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Corticosterone - an anxiogenic or an anxiolytic agent?

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Corticosterone (3–12 mg kg^{-1}, i.p., giving rise to plasma corticosterone concentrations from 26·7 to 89·0 μg/100 ml) failed to have a significant anxiogenic action. Instead, corticosterone (3 mg kg^{-1}) had a significant anxiolytic effect in the social interaction test of anxiety. Adrenalectomized rats had very low levels of social interaction; but adrenalectomized rats that had been given replacement corticosterone therapy did not differ from the sham-operated controls. Thus, corticosterone appears to have the opposite effect to that previously reported for ACTH. Possible mechanisms for the observed results are discussed.

EXPERIMENT 1: DOES CORTICOSTERONE HAVE AN ANXIOGENIC ACTION?

File & Vellucci (1978) reported that injected corticotrophin (ACTH, 5 and 7·5 μg/100 g) produced a significant decrease in social interaction, when rats were tested in the various conditions of the social interaction test of anxiety. Since ACTH did not produce sedation this result could be interpreted as ACTH having an anxiogenic action. A similar suggestion was made by Weiss et al (1970) on the basis of the avoidance performance of adrenalectomized and hypophysectomized rats.

The effects on social interaction of ACTH were maximal when the rats were tested 3 min after injection, but were no longer significant after 30 min. This made it unlikely that the effect was caused by released corticosterone, but the possibility could not be excluded, and, indeed, on the basis of a review of clinical studies and animal experiments, Warburton (1974) suggested that corticosteroids might be anxiogenic. Our first experiment (1) was designed to test this possibility by examining the effects of three doses of corticosterone at two time-intervals between injection and testing. ACTH reduced social interaction in all the test conditions, but it is only necessary to test the rats in one condition to demonstrate an anxiogenic action (whereas several conditions are needed to show an anxiolytic action). ACTH had the greatest effect when the rats were tested under low light, in a box with which they were familiar. This test condition was therefore selected for condition 1.

In order to relate any behavioural effects to changes in plasma corticosterone concentrations, the latter were determined 20 min after injections of the drug.

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Methods

Animals. Male hooded rats, 200–250 g, from Olac Ltd. (Bicester) were housed singly for 5 days before testing, in a room lit by an 11 h light: 13 h dark cycle (lights on at 0700 h). Food and water were freely available.

Drugs. Corticosterone (Sigma Chemical Co.) was suspended in a 5% ethanol solution in distilled water (v/v) and administered i.p., the 3 and 6 mg kg^{-1} doses in 1·5 ml kg^{-1}, and the 12 mg kg^{-1} dose in 3 ml kg^{-1}. Half the control rats received 1·5 ml kg^{-1} and half 3 ml kg^{-1} of 5% ethanol.

Apparatus. The test box was 66 × 66 × 47cm with wooden walls and floor. The illuminance on the floor was 13·3 scotopic lux. A camera was mounted 150 cm above the box and the rats were observed on a video screen in an adjacent room.

Procedure. (a) Social Interaction Test. Six pairs of rats were randomly assigned to each of the dose levels (control, 3, 6 and 12 mg kg^{-1} corticosterone) and to the two time-intervals (15 and 30 min after injection). On the two days before testing, each rat was placed alone in the test box for 10 min to familiarize him with the box. Rats were assigned to pairs such that each had a partner that did not differ in weight by more than 5 g and was in the same drug condition (dose and time-interval).

On the day of testing the rats were given the appropriate dose and were tested in a random order between 0730 and 1130 h. Each pair of rats was placed in the centre of the box and their behaviour was observed for 10 min by an observer who was unaware of the drug condition of the rats and who scored and timed all the pairs for boxing, wrestling, kicking, following, sniffing, grooming, mounting, jumping on, crawling under or over the partner.

Passive contact, where the rats were sitting or lying with their bodies in contact, was scored separately. For further details see File & Hyde (1978).

(b) Determination of plasma corticosterone. The rats were from the same delivery as those tested in (a) and were similarly housed and handled. Six rats were randomly assigned to each of the four dose levels described in (a). Injections were given i.p. 20 min before decapitation which was between 0900 and 1100 h. Trunk blood was collected in chilled heparinized plastic tubes, centrifuged at 3000–3500 rev min^{-1} and the plasma stored at −10 °C. Plasma corticosterone concentrations were determined fluorimetrically (Zenker & Bernstein 1958).

