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Avoidance learning during antidepressant withdrawal in mice

MARIO SANSONE, MARIO BATTAGLIA, JONAS EKBLOM*, IRENA NALEPA**, JERZY VETULANI**, *Istituto di Psicobiologia e Psicofarmacologia, CNR, Roma, Italy, *Department of Biochemistry, University of Stockholm, Sweden and **Institute of Pharmacology, PAN, Krakow, Poland*

Abstract—Shuttle-box avoidance acquisition, locomotor activity and density of adrenoreceptors in the cerebral cortex have been evaluated, in CD-1 mice, during withdrawal from repeated treatment with desipramine or mianserin (5 or 14 daily injections of antidepressant drug, 10 mg kg^{-1}). Withdrawal from mianserin did not produce any behavioural or neurochemical change. Mice withdrawn from desipramine exhibited avoidance facilitation, when training started 24 h (but not 72 or 120 h) after the last injection. Locomotor activity was not affected and no change was found in the density of β-adrenoreceptors. An up-regulation of α_2- and, to a lesser extent, of α_1-adrenoreceptors, occurred 72 h following desipramine withdrawal. However, the assessment of the role played by these neurochemical changes in the avoidance facilitation observed during withdrawal from the antidepressant treatment requires further study.

A variety of behavioural tests has been employed to investigate the effects of acute and chronic administration of antidepressant drugs (File & Tucker 1986; Tucker & File 1986), while behavioural consequences of withdrawal from antidepressant treatment have mainly been examined by measuring locomotor activity, a test which indicates absence of behavioural changes or withdrawal hyperactivity (see Tucker & File 1986). However, other behavioural tests may reveal antidepressant withdrawal effects: rats withdrawn from chronic treatment with desipramine showed enhanced amphetamine anorexia (Willner & Montgomery 1981) and increased resistance to extinction of food rewarded performance (Willner et al 1981). Moreover, our preliminary findings (Sansone et al 1990) indicate that mice subjected to shuttle-box training, during withdrawal from desipramine, may exhibit higher levels of avoidance performance in comparison with controls.

In the present study a closer examination of the avoidance learning, following withdrawal from repeated antidepressant treatment, was carried out by testing desipramine and mianserin, typical and atypical antidepressants, with different pharmacological profiles (see File & Tucker 1986). Withdrawn mice were also tested for spontaneous locomotor activity.

Biochemical experiments were also carried out to evaluate the density of adrenoreceptors in the cerebral cortex of mice during antidepressant withdrawal, since previous studies have demonstrated that repeated administration of antidepressant drugs in rats produces complex changes in densities (though not affinities) of various subclasses of adrenoreceptors (Campbell & McKernan 1982; Vetulani et al 1984; Vetulani & Antkiewicz-Michaluk 1985) and increased responsiveness of postsynaptic α_1-adrenoreceptors (Maj 1984).

Materials and methods

Animals. Behavioural experiments were carried out on naive male mice, 28–33 g, of the randomly bred CD-1 strain (Charles River, Calco-Como, Italy). Upon their arrival in the laboratory (7–10 days before the experiment) the mice were housed in standard transparent plastic cages (8 per cage) under standard animal room conditions (free access to food and water, 12 h light/dark cycle, ambient temperature of 23°C). The experiments were carried out between 0900–1400 h, using different animals for different tests.

Biochemical experiments were carried out on male CD-1 mice obtained from Charles River, Sulzfeld, Germany.

Drug treatment. Treatment consisted of 5 or 14 daily injections of saline solution (0·9% NaCl), desipramine hydrochloride (Ciba-Geigy; 10 mg kg^{-1}) or mianserin hydrochloride (Organon; 10 mg kg^{-1}). All injections were made i.p., in a volume of 10 mL kg^{-1}.

Active avoidance. The apparatus consisted of 8 automated shuttle-boxes, each divided into two 20 × 10 cm compartments, connected by a 3 × 3 cm opening. A light (10 W) was switched on alternately in the two compartments and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s and overlapped it for 25 s. The US was an electric shock (0·2 mA) applied continuously to the grid floor. The intertrial interval was 30 s. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 5 s after the onset of the CS. If animals failed to avoid the shock they could escape it by crossing during the US. Spontaneous crossings from the dark to the light compartment were punished and recorded as intertrial responses.

