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Alterations in Shock-Induced Fighting and Locomotor Activity Following Intracerebroventricular Injection of Hydrocortisone in the Rat

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Three experiments were conducted in an attempt to clarify the facilitatory influence of hydrocortisone on shock-induced fighting in rats. Results of the first experiment indicated a biphasic, dose-dependent action of intraventricularly-administered hydrocortisone. Low (25 μg) and intermediate (50 μg) doses both facilitated fighting whilst the high (100 μg) dose exerted a potent suppressant effect. Two control tests were performed to determine whether alterations in pain reactivity or locomotor activity could have accounted for the observed changes in fighting behaviour. None of the treatments altered shock thresholds (Experiment 2) but whilst neither low nor intermediate doses affected activity measures, the high dose preferentially reduced vertical activity (Experiment 3).

Key words: shock-induced fighting, activity, pain thresholds, hydrocortisone, rats

INTRODUCTION

Over the past decade, considerable experimental evidence has accumulated for pituitary-adrenocortical involvement in agonistic behaviour [Brain, 1972; Leshner, 1975]. Bilateral adrenalectomy reduces isolation-induced fighting in mice [Brain et al, 1971; Harding and Leshner, 1972; Leshner et al, 1973], an effect also observed following hypophysectomy [Sigg et al, 1966]. Corticotrophin (ACTH) acts

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differently on fighting behaviour according to treatment regimen. Chronic administration of ACTH suppresses isolation-induced fighting in mice [Brain et al, 1971; Brain and Poole, 1974; Leshner et al, 1973; Poole and Brain 1975a] whilst acute ACTH treatment augments this behaviour [Brain and Evans, 1977; Poole and Brain, 1975b]. Although the former effect appears to correspond to an extra-adrenal action of ACTH, the latter response has been tentatively related to enhanced glucocorticoid release [Brain and Evans, 1977]. Glucocorticoid injections (natural and synthetic) have been reported to increase isolation-induced fighting [Banerjee, 1971; Brain et al, 1971; Kostowski et al, 1970; Leshner et al, 1973] and shock-induced fighting [Kostowski, 1967] in mice and to restore aggressive behaviour in adrenalectomized mice [Walker and Leshner, 1972]. This treatment also increases muricidal behaviour [Kostowski, 1967] and maternal aggression [Endroczi et al, 1958] in rats. In spite of this wealth of data it has been argued that corticosterone treatment has no effect on murine aggression independent of its effects on ACTH levels [Leshner et al, 1973].

In a recent extension of the above research, Rodgers and Semple [1978] reported that shock-induced fighting was suppressed by adrenalectomy, hypophysectomy and high doses of ACTH. In contrast, fighting was enhanced by hydrocortisone and low ACTH doses. The present report attempted to investigate the facilitative influence of hydrocortisone on shock-induced fighting by using intraventricular injections. This method has the advantage of ensuring that the hormone reaches brain tissue in significant concentrations, thus reducing the possibility of peripherally-mediated behavioural changes. Since the frequency of fighting in this paradigm can easily be influenced indirectly, two control experiments were performed to assess the possibility of treatment-induced alterations in locomotor activity and pain reactivity.

GENERAL METHODS

Animals and Surgery

Adult male Sprague-Dawley rats (260–300 gm) from Bradford University colony, were used as subjects. Animals were individually housed with food and water available ad libitum and maintained on a 12 hour light/dark cycle (7 am–7 pm). All testing was performed under red light during the dark phase, ie, 7 pm onwards.

Under Equithesin anaesthesia (Jensen-Salsbery Lab., Inc.), experimental animals were implanted unilaterally with guide cannulae terminating 1 mm dorsal to the right lateral ventricle (A/P: – 2.0, L: 1.8, V: 2.0 mm, calculated with reference to Bregma). Cannulae consisted of 23 gauge stainless-steel guides fitted with 31 gauge stylets. The animals received a single intramuscular injection of penicillin

(50,000 units) following surgery and were allowed at least seven days recovery before behavioural testing.

Injection Technique and Hormone Doses

During injection, stylets were replaced with 31 gauge injection units which extended 1 mm ventral to the guide tips. Single unilateral injections were performed whilst the animals were hand held. A 10 μl Hamilton microsyringe (model 710N) with a polythene tubing connection to the injection unit was employed to deliver the solutions. Ten μl injections were made at a rate of 1 μl/15 seconds, with a total injection time of approximately 150 seconds. An uptake time of one hour was adopted in the present study, to allow for widespread hormone diffusion throughout the ventricular lumen and adjacent tissue.