Results

Because most of the time was spent in investigatory behaviour and other behaviours contributed little to the total score, the various forms of active interaction were combined into one score (Fig. 1). As the behaviour of one rat cannot be considered to be independent of its partner's behaviour, pair scores were used giving a maximum score of 1200 s. Although the drug-injected rats generally had slightly lower scores, these never differed significantly from those of the controls (Fig. 1). The incidence of aggressive behaviour was low and did not differ among the various drug groups.

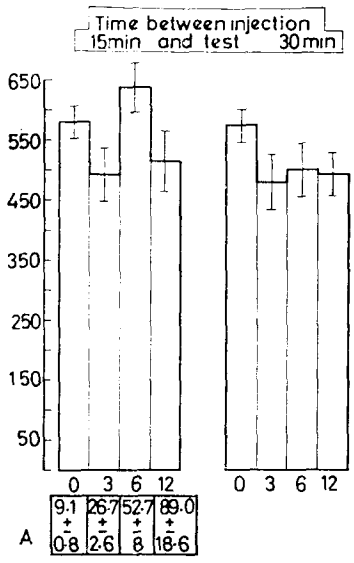

FIG. 1. The mean (and s.e.m.) time (s) (ordinate) spent in active social interaction for rats injected with vehicle (5% ethanol), 3, 6 or 12 mg kg^{-1} corticosterone, either 15 or 30 min before the start of the 10 min test. The Table (A) shows the mean plasma corticosterone concentrations (μg 100 ml^{-1}) 20 min after injection with vehicle or 3, 6 or 12 mg kg^{-1} corticosterone (abscissa: corticosterone mg kg^{-1}).

Fig. 1 also shows the mean plasma corticosterone concentrations 20 min after the various injections. Since the social interaction test lasted 10 min this would correspond to the mid-point of the test for those tested 15 min after injection. All doses of corticosterone produced significant increases in plasma corticosterone concentrations compared with the controls given 5% ethanol (ts = 6·5, 5·1 for 3 and 6 mg kg^{-1}, $P < 0.001$, and t = 4·3, $P < 0.01$ for 12 mg kg^{-1}).

Discussion

As none of the doses of corticosterone significantly reduced the level of social interaction it is unlikely that the anxiogenic action of $ACTH_{1-24}$ can be attributed to the effect of released corticosteroids. Although Warburton (1974) proposed that anxiety resulted from the neurochemical action of corticosteroids, most of the evidence he cites in support could equally well be interpreted as evidence that ACTH is anxiogenic.

It is unlikely that the failure to find a significant reduction in social interaction is because peripheral injections failed to raise plasma concentrations of the drug sufficiently as these were significantly elevated by all the doses and were in the range produced by a variety of stressors, from the mild one of a novel environment (Seggie & Brown 1975) to the severe stress of sham adrenalectomy (Buckingham & Hodges 1974). If corticosterone were anxiogenic then it should result in the emotional state called anxiety and in the concomitant behavioural changes, whether its concentration in the c.n.s. is raised by external stressors or by its administration.

EXPERIMENT 2: DOES CORTICOSTERONE HAVE AN ANXIOLYTIC ACTION?

File & Vellucci (1978) found that when the interval between injection and test was 30 min, ACTH failed to have a significant effect in the social interaction anxiety test. By this time, plasma corticosterone concentrations would have reached a peak (Hodges & Mitchley 1971) and one possible explanation for the social interaction result is that corticosterone was counteracting the effects of ACTH. There are examples from other situations where corticosterone has been found to exert the opposite effect to ACTH; e.g. on avoidance responding (Weiss et al 1970), on neuronal firing (Steiner et al 1969), and on the rate of uridine incorporation into protein (Gispen et al 1977).

