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The Effect of Psychoactive Drugs on Plasma Corticosterone Levels and Behaviour in the Bulbectomised Rat[1]

K. D. CAIRNCROSS,* A. WREN, C. FORSTER, B. COX AND H. SCHNIEDEN

Department of Pharmacology, Materia Medica and Therapeutics
University of Manchester, Manchester M13 9PT, England and School of Biological Sciences*
Macquarie University, North Ryde, N.S.W. 2113, Australia

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CAIRNCROSS, K. D., A. WREN, C. FORSTER, B. COX AND H. SCHNIEDEN. *The effect of psychoactive drugs on plasma corticosterone levels and behaviour in the bulbectomised rat.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 355–359, 1979.—Bilateral olfactory bulbectomy (OB) in the rat produces a rise in circulating 11-hydroxycorticosterone (11-OHCS) to intermediate levels (40–50 μg/100 ml plasma). Following footshock extreme corticosterone elevation occurs (65–80 μg/100 ml plasma). Bulbectomy also produces behavioural changes which include hyper-reactivity and an acquisition deficit in a step-down passive avoidance test. Treatment of the bulbectomised rat with amitriptyline (5 and 10 mg/kg), mianserin (5 and 10 mg/kg) and viloxazine (2 and 5 mg/kg) administered IP for at least 7 days corrected the acquisition deficit, reduced the hyper-reactivity and the elevated corticosterone levels in a reproducible manner. This reduction in 11-OHCS concentrations occurred in bulbectomised rats with and without footshock. In contrast, the antidepressant drugs did not produce these changes in sham-operated controls (SO). The central stimulant, amphetamine (1 and 3 mg/kg/day for 7 days, IP), increased 11-OHCS concentrations in unstressed OB and SO rats. There was no further elevation in the 11-OHCS concentrations of stressed rats of both OB and SO groups. This drug further impaired the acquisition of both OB and SO rats and increased the reactivity scoring of both groups. The major tranquillizer, chlorpromazine (1 and 3 mg/kg IP for 7 days), reduced plasma 11-OHCS levels and the hyperreactivity of both OB and SO groups. It did not reduce the acquisition deficit exhibited by the OB rats. Chlordiazepoxide (5 and 15 mg/kg IP for 7 days), had a profile similar to that of chlorpromazine except that it impaired acquisition in the SO group. Thus using the techniques described above it is possible to separate the antidepressants from other major classes of psychotropic drugs.

Bulbectomy Corticosterone Passive avoidance Antidepressants Tranquillizers Central stimulants

IT HAS been established that in addition to the changes in behaviour which accompany bilateral olfactory bulbectomy (OB), [17], there occurs an elevation in plasma 11-hydroxycorticosterone (11-OHCS) concentration [7]. Two levels of 11-OHCS elevation were noted: an intermediate level (40–50 μg/100 ml plasma) in unstressed OB rats and in stressed sham operated (SO) rats and an extreme level (65–80 μg/100 ml plasma) in stressed OB rats. These same two levels were observed when unoperated rats were exposed to a variety of experimental parameters, including novelty, foot-shock predictability and response contingency [2].

The fact that 11-OHCS elevation occurred after bulbectomy suggested that this physiological parameter could be included to increase the number of tests used to evaluate the OB syndrome [7]. Further, since it was demonstrated that the tricyclic antidepressant drug, amitriptyline reversed the learning deficit observed after bulbectomy [6] it was decided to determine if this drug would reduce the changes in 11-OHCS concentrations associated with bulbectomy. The structurally dissimilar, but clinically effective antidepressant drugs viloxazine and mianserin have been shown to reduce the behaviour deficit after bulbectomy [4,13]. Therefore it was decided to include these drugs in the study, to further test the possible use of bulbectomy as a potential screen for antidepressant drugs [3,17].

The OB rat would only be an effective model if other classes of psychotropic drug, (the major tranquillizers, the anti-anxiety agents and the central stimulants) had different effects on the behavioural syndrome, and perhaps on the 11-OHCS elevation. Accordingly examples of these drugs were also included in the study.

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METHOD

Male Sprague-Dawley rats (Manchester strain, 200–250 g at the start of the experiment) were used. The animals were housed in groups of 5, under conditions of constant temperature and humidity (21 ± 0.5°C, 45% humidity). The animals were subjected to a 14 hr light, 10 hr dark cycle, the light period running from 6.00 to 20.00 hr. Food and water were provided ad lib. The animals were handled daily during the second week of a 14 day postoperative recovery period.