The mice were subjected to 5 daily 100-trial training sessions, starting 24 h after the last injection of saline, desipramine or mianserin, given for 5 or 14 days. Each experimental group included 16 subjects.

In additional experiments the trials started 72 or 120 h after the last injection.

Locomotor activity. Spontaneous locomotor activity was measured using the same apparatus employed to measure active avoidance. For this purpose the lamps of the shuttle-boxes were switched off and no electric shock was applied to the floor. For each mouse, the number of crossings from one compartment to the other was recorded for 60 min.

Mice were subjected to the activity test 24, 72 or 120 h after the last of 14 daily injections of saline, desipramine or mianserin. Each group comprised 8 animals.

Biochemistry. Mice were killed by cervical dislocation and decapitation 72 h after the last of 14 daily injections. The cerebral cortex was prepared on an ice-chilled glass plate. The membrane preparation (crude synaptosomal fraction) was prepared as previously described (Vetulani et al 1984) and the membrane pellets were stored at −18°C for no more than 48 h. Binding assays were carried out using a single concentration of an appropriate radioligand, approximating the previously determined K_d value. The radioligands used were: [^{3}H]prazosin (NEN, 24·4 Ci $mmol^{-1}$, 0·1 nmol L^{-1}) for α-adrenoreceptors, [^{3}H]clonidine (NEN, 25·5 Ci $mmol^{-1}$, 0·5 nmol L^{-1}) for α_2-adrenoreceptors, [^{3}H]dihydroalprenolol (Amersham, 73·9 Ci $mmol^{-1}$, 1 nmol L^{-1}) for β-adrenoreceptors. Prazosin, clonidine and propranolol at concentrations of 10 μmol L^{-1} served as appropriate displacers. Incubations were carried out as previously described (Vetulani et al 1984). Results are given as

Correspondence to: M. Sansone, Istituto di Psicobiologia e Psicofarmacologia, via Reno 1, 00198 Roma, Italy.

radioligand binding in fmol (mg protein)$^{-1}$. Protein was assayed according to Lowry et al (1951).

Results

Active avoidance. Fig. 1 reports the mean percent avoidance responses for each daily shuttle-box session and for each experimental group. Escape and intertrial responses are not reported, since escape failure never occurred, while intertrial responses (spontaneous crossings from the dark to the light compartment), which were punished by electric shock, were always at low levels.

When the training started 24 h after the fifth injection of an antidepressant, in mice treated with desipramine, but not mianserin, avoidance acquisition was improved to some degree (Fig. 1A). The two-way ANOVA showed a significant main effect of training ($F(4,120)=43{\cdot}13$, $P<0{\cdot}001$), in mianserin-treated animals, but not of treatment ($F(1,30)=1{\cdot}24$, $P>0{\cdot}05$) and no significant treatment × sessions interaction ($F(4,120)=0{\cdot}65$, $P>0{\cdot}05$). Desipramine had no significant effect during the whole of the five training sessions ($F(1,30)=3{\cdot}84$, $P>0{\cdot}05$), but there was a significant effect of training ($F(4,120)=59{\cdot}37$, $P<0{\cdot}001$) and a significant treatment × session interaction ($F(4,120)=4{\cdot}96$, $P<0{\cdot}01$) allowing a further analysis with Duncan's test, which demonstrated a significant increment in avoidance responses in the fifth session.

Even after a more prolonged antidepressant treatment mice withdrawn from mianserin did not show any facilitation in acquisition of conditioned avoidance response, while desipramine-treated mice performed better than controls (Fig. 1B). In the mianserin group, a two-factor analysis of variance showed a significant effect of training ($F(4,120)=23{\cdot}78$, $P<0{\cdot}001$), but not of treatment ($F(1,30)=0{\cdot}44$, $P>0{\cdot}05$), and no significant treatment × sessions interaction ($F(4,120)=1{\cdot}04$, $P>0{\cdot}05$). In the desipramine group, a two-factor ANOVA (treatment × sessions) showed significant treatment ($F(1,30)=5{\cdot}58$, $P<0{\cdot}05$) and training ($F(4,120)=26{\cdot}55$, $P<0{\cdot}001$) effects and a significant two-factor interaction ($F(4,120)=4{\cdot}89$, $P<0{\cdot}01$): a further analysis for between-group comparisons (Duncan's test) indicated that the number of avoidance responses was significantly higher, starting from the second training session, in the drug withdrawn group.