Experiment 2 was therefore designed to test whether corticosterone has an anxiolytic action in the social interaction test of anxiety. This was done by testing the effect of corticosterone in all four of the test conditions (low light, familiar (LF); high light, familiar (HF); low light, unfamiliar (LU); high light, unfamiliar (HU)). A dose of 3 mg kg^{-1} was chosen, as Experiment 1 had shown that this dose resulted in plasma corticosterone values similar to those found in mild to moderate stress, i.e. they were within the physiological range (Seggie & Brown 1975). To test further the role of corticosterone, one group of adrenalectomized rats, and one group of adrenalectomized rats given replacement corticosterone therapy were also tested.

Lastly, to see whether blocking the release of ACTH produces behavioural changes in the test, a group of rats were treated with betamethasone which blocks the release of ACTH following ether stress (Buckingham & Hodges 1976), although the ACTH release to some stresses is only partially blocked by synthetic glucocorticoids (Dallman & Yates 1968). To ensure that the dose of betamethasone was sufficient to block the release of ACTH it was administered in the drinking water, which is a non-stressful means of drug administration (Purves & Sirett 1965; Hodges & Mitchley 1970a,b). for 2 days before the test.

Methods

Animals and Surgery. The source of animals and housing conditions were as described in Exp. 1.

Bilateral adrenalectomy was performed by the dorsal approach, under sodium pentobarbitone anaesthesia (60 mg kg^{-1}). In similar sham operations the adrenals were exposed and touched. A single dose of corticosterone (400 μg in 5% ethanol, in a volume of 0·25 ml/100 g) was given to the adrenalectomized rats immediately after operation, as pilot studies showed that this aided post-operative recovery. The animals were housed in pairs for 10 days post-operatively; they were then housed singly for 5 days before the social interaction test.

One group of adrenalectomized rats was maintained on 0·9% sodium chloride solution, the other received corticosterone in the drinking water at 160 μg ml^{-1} (the solution also contained 1% ethanol and 2% glucose). The fluid intake was constant (approximately 20 ml/100 g day^{-1}) and was sufficient to maintain the plasma corticosterone at concentrations not significantly different from those of the sham-operated controls (see Results). The sham-operated group received tap water.

Drugs. Corticosterone for injection was suspended in a 5% ethanol solution, as described in Experiment 1.

Betamethasone sodium phosphate (Glaxo Laboratories Ltd.) was administered in the drinking water, at 20 μg ml^{-1} for 48 h before the test. Pilot experiments showed that the volume drunk remained constant and that the rats ingested approximately 450 μg/100 g day^{-1}.

Apparatus. This was as described in Experiment 1, but two light levels were used. The low light was 13·3 and the high light 333 scotopic lux.

Procedure. (a) Social Interaction Test. Unoperated rats were randomly allocated to the four test conditions (LF, HF, LU, HU) and to the four drug groups (corticosterone, 3 mg kg^{-1}; controls receiving 5% ethanol; betamethasone; uninjected controls) such that 6 pairs of rats in each drug group were tested in each condition.

The adrenalectomized rats were randomly allocated to the untreated and corticosterone-treated groups and to three test conditions (LF, LU and HU), such that there were 5 pairs in each group tested in each condition. (Because of post-operative losses in the adrenalectomized rats there were insufficient rats to test in all four test conditions.) The sham-operated controls were randomly assigned, 6 pairs to each test condition.

The rats allocated to the LF or HF test conditions were placed singly in the test box, under the appropriate light level, for a 10 min period in the 2 days before the social interaction test. Those in the LU and HU conditions were given two 10 min periods in the test room, but they remained in their home cages.

On the day of the test, rats in the corticosterone group and their vehicle controls were given i.p. injections 20 min before the start of the test and were tested in a random order from 0730 to 1130 h. Each pair was placed in the centre of the box and observed for 10 min, as described in Experiment 1.

(b) Plasma Corticosterone Assay. Six adrenalectomized rats that had been maintained on saline, 16 given replacement corticosterone therapy and 11 sham-operated controls selected at random were decapitated 1 h after behavioural testing (between 0900 and 1100 h) and trunk blood was collected. Plasma corticosterone concentrations were determined as in Experiment 1.

Statistics. For the groups of rats tested in only three conditions, the data were analysed by two-way analysis of variance with the drug treatment as one factor and the test condition as the other. Anxiolytic drugs produce less change in social interaction across the test conditions than is seen in control animals;

this is revealed by a significant drug × test condition interaction. A drug treatment resulting, say, in lower scores in all the conditions, as is seen with an anxiogenic agent, would produce a significant main effect.