Surgical Procedure

Rats were anaesthetised with Equithesin (0.33 ml/100 g body weight, IP). The head was shaved and placed in a stereotaxic frame and the skull exposed by a mid-line incision. Two holes were drilled, 1–2 mm on either side of the mid-line at a point 5 mm anterior to bregma. The olfactory bulbs were sectioned and removed by aspiration. The holes were plugged with gel foam (Spongostan) and the wound sutured. SO rats underwent the same surgical procedure, but the sectioning and aspiration of the bulbs was omitted. Bulbectomy was confirmed as described previously [5,13].

Footshock

Rats were placed in a clear perspex box 55 cm^2 with a stainless steel grid floor set at 1.5 cm centres. Footshock was delivered through a grid floor (as a 1.0 mA, 50 pulses/sec square wave) for 5 sec every 55 sec over a 30 min period. This procedure ensured that corticosterone elevation was maximal [2].

Passive Avoidance Testing

The apparatus was the same as that used for footshock except that a wooden platform 19 cm square and 4 cm above the grid was placed in the centre of the floor. The paws of the rat were dampened and it was placed on the central wooden platform. The latency time for the rat to step off the platform and on to the grid with all four paws was measured. When this occurred the rat was immediately removed and the procedure repeated with an intertrial interval of 30 sec until the rat remained on the platform for one minute. The rat was then considered to have acquired the appropriate avoidance response and the number of trials needed by each rat to reach this criterion was recorded.

Reactivity Testing

The reactivity of a rat was scored by means of an arbitrary numerical system similar to that of King [10] and Nurimoto, Ogawa and Ueki [12] (Table 1). The stimuli used were: (a) a puff of air blown sharply onto the back of the rat when it was facing away from the experimenter, immediately followed by (b) a loud click delivered close to and in front of the rat's nose.

TABLE 1

SCORING SYSTEM* USED IN REACTIVITY TESTING

Score	Response
0	No response
1	Slight - rat raises head and moves away from stimulus.
2	Moderate - rat jumps and 'freezes' for 0–2 sec.
3	Marked - rat jumps and 'freezes' for 3–15 sec.
4	Extreme - rat jumps and 'freezes' for 15 sec.

The maximum score attainable for two stimuli was 8.

*Similar to that of King [10].

11-Hydroxycorticosterone (11-OHCS) Assay

Blood samples for assay of 11-OHCS were taken in the late morning or around noon immediately following behavioural testing, which was always carried out between 10.00 and 12.00 hr. Two groups of rats were used. In one group the rats were placed in the perspex box used in the passive avoidance test after removal of the wooden platform. The rats were subjected to 5 sec of footshock every min for 30 min. Immediately following this procedure, the rats were sacrificed by cervical dislocation and exsanguinated. The footshock procedure was omitted for the second group of rats who were sacrificed immediately following step-down avoidance testing. Blood samples were collected in heparinised tubes and centrifuged at 1000 × g for 30 min to obtain cell-free plasma which was stored at −20°C. Plasma 11-OHCS was determined by the fluorimetric method of Mattingly [11] which is specific for free 11-hydroxycorticoids.

Drug Treatment

Drug or saline injections were given IP to both OB and SO rats daily from the fifteenth post-operative day, for a period of 10 days. On the 8th day of treatment, both OB and SO rats were tested for irritability, on Day 9 for passive avoidance. In those animals which received footshock (Day 10), treatment was administered one hour prior to testing. Animals not receiving foot-shock were sacrificed on Day 9 immediately after passive avoidance testing, which was one hour after drug or saline treatment. The drugs used were amitriptyline (5 and 10 mg/kg), mianserin (5 and 10 mg/kg), viloxazine (2 and 5 mg/kg), amphetamine (1 and 3 mg/kg), chlorpromazine (1 and 3 mg/kg) or chlordiazepoxide (5 and 15 mg/kg).

Each drug or saline was administered to 10 OB animals and to 10 SO animals. All drugs were tolerated uneventfully by both OB and SO groups, apart from OB amphetamine at the 3 mg/kg dose. Three animals from this group had to be destroyed prior to Day 9 of treatment due to intraspecies aggression. Thus n=10 for all groups apart from OB, 3 mg/kg amphetamine where n=7.