Hydrocortisone sodium succinate (Organon NV, Oss, Holland) was used in three dose levels (25 μg/10 μl; 50 μg/10 μl; 100 μg/10 μl) with injection water serving as both hormone solvent and placebo solution. Each animal received one injection only and was used only once in behavioural testing.

Histology

Prior to sacrifice, experimental animals received a unilateral injection of trypan blue dye (10 μl) into the right lateral ventricle. One hour later animals were given an overdose of Nembutal and perfused intracardially with normal saline followed by 10% formal saline. Brains were removed, hardened in formal saline and sectioned on a freeze microtome. In all but three cases (where data were discarded), dye was observed in maximum concentration around the site of injection (ventriculus lateralis) with widespread distribution noted throughout the ventricular system. Most notably, significant staining was seen at the medial portion of the basal hypothalamus, in the area of the median eminence. Although these observations do not in any sense quantify hormonal distribution, they nevertheless indicate that one hour after unilateral intraventricular injection of a 10 μl volume significant staining occurs throughout the ventricular system and adjacent tissues.

Apparatus

Shock-induced fighting and flinch-jump tests. A modified rat operant station (manipulanda removed, flat interface) measuring 23.5 X 22 X 22 cm served as the test chamber. The chamber was opaque apart from the perspex door, which also served as an observation window. An Aim Bioscience shock generator (Model 507) supplied electric shock of specified intensity, duration, and frequency to the grid floor of the test chamber.

Activity test. A square enclosure with plywood floor (90 X 90 cm) and walls (30 cm high) painted flat white was used. Embedded in two adjacent walls were

two banks of six red light sources. The lower bank was positioned at 3 cm above the floor level (to monitor horizontal activity) with the upper bank at 12.5 cm (to monitor vertical activity). On the opposing walls, two banks of six sensors each were arranged to receive light beams from the corresponding sources. Sensors from lower and upper banks were connected to separate counters which registered one count each time an appropriate light beam was interrupted.

Procedure

Shock-induced fighting. Operated animals and unoperated stimulus opponents were matched for weight, and fighting pairs were randomly assigned to treatment conditions. Pairs were placed in the test chamber one hour after intraventricular hormone or placebo injection to the experimental animal; after two minutes' habituation, the animals were exposed to 60 electric shocks delivered to the grid bars (shock intensity: 2 mA, duration: 0.5 sec, frequency: 6/min). An attack response was recorded when one animal made a directed forward lunge either with the forepaws or the whole body. The upright boxing posture itself did not, in this study, constitute an attack response. Only the behaviour of the injected animals was used in data analysis.

Flinch-jump test. Pain reactivity was measured using a modification of the flinch-jump threshold test (Evans, 1961; Rodgers, 1977). One hour after either hydrocortisone or placebo injection, individual animals were placed in the test chamber where they received six series of eight electric shocks (0.5 sec duration) delivered at ten-second intervals to the grid floor. Shock series were administered in alternating ascending and descending order with intensities ranging between 0.13 and 1.00 mA in eight steps. Jump thresholds (the intensity at which the hind paws leave the grid floor) were recorded for each series and an overall mean value calculated to provide an estimate of pain reactivity.

Activity test. One hour following intraventricular treatment, individual animals were placed in the activity box and horizontal activity, vertical activity, and defecation scores were recorded over a ten-minute observation period.

In all behavioural tests, the apparatus used was cleansed thoroughly after each animal in order to minimize the effects of any olfactory cues from the preceding subjects.

EXPERIMENT 1

This experiment was designed to examine the effect of intraventricular hydrocortisone injection on shock-induced fighting. Prior to treatment allocation, 34 operated animals were weight-matched with unoperated stimulus opponents. Four treatment conditions were used: placebo (n of pairs = 10), 25 μg hydrocortisone (n = 8), 50 μg hydrocortisone (n = 8) and 100 μg hydrocortisone

(n = 8). One hour following injection, each pair was placed in the test chamber and the frequency of fighting behaviour was recorded.

Results

Results of this experiment are presented in Figure 1. ANOVA revealed an overall significance in attack frequency ($F = 18.4$, $F_{.999}(3, 30) = 7.05$, $P < 0.001$). Statistical follow up, using Newman Keuls test for comparison between treatment means, indicated that the 25 μg and 50 μg doses of hydrocortisone resulted in significantly increased fighting behaviour ($P < 0.01$) compared with placebo treatment, and that the 100 μg dose of hydrocortisone significantly depressed fighting ($P < 0.01$) compared with all the other treatments. It would thus appear that intraventricular hydrocortisone produces a dose-dependent biphasic alteration in shock-induced fighting in rats. Casual observation suggested that, whilst animals receiving doses of 25 μg or 50 μg initiated and maintained fight encounters, those receiving 100 μg doses did not do so, and were more actively engaged in overt avoidance and submissive/defensive behaviours.