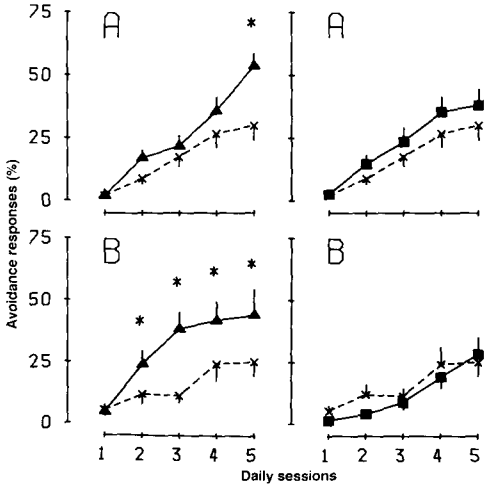

FIG. 1. Shuttle-box avoidance acquisition in mice withdrawn from a treatment consisting of 5 (A) or 14 (B) daily injections of saline solution (×- - -×), desipramine hydrochloride 10 mg kg^{-1} (▲——▲) or mianserin hydrochloride 10 mg kg^{-1} (■——■). Training started 24 h after the last injection and consisted of five 100-trial daily sessions. Mean percent avoidance responses in groups of 16 animals. Vertical lines indicate s.e.m. Asterisks denote a significant difference ($P<0{\cdot}05$; Duncan's test) between antidepressant and saline withdrawn groups.

In the experiments in which the training began 72 or 120 h after the last injection of either antidepressant no difference from controls was observed (results not shown).

Locomotor activity. Withdrawal from antidepressant treatment did not affect spontaneous locomotor activity. A two-factor ANOVA, concerning the activity crossings exhibited during 60 min by mice treated with saline or desipamine (Table 1), showed no significant effect of the treatment ($F(1,42)=0{\cdot}20$, $P>0{\cdot}05$) or of the interval (24, 72 or 120 h) between the last injection and the activity test ($F(2,42)=0{\cdot}27$, $P>0{\cdot}05$) and no significant two-factor interaction ($F(2,42)=2{\cdot}68$, $P>0{\cdot}05$). Similarly, no significant effect was observed during withdrawal from mianserin.

Biochemistry. In animals withdrawn from desipramine, the specific binding to α_2-adrenoreceptors was significantly augmented (by 60%, $P<0{\cdot}05$). A similar increase (by 58%), but not reaching the level of statistical significance ($P<0{\cdot}1$), was observed for α_1-adrenoreceptors. No significant change concerning binding to β-adrenoreceptors was observed. Withdrawal from mianserin resulted in no significant change in binding to adrenoreceptors (Table 2).

Table 1. Locomotor activity during withdrawal from antidepressant treatment.

Hours after the last injection	Treatment		
	Saline	Desipramine	Mianserin
24	131·75 ± 4·69	105·25 ± 11·16	128·75 ± 12·72
72	117·25 ± 5·40	116·00 ± 9·34	113·50 ± 10·56
120	114·75 ± 12·30	132·00 ± 11·10	109·75 ± 14·14

Mean ± s.e.m. activity crossings, during 60 min, in groups of 8 mice. The animals received 14 daily injections i.p. of saline solution, desipramine hydrochloride 10 mg kg^{-1} or mianserin hydrochloride 10 mg kg^{-1} and were tested for locomotor activity 24, 72 or 120 h after the last injection.

Table 2. Specific binding of adrenoreceptor ligands to cerebral cortical membranes of CD-1 mice during withdrawal from antidepressant treatment.