RESULTS

Passive Avoidance

The results of this test are illustrated in Fig. 1. OB rats required a significantly greater number of trials to acquire the passive avoidance response than SO controls. The performance of OB rats treated with the antidepressant drugs amitriptyline, mianserin and viloxazine was significantly im-

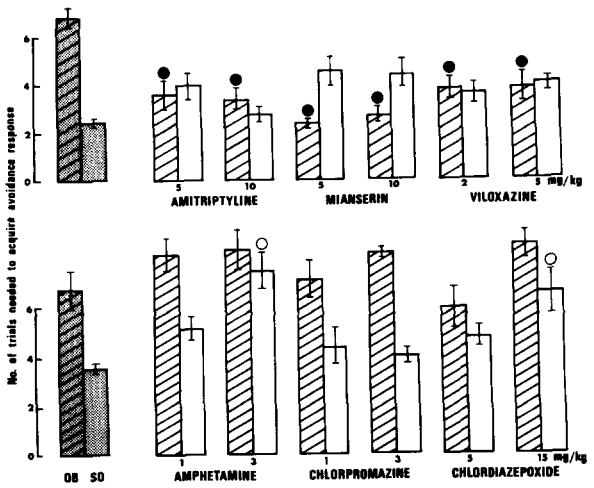

FIG. 1. The effect of chronic drug treatment on the performance of olfactory bulbectomised (hatched columns) and sham-operated (non-hatched columns) rats in a step-down passive avoidance test. ● represents significant difference from saline-treated bulbectomised group and ○ represents significant difference from saline-treated sham-operated group ($p<0.05$).

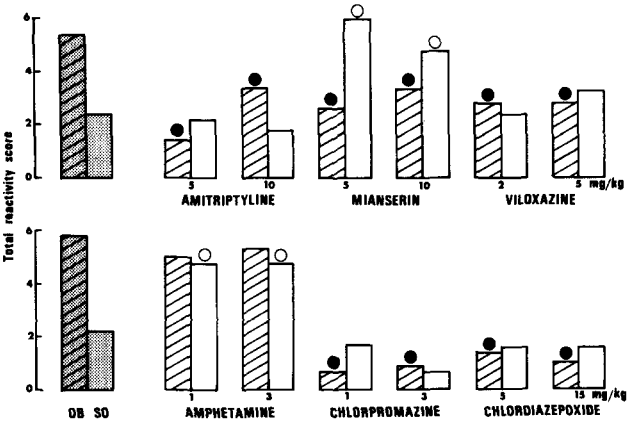

FIG. 2. The effect of chronic drug treatment on the mean reactivity score of bulbectomised (hatched columns) and sham-operated (non-hatched columns) rats. ● represents a significant difference from saline-treated bulbectomised group and ○ represents significant difference from saline-treated sham-operated group ($p<0.05$, Mann Whitney U test).

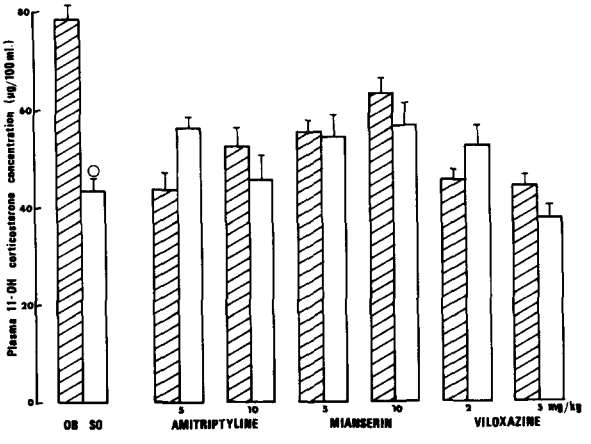

FIG. 3. The effect of chronic antidepressant treatment on mean plasma 11-hydroxycorticosterone concentration (± s.e.) of bulbectomised (first hatched column of each pair) and sham-operated (second column of each pair) rats receiving footshock. ○ represents significant difference between saline injected OB and SO rats ($p<0.01$).

proved. These drugs had no significant effect on the acquisition of the response by SO rats, although mianserin appeared to increase the number of trials required.

In comparison to the antidepressants, the central stimulant amphetamine produced an increase in the number of trials needed to acquire the avoidance response in both the OB and SO groups. The major tranquillizer, chlorpromazine, brought about an impairment in the acquisition of OB rats which became significant ($p<0.05$, students t-test, two-tailed) at the higher dose. It had no effect on SO groups. The anxiolytic, chlordiazepoxide, had a similar effect to chlorpromazine on OB rats but at 15 mg/kg it caused a significant ($p<0.05$) impairment in the acquisition of SO rats.