EXPERIMENT 2

It is well established that alterations in shock sensitivity can indirectly modify levels of fighting in the present test paradigm [Rodgers, 1977]. In order to control for this possible explanation of the above results, a second experiment was conducted in which shock thresholds were estimated under the treatment conditions employed in Experiment 1. Twenty naive animals, equipped with unilateral intraventricular cannulae, were randomly assigned to one of four equal treatment

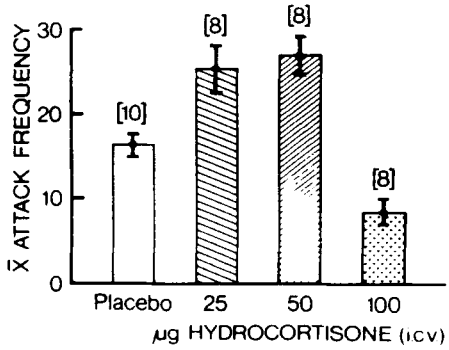

Fig. 1. The effects of intraventricular hydrocortisone and placebo injections on attack frequency ($\bar{x} \pm$ SEM) in Experiment 1. Figures in brackets refer to the number of pairs in each condition.

groups (placebo, 25, 50 and 100 μg hydrocortisone). One hour posttreatment, each animal was placed in the test chamber and allowed two minutes habituation before reactivity to electric shock was determined using the flinch-jump technique.

Results

Results of this experiment are presented in Figure 2. It would seem that hydrocortisone injection at three dose levels does not alter the animal's reactivity to the shock stimulus since ANOVA revealed no overall significance in jump thresholds with treatment ($F = 2.99$, $F_{.95}$ (3, 16) = 3.24, ns).

EXPERIMENT 3

Another possibility for the results of Experiment 1 is that hormone administration alters motor activity/co-ordination, thus indirectly influencing fighting behaviour. To test for this possibility, 40 naive animals, equipped with intraventricular cannulae, were randomly assigned to one of four treatment conditions [placebo (n = 9), 25 μg (n = 10), 50 μg (n = 10), 100 μg (n = 11) hydrocortisone]. One hour following treatment, individual animals were placed in the activity box and behaviour was recorded over a ten-minute session.

Results

Results of this experiment are presented in Figure 3. ANOVA revealed no overall significance either on measures of horizontal activity [$F = 0.82$, $F_{.95}$(3, 36) = 2.86, ns] or defecation [$F = 1.81$, $F_{.95}$ (3, 36) = 2.86, ns]. However, ANOVA did reveal significance on vertical activity scores [$F = 7.62$, $F_{.999}$ (3, 36) = 6.74, $P < 0.001$]. Statistical follow-up, using Newman-Keuls test, indicated

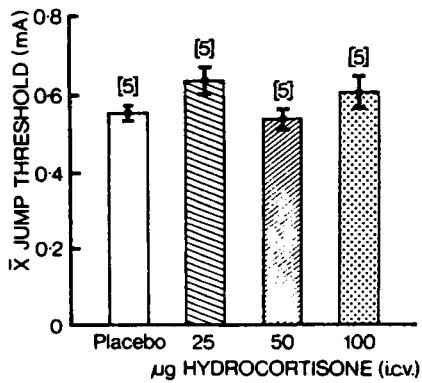

Fig. 2. The effects of intraventricular hydrocortisone and placebo injections on shock thresholds ($\bar{x} \pm$ SEM) in Experiment 2. Figures in brackets refer to the number of animals in each condition.