Radioligands (concn)	Treatment		
	Saline	Desipramine	Mianserin
[^{3}H]Prazosin (0·1 nM)	4·0 ± 0·3 (8)	6·3 ± 1·3† (6)	4·1 ± 0·4 (6)
[^{3}H]Clonidine (0·5 nM)	12·6 ± 0·7 (6)	20·3 ± 1·9* (6)	17·2 ± 1·3 (6)
[^{3}H]Dihydro-alprenolol (1·0 nM)	4·4 ± 0·7 (4)	5·6 ± 1·0 (5)	2·8 ± 0·6 (6)

Means ± s.e.m. (n) of specific binding in fmol (μg protein)$^{-1}$. The mice were killed 72 h after the last injection. Desipramine and mianserin were given in a dose of 10 mg kg^{-1} i.p. once daily for 14 days. The controls received saline solution, 10 mL kg^{-1} i.p., according to the same schedule. † $P<0{\cdot}10$; * $P<0{\cdot}05$ (Dunnet's test).

Discussion

In the present study, mice receiving 5 or 14 daily injections of desipramine (10 mg kg^{-1}) performed better than controls, when subjected to 5 daily shuttle-box avoidance sessions, starting 24 h after the last injection. The avoidance facilitating effect was apparently a short-lasting one, as it disappeared when shuttle-box training began 72 or 120 h after the last injection of the antidepressant drug. No avoidance change occurred during withdrawal from treatment with the atypical antidepressant mianserin. Neither desipramine nor mianserin affected spontaneous locomotor activity measured 24, 72 or 120 h after the last of 14 daily injections. Thus, it seems that the avoidance facilitation induced by desipramine withdrawal cannot be ascribed to increased general activity.

In agreement with previous findings obtained with Swiss mice (Vetulani & Antkiewicz-Michaluk 1985), the present results indicate that in CD-1 mice, chronic treatment with antidepressants does not produce down-regulation of β-adrenoreceptors. These results also suggest that in contrast with the rat, in which a prolonged administration of antidepressants produces down-regulation of α_2-adrenoreceptors (Vetulani et al 1984; Vetulani & Antkiewicz-Michaluk 1985), an opposite effect might be expected in the mouse. In fact, in mice withdrawn from desipramine for 72 h, the density of α_2- and probably also of α_1-adrenoreceptors was increased. It might be thus supposed that the animals withdrawn from desipramine and trained from the first day after withdrawal had elevated α_2- possibly also α_1-adrenoreceptor density during the first half of the training period. Avoidance facilitation was not observed in mice withdrawn from mianserin, in which no receptor change was detected.

Acute administration of antidepressants produces sedative effects in rodents and several findings indicate that tolerance either develops only slowly or not at all to the depressant effects of these drugs (Tucker & File 1986). An inhibitory action of antidepressants can also be observed in active avoidance tasks, with impairment of avoidance acquisition produced by acute treatment (Herr et al 1961; Sansone 1978; Telegdy et al 1983; Lucki & Nobler 1985). In a previous study (Hano et al 1981), desipramine and mianserin, given daily (10 mg kg^{-1}) during training without pretreatment or after a short pretreatment (1 week), impaired shuttle-box avoidance learning in mice, but the learning of mice receiving a longer pretreatment (4 weeks) remained unimpaired or was even facilitated, in the case of desipramine. This last finding indicates that tolerance to the inhibitory action of desipramine on avoidance behaviour may develop during chronic treatment and that facilitating effects of the drug may be unmasked. A short persistence of a stimulatory effect, accompanied by attenuation or disappearance of the inhibitory action, might also explain the present results, showing avoidance improvements when training began 24, but not 72 or 120 h, after withdrawal from desipramine. It has been previously suggested that an effect which develops during chronic treatment with desipramine may be masked by the presence of the drug and unmasked during withdrawal (Willner & Montgomery 1981).

The temporary coincidence of the avoidance facilitation with changes in α-adrenoreceptor density, appearing during withdrawal from desipramine, suggests that the increase in density of these receptors may be related to the observed behavioural effect. However, since the neurochemical changes observed after chronic antidepressant treatment are not confined to the α-adrenergic system and because various neurotransmitter systems may be involved in avoidance learning, the conclusions about the role of α-adrenoreceptors in the observed phenomena should be drawn with caution.

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