The data for the rats tested in all four conditions were also subjected to a three-way analysis of variance, with the drug treatment, the light level and the level of familiarity as the three factors.

Results

From Fig. 2 it can be seen that the corticosterone-injected rats showed a lower level of interaction in LF than their vehicle-injected controls, but that they

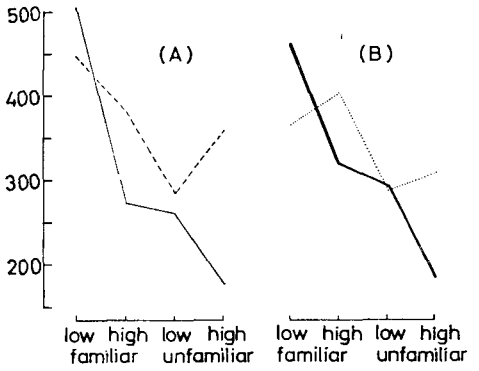

FIG. 2. The mean time (s) (ordinate) spent in active social interaction for rats tested under low or high light, in a box with which they were either familiar or unfamiliar. A, shows the data for rats injected with corticosterone (3 mg kg^{-1}) (- - - -) and for their vehicle injected controls (5% ethanol) (——). B, shows the data for rats given betamethasone in their drinking water (· · · · · · ·), and for their uninjected controls (——). Abscissa: light level and familiarity.

then showed significantly less decrease in the other test conditions (drug × test condition interaction, F (3, 40) = 9·2, $P < 0{\cdot}001$). The 3-way analysis of variance revealed that this effect was significant for both the light level (F (1, 40) = 19·2, $P < 0{\cdot}001$) and for familiarity (F (1, 40) = 6·9, $P < 0{\cdot}01$).

The betamethasone treatment (Fig. 2) resulted in significantly less change in social interaction when the light level was manipulated (F (1, 40) = 11·8, $P < 0{\cdot}002$), but the change with manipulation of the level of familiarity was not significantly different from that shown by the uninjected controls (F (1, 40) = 2·1, $P > 0{\cdot}10$).

The social interaction data for adrenalectomized rats given corticosterone therapy in no way differed from the sham-operated controls (Fig. 3). However, the adrenalectomized rats showed significantly lower

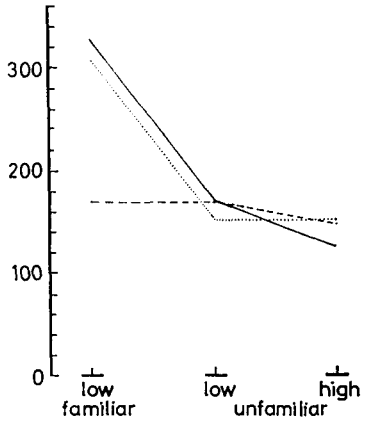

FIG. 3. The mean time (s) (ordinate) spent in active social interaction for rats tested in the LF, LU and HU test conditions. Sham-operated controls (——); adrenalectomized rats (- - - -); adrenalectomized rats given replacement corticosterone therapy (· · · · · · ·). Abscissa: light level and familiarity.

levels of social interaction in the LF condition (F (1, 27) = 5·3, $P < 0{\cdot}05$) and little further decline in the other test conditions (drug × test condition interaction F (2,27) = 9·3, $P < 0{\cdot}001$). Rats adrenalectomized and given replacement corticosterone, differed significantly from adrenalectomized rats in the overall level of social interaction (F (1, 24) = 9·9, $P < 0{\cdot}005$) and in their pattern of change across the test conditions (F (2, 24) = 5·7, $P < 0{\cdot}01$).

Comparison of Figs 2 and 3 shows that the scores from the operated rats were much lower than those for the unoperated animals. There are two reasons for this. The rats were 200–250 g at surgery and were thus heavier than unoperated rats at the time of test by at least 50 g. It has been shown previously that the weight of the rats determines the overall level of interaction (File & Hyde 1978). Secondly, we have consistently found that operated animals (whether undergoing a c.n.s. lesion or, as in this case, the removal of endocrine glands) have lower scores than unoperated animals.