Reactivity

The results of this test are shown in Fig. 2. OB rats had a significantly ($p<0.05$, Mann Whitney U test) higher total reactivity score than SO controls. The three antidepressant drugs significantly reduced the reactivity of OB rats and, with the exception of mianserin, had no effects on SO rats. Amphetamine had no significant effect on OB rats but increased the reactivity of SO groups. Chlorpromazine and chlordiazepoxide reduced reactivity in all groups. This reached significance ($p<0.01$) for OB groups only.

Plasma 11-Hydroxycorticosterone Concentrations 11-OHCS

Saline treated OB rats had a plasma 11-OHCS level of 39.1 ± 1.03 (Fig. 6) or 42.9 ± 3.01 (Fig. 4) which increased by the footshock procedure to 79.3 ± 3.04 (Fig. 3) or 76.4 ± 4.46 (Fig. 5) respectively. These observations confirmed the concept of intermediate and extreme 11-OHCS elevation discussed previously [2,7]. All doses of the antidepressants significantly ($p<0.01$, students t-tests, two-tailed) decreased the 11-OHCS plasma level in footshocked OB rats (Fig. 3). Similarly, all doses except 5 mg/kg viloxazine significantly decreased the 11-OHCS levels in non-footshocked OB rats (Fig. 4). None of these drug treatments significantly altered 11-OHCS levels in the SO groups.

The effects of other psychotropic drugs on plasma (11-OHCS) are shown in Figs. 5 and 6. Both doses of chlorpromazine and chlordiazepoxide significantly ($p<0.01$) reduced 11-OHCS in footshocked and non-footshocked OB and SO rats. At 1 mg/kg amphetamine significantly ($p<0.05$) decreased 11-OHCS in footshocked OB rats. At 3 mg/kg, it significantly ($p<0.01$) increased 11-OHCS in footshocked and non-footshocked SO rats and in non-footshocked OB rats.

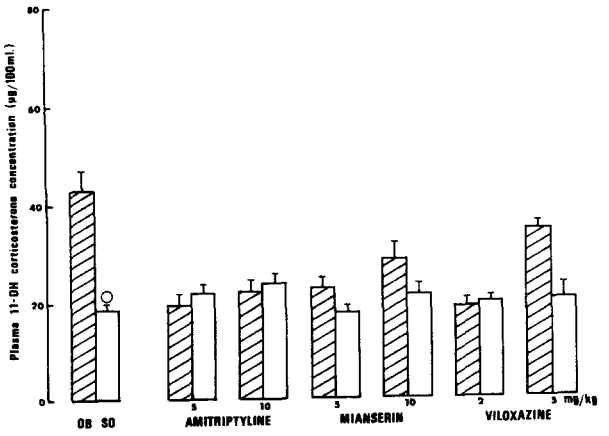

FIG. 4. The effect of chronic antidepressant treatment on mean plasma 11-hydroxycorticosterone concentration (± s.e.) of bulbectomised (first hatched column of each pair) and sham-operated (second column of each pair) rats receiving no footshock. ○ represents significant difference between saline injected OB and SO rats ($p<0.01$).

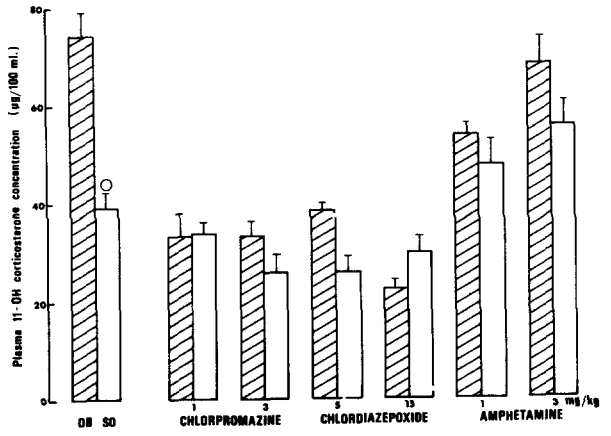

FIG. 5. The effect of chronic psychotropic drug treatment on mean plasma 11-hydroxycorticosterone concentration (± s.e.) of bulbectomised (first hatched column of each pair) and sham-operated (second column of each pair) rats receiving footshock. ○ represents significant difference between saline-treated OB and SO rats.

