produced a significant increase (by 20–40%) of the density of [³H]DADLE binding sites and it produced a similar effect in the rats treated with reserpine. The increase in the density of [³H]DADLE binding sites in chemosympathectomized rats did not reach the level of $p<0.05$ (Table 1).

In the second series of experiments imipramine treatment caused an increase in the [³H]DADLE binding sites density in rats with the central monoaminergic systems intact, but not in either reserpinized or centrally chemosympathectomized rats (Table 2).

DISCUSSION

This experiment confirms our previous findings [1] and those of Holaday *et al.* [6] that in intact rats the electroconvulsive shock treatment increases the density of [³H]DADLE binding sites in the rat cerebral cortex. We have also observed a statistically significant elevation of the K_D value for [³H]DADLE binding, but the physiological meaning of this change is difficult to assess and rather doubtful. Our results demonstrate also that chronic imipramine treatment, which was previously found to increase the density of μ opiate receptors [2], is also able to increase the density of [³H]DADLE binding sites, representing opiate δ receptors.

The treatment with reserpine and 6-hydroxydopamine resulted in a complete inhibition of postdecapitation convulsions. We have demonstrated earlier that this phenomenon reflects the functional state of the central noradrenergic system employing α_1-adrenoceptors [17]. Neither electroconvulsive shock, nor imipramine treatment, administered in a manner producing definite receptor changes in the adrenergic system [14, 15, 19, 21] did restore the activity of the central α_1-adrenergic system to the degree enabling the appearance of decapitation convulsions, and therefore it might be assumed that the pretreated animals had the central noradrenergic transmission impaired. However, the electroconvulsive shock treatment in reserpinized rats still re-

tains its ability to elevate the density of δ opiate receptors. A similar tendency was observed in centrally chemosympathectomized rats, but, as the results were slightly below the generally accepted level of statistical significance ($0.1>p>0.05$), further studies are needed to find out if the null hypothesis can be rejected. As it has been found previously that the electroconvulsive shock treatment-induced increase in the density of α_1-adrenoceptor may be observed not only in normal, but also in reserpinized rats [10], it might be suggested that presynaptic catecholaminergic system is not necessary for the induction of receptor changes by electroconvulsive shock treatment.

In contrast, the imipramine effect of opioid δ receptors is inhibited both after reserpinization and chemosympathectomy. This suggests that this action of imipramine requires the integrity of catecholaminergic transmission. In this respect the effects of antidepressant treatments on opioid receptors are similar to those on α_1-adrenoceptors [16]. It might be speculated that the effectiveness of electroconvulsive therapy in some cases of drug-resistant depression may be related to its independence of the state of presynaptic catecholaminergic terminals.

The role of the opioid system in mood regulation is still a matter of dispute, but several observations, among them the use of "opioid cure" in depression, suggest its importance (see [5] for discussion). The present data confirm that antidepressant treatments may affect the δ-opioid receptor population, and that the effectiveness of imipramine, but not of electroconvulsive shock treatment, depends on the integrity of presynaptic catecholaminergic mechanisms. However, further studies are required to find out the relation of this biochemical change to possible therapeutic action of imipramine or electroconvulsive shock treatment in depression.

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