Although the plasma corticosterone concentrations in the adrenalectomized rats were significantly lower than those of the other groups (Table 1), corticosterone was apparently still detectable by the assay method, which detects a residual fluorescence in the plasma in the absence of corticosterone. This has also been observed by Hodges & Jones (1964) and Buckingham & Hodges (1974). The plasma corticosterone values in the adrenalectomized rats were

Table 1. Mean (and s.e.m.) plasma corticosterone concentrations (μg/100 ml) for adrenalectomized rats, adrenalectomized rats given replacement corticosterone, and for sham-operated controls.

Groups	Mean plasma corticosterone concentrations
Adrenalectomized	3·5 ± 0·32
Adrenalectomized and replacement corticosterone	8·8 ± 0·59
Sham operated controls	8·4 ± 0·67

significantly lower than the controls ($t = 5{\cdot}21$, $df = 15$, $P < 0{\cdot}001$). The plasma corticosterone concentration for the group of rats given replacement corticosterone therapy was not significantly different from that for the sham-operated controls. The values for the sham-operated controls were not higher than those for the controls in Experiment 1, hence by the time they were tested there was no evidence of any remaining post-operative stress.

DISCUSSION

The anxiolytic action of corticosterone (as seen when injected into intact rats) could be due to its exerting effects on the c.n.s. opposite to those produced by ACTH (Steiner et al 1969; Pfaff et al 1971). The effects of ACTH and corticosterone on tryptaminergic function would support this suggestion. We found that $ACTH_{1-24}$ (5 μg/100 g) increased concentrations of 5-hydroxyindoleacetic acid in the midbrain and hypothalamus (File & Vellucci 1978), whereas, corticosterone, in a dose range similar to that used here, increased 5-HT values (Telegdy et al 1976). Increases in 5-HT were also produced by chlordiazepoxide in a dose regime that produced an anxiolytic profile (5 mg kg^{-1} for 5 days) (File & Vellucci 1978). ACTH and corticosterone have been reported to exert opposite behavioural effects on avoidance responding (Weiss et al 1970) and on the extinction of active avoidance responses in the rat (Bohus et al 1968; Van Wimersma Greidanus 1970; de Wied 1974). However, ACTH and corticosterone do not always exert opposite effects; corticosterone at 10 mg kg^{-1}, delays extinction, as does ACTH, and at this high dose corticosterone reduces 5-HT concentrations (Telegdy et al 1976), the opposite effect to that seen in low doses. Lastly, both $ACTH_{4-10}$ and corticosterone have anti-amnestic effects (Flood et al 1976, 1978). Thus, although corticosterone produced opposite behavioural effects to ACTH in the social interaction test, and other examples of such differences can be found, this is by no means a universal finding.

An alternative explanation for the results with corticosterone is that it is inhibiting, via a feedback mechanism (Jones et al 1972), the release of ACTH to stressful environmental stimuli. Whether this is so and/or whether the behavioural effects are due to corticosterone exerting an action on c.n.s. sites (e.g. 5-HT pathways in the midbrain and hypothalamus) opposite to the action of ACTH is not resolved.

The results with betamethasone demonstrate that blocking ACTH release can prevent the decrease in social interaction that normally occurs under high light levels. However, when the familiarity of the box was manipulated, betamethasone failed to modify the decrease in social interaction that normally occurs in unfamiliar situations. It is unlikely that the dose used was too low to be effective in blocking ACTH release as it has been shown to prevent ACTH release to ether stress in the rat (Hodges & Mitchley 1970b; Buckingham & Hodges 1976) and to prevent the depletion of hypothalamic noradrenaline that normally occurs in response to ether stress (Vellucci 1977). Although synthetic glucocorticoids block ACTH release to several stressors (Feldman et al 1973; Buckingham & Hodges 1976), not all stress-induced ACTH release can be inhibited in this way (Dallman & Yates 1968), and it is possible that betamethasone does not block ACTH release to the stress of a novel environment.