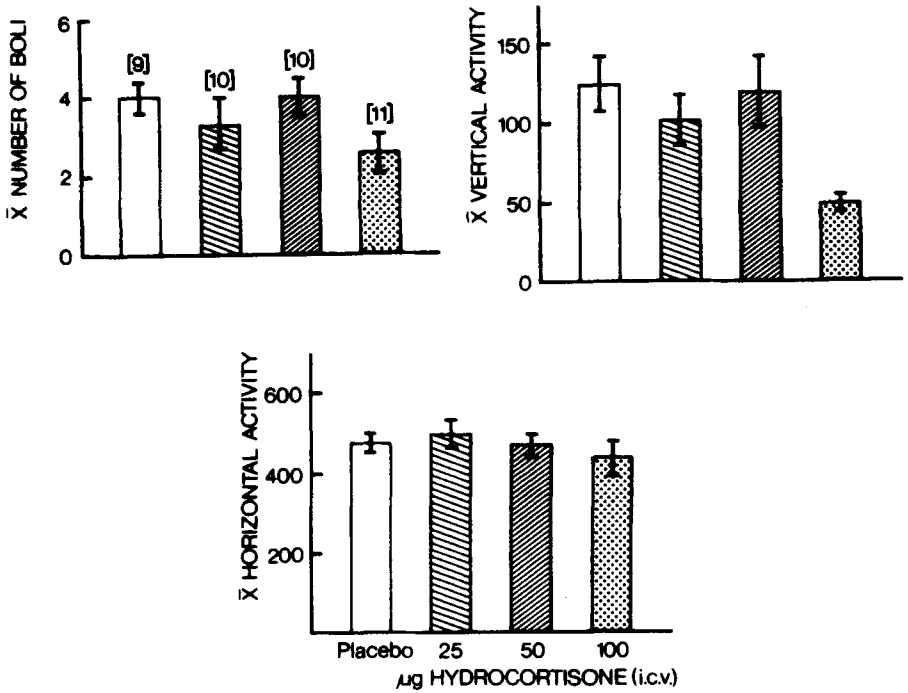

Fig. 3. The effects of intraventricular hydrocortisone and placebo injections on three behavioural measures ($\bar{x} \pm$ SEM) in Experiment 3. Figures in brackets refer to the number of animals in each condition.

that the 100 μg dose hydrocortisone significantly depressed vertical activity compared to placebo ($P < 0.01$), 25 μg hydrocortisone ($P < 0.05$) and 50 μg hydrocortisone ($P < 0.01$).

DISCUSSION

The results of the present investigation confirm and extend previous observations that glucocorticoids can modify levels of agonistic behaviour in rodents [Banerjee, 1971; Brain et al, 1971; Brain and Evans, 1977; Endroczi et al, 1958; Kostowski, 1967; Kostowski et al, 1970, Leshner et al, 1973; Rodgers and Semple, 1978; Walker and Leshner, 1972]. Intraventricular injection of both low (25 μg) and intermediate (50 μg) doses of hydrocortisone facilitated shock-induced fighting behaviour in the rat whilst a high dose (100 μg) exerted a potent suppressant effect (Experiment 1). In the dose range currently employed, hydrocortisone failed to alter shock thresholds, thus eliminating the possibility that the effect on

fighting was indirectly mediated via changes in pain reactivity (Experiment 2). This finding confirms the previous observation that shock thresholds remain unaltered following subcutaneously-administered hydrocortisone [Rodgers and Semple, 1978]. Whilst neither low nor intermediate doses of steroid altered behaviour in the activity test, the high dose preferentially decreased vertical activity (Experiment 3). Thus the possibility exists that the reduced levels of fighting at the high dose reflect a diminished ability to form the upright posture characteristic of fighting in this test. However, casual observation of these animals in Experiment 1 (see Results) indicated that they were capable of forming the posture but that their behaviour was characterized more by increased avoidance of, and submission to, the opponent. A similar biphasic relationship has previously been reported in murine aggression following peripheral corticosterone treatment [Candland and Leshner, 1974].

Since Erskine and Levine [1973] have reported that complete suppression of pituitary-adrenal activity (via implants of hydrocortisone into the median eminence) has little effect on shock-induced fighting in this species, it is tentatively suggested that the current positive results may reflect an ACTH-independent action of the glucocorticoids on neural mechanisms mediating avoidance and attack. Indeed, the finding that peripherally-administered hydrocortisone can restore fighting in hypophysectomized animals to near normal levels (unpublished observations) would seem to support this suggestion. It is of interest to note in this context that McEwen et al [1970] have found that cells within the limbic system (including areas traditionally associated with avoidance and attack) selectively accumulate glucocorticoids.

The potentiation of attack produced by both low and intermediate doses of hydrocortisone would appear to contradict the recent suggestion that glucocorticoids are selectively involved in mediating "avoidance-of attack" [Moyer and Leshner, 1976; Nock and Leshner, 1976]. However, it should be emphasized that the present paradigm differs from that of Leshner's group in several important respects: species (rats vs mice), schedule and route of injection (acute intraventricular vs chronic subcutaneous) and steroid preparation (hydrocortisone vs corticosterone). These methodological differences make a direct comparison of findings difficult. We are currently investigating the effects of chronic vs acute intraventricular injections of glucocorticoids (hydrocortisone and corticosterone) on levels of shock-induced fighting.

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