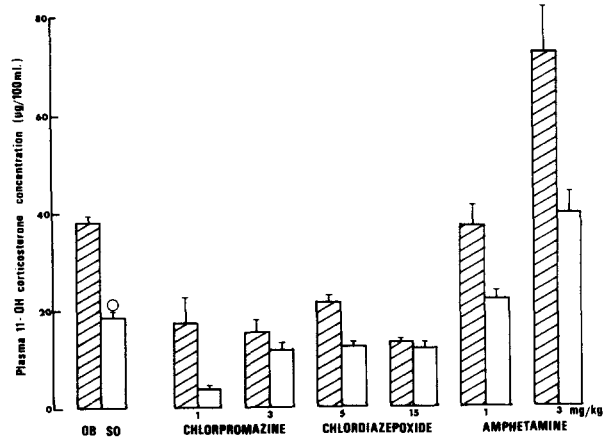

FIG. 6. The effect of chronic psychotropic drug treatment on mean plasma 11-hydroxycorticosterone concentration (± s.e.) of bulbectomised (first hatched column of each pair) and sham-operated (second column of each pair) rats receiving no footshock. ○ represents significant difference between saline-treated OB and SO rats.

DISCUSSION

The results obtained suggest that the increase in plasma 11-OHCS which occurs after bilateral olfactory bulbectomy can be used, together with behavioural testing, to predict antidepressant efficacy. The results indicate further, that it is not necessary to subject the animals to the stress of footshock to obtain such a predictive differentiation. Indeed, comparison of the results obtained in the footshocked and the non-footshocked situation, suggest that the extreme steroid elevation obtained after footshock can mask the effects of drugs like amphetamine, which themselves increase 11-OHCS concentration. It would appear, as previously suggested, that olfactory bulbectomy acts as a form of stressor itself, and that the superimposition of a second stressor, such as footshock, which produces extreme steroid elevation [2], is not further enhanced by a stimulant drug. This would indicate that the 11-OHCS of 80–90 μ/100 ml blood plasma, described by Bassett and Cairncross [1], as 'extreme' is in fact a physiological maximum. Exposure of SO groups to footshock produces an intermediate elevation of 11-OHCS, which can mask a pharmacological action. This is illustrated by the effect of the low dose of chlorpromazine on steroid elevation in the nonfootshocked SO group, when compared with the same response in the footshocked SO group. The conclusion must be therefore, that it is not necessary to subject the OB rats to further stress in the form of footshock, because bulbectomy per se creates an experimental situation which adequately differentiates between classes of psychotropic drugs when combined with the other experimental parameters. Thus, the antidepressants tested 'normalize' the behaviour and lower the 11-OHCS in OB rats without significantly affecting SO rats. The only exception being mianserin in the reactivity test, which caused an increase in reactivity. This response might reflect a mild stimulant action of mianserin. Neither amphetamine, chlorpromazine nor chlordiazepoxide produce the pattern of responses shown by the antidepressants and therefore can be distinguished from the antidepressants. Results obtained with chlorpromazine and chlordiazepoxide were as expected for drugs with a sedative action. Thus, although reactivity was reduced, passive avoidance acquisition was impaired. It is not suggested that the behavioural tests used in this study are in any way specific to the screening of potential psychotropic agents. Rather, the tests should be looked on as providing a readily quantifiable paradigm which allows the selection of possible antidepressant drugs.

The effect of bulbectomy could be considered as the initiation of a 'hyperactivity syndrome.' Thus the apparent loss of learning in a passive avoidance task following bulbectomy might not reflect a learning deficit but rather the loss of an inhibitory mechanism. The exaggerated response of the OB rat in the reactivity test can also be explained in this way. Such a concept was considered by Ellison [9] who suggested that in the rat two contrasting drive mechanisms exist. One is dominant when the animal is vigilant and out of its home

environment, which Ellison suggests is related to a noradrenergic neuronal dominance. The other mechanism exists when the animal is in its home environment, which Ellison considered to be a serotonergic neuronal dominance. It is possible therefore that the hyperactivity syndrome of the OB rat is due either to noradrenergic dominance, or to loss of an inhibitory serotonergic input, which indirectly produces noradrenergic dominance. The results obtained with the psychotropic drugs provide some support for this hypothesis. Thus chlorpromazine lowers the hyperactivity and amphetamine increases it, which is consistent with their known actions in inhibiting or stimulating respectively catecholamine systems.

The mode of action of the antidepressants in reversing the OB syndrome is less easily explained using Ellison's model. Indeed, their known actions on noradrenaline uptake should in theory exacerbate the syndrome [14, 15, 16]. However the antidepressants also interact with 5-HT uptake mechanisms [8,15], an action consistent with their ability to normalise the syndrome.

In conclusion therefore, the work presented in this paper supports the contention that the OB rat is an effective animal model for selecting antidepressant drugs. It is possible that further work will elucidate the neuronal mechanisms subserving the OB syndrome, which in turn would lead to a better understanding of the mode of action of antidepressant drugs.

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