The low level of social interaction shown by the adrenalectomized rats could have been due to the high concentrations of ACTH in plasma and pituitary which are present two weeks after adrenalectomy (Mims 1973; Buckingham & Hodges 1974), or to the low concentrations of corticosterone, or both. Since we found an anxiogenic action with injected $ACTH_{1-24}$ (File & Vellucci 1978) and an anxiolytic action for injected corticosterone (Experiment 2) it seems likely that both hormone effects were contributing to the pattern seen in the adrenalectomized rats; their failure to show any further decrease in social interaction in the LU and HU test conditions could be because the level of social interaction was so low that a further significant decrease was not possible. Alternatively, the high concentration of ACTH may have been blocking further release of ACTH to the comparatively mild stress of an unfamiliar environment. Although it has been shown that adrenalectomized rats can exhibit further increases in ACTH to more severe stress (such as ether, Buckingham & Hodges 1974; or combined effects of ether anaesthesia, laparotomy, aortic cannulation and exsanguination, Mims 1973), this may not necessarily be the case with the

relatively mild stress of a novel environment. The adrenalectomized rats that received replacement corticosterone therapy had plasma corticosterone concentrations and a behavioural pattern that were indistinguishable from those shown by the sham-operated controls.

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REFERENCES

Bohus, B., Nyakas, C., Endroczi, E. (1968) Int. J. Neuropharmacol. 7: 307–314

Buckingham, J. C., Hodges, J. R. (1974) J. Endocrinol., 63: 213–222

Buckingham, J. C., Hodges, J. R. (1976) Br. J. Pharmacol. 56: 235–239

Dallman, M. F., Yates, F. E. (1968) Mem. Soc. Endocrinol., No. 17: 39–72

De Wied, D. (1974) Excerpta Med. Int. Congr. Ser. 132: 945–951

Feldman, S., Conforti, N., Chowers, I. (1973) Acta Endocrinol. Copenhagen 73: 660–664

File, S. E., Hyde, J. R. G. (1978) Br. J. Pharmacol. 62: 19–24

File, S. E., Vellucci, S. V. (1978) J. Pharm. Pharmacol. 30: 105–110

Flood, J. F., Jarvik, M. E., Bennett, E. L., Orme, A. E. (1976) Pharmacol. Biochem. Behav. 5: 41–51

Flood, J. F., Vidal, D., Bennett, E. L., Orme, A. E., Vasquez, S., Jarvik, M. E. (1978) Ibid. 8: 81–87

Gispen, W. H., Reith, M. E. A., Schotman, P., Weigant, V. M., Zwiers, H., De Wied, D. (1977) in: Miller, L. H., Sandman, C. A., Kastin, A. J. (eds) Neuropeptide influences on the brain and behaviour, Raven Press, New York

Hodges, J. R., Jones, M. T. (1964) J. Physiol. 173: 190–200

Hodges, J. R., Mitchley, S. E. (1970a) Br. J. Pharmacol. 40: 732–739

Hodges, J. R., Mitchley, S. E. (1970b) Ibid. 38: 719–724

Hodges, J. R., Mitchley, S. E. (1971) Ibid. 41: 640–647

Jones, M. T., Brush, F. R., Neame, R. L. B. (1972) J. Endocrinol. 55: 489–499

Mims, R. B. (1973) Horm. Metab. Res. 5: 368–371

Pfaff, D. W., Silva, M. T. A., Weiss, J. M. (1971) Science, 172: 394–395

Purves, H. D., Sirett, N. E. (1965) Endocrinology 77: 366–374

Seggie, J. R., Brown, G. M. (1975) Can. J. Physiol. Pharmacol. 53: 629–637

Steiner, F. A., Ruf, K., Akert, K. (1969) Brain Res. 12: 74–85

Telegdy, G., Kovacs, G. L., Vermes, I. (1976) Acta Physiol. Acad. Sci. Hung. 48: 417–419

Van Wimersma Greidanus, Tj. (1970) Prog. Brain Res. 32: 185–191

Vellucci, S. V. (1977) Br. J. Pharmacol. 60: 601–605

Warburton, D. (1974) Int. Pharmacopsychiatry 9: 189–205

Weiss, J. M., McEwen, B. S., Silva, M. T., Kalkut, M. (1970). Am. J. Physiol. 218: 864–868

Zenker, N., Bernstein, D. E. (1958) J. Biol. Chem. 231: 695–701