Pharmacology Biochemistry & Behavior, Vol. 7, pp. 425–433.

Acute Influences of Some ACTH-Related Peptides on Fighting and Adrenocortical Activity in Male Laboratory Mice[1]

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(Received 2 November 1976)

BRAIN, P. F. AND A. E. EVANS. *Acute influences of some ACTH-related peptides on fighting and adrenocortical activity in male laboratory mice.* PHARMAC. BIOCHEM. BEHAV. 7(5) 425–433, 1977. – A number of corticotrophin preparations when given acutely enhanced fighting behavior, an event which may be consequent upon elevation of circulating corticosterone. A series of short ACTH analogues (ACTH 11–24, ACTH 4–10) had slight influences on circulating plasma corticosterone values and on fighting behavior. However, 20 μg ACTH 1–10 given 12 hr before testing did increase fighting c.f. controls. As this compound only caused a slight stimulation of plasma corticosterone titers, the possibility exists of an extra-adrenal influence. One cannot, it appears, eliminate glucocorticoid influences using these preparations in the mouse. Acutely, ACTH 1–24 had virtually no influences on Animex-assessed motor activity but did enhance fighting in castrated mice maintained with either androgen or estrogen. It is tentatively concluded that the ACTH influences on aggression in the mouse are partially mediated via glucocorticoid influences on the CNS combined with an extra adrenal influence of part of the ACTH molecule. A possible reason for the differing acute and chronic influences of ACTH on fighting in mice is presented.

ACTH Activity Adrenal Aggression Analogues Androgens Corticosterone Estrogens Isolation

CHRONICALLY administered corticotrophin (ACTH) suppresses fighting behavior in individually-housed albino laboratory mice [3, 7, 23]. It has been suggested that this influence may involve an extra-adrenal action. Leshner *et al.* [24] initially related the apparent suppressive actions of ACTH on fighting to influences on fear-of-being-attacked, i.e. they suggest that this hormone influences the animals by making them more fearful in aggressive encounters. However, irrespective of the precise way in which it brings about this action, it seems likely that chronic stress (with elevated ACTH) suppresses fighting behavior in the mouse.

The other major series of studies, in which extra-adrenal behavioral influences of ACTH and ACTH-related peptides have been postulated, concern the influences of these factors on acquisition and extinction of active and passive conditioned avoidance reactions (CAR's) by hypophysectomized or intact rat (e.g. [10,11]). Brain [2] has drawn a parallel between these two situations, suggesting that the stressed subordinate mouse may react to the attacking dominant mouse by rapidly acquiring fleeing or submissive responses, which is analogous to the relationship between the test rat in the CAR situation and the electric shock. A major methodological variation between these two areas of study, is the fact that most rat CAR investigations involved behavioral testing within hours after administration of peptide, whereas behavioral testing in the mouse fighting tests was employed some 17 days after the commencement of treatment. Preliminary studies by Brain and Evans [4] have indicated that acutely administered ACTH may stimulate spontaneous fighting in isolated male mice. Various workers (e.g. [1,20]) have obtained evidence that injection of glucocorticoid preparations stimulate murine fighting. Consequently it seems reasonable to suggest that the acute influences of ACTH on this behavior, are a consequence of the production of endogenous glucocorticoids. Further circumstantial evidence includes the findings that glucocorticoids may be preferentially accumulated by various neural regions in the rat (e.g. [25, 26, 27]) and it has been shown that such binding is modified by housing conditions which also alter fighting frequencies [12]. Steroid and ACTH-sensitive neurones have also been described in the hypothalamus and midbrain of the rat [31, 32, 34, 35].

It is interesting to note that McEwen *et al.* [27] have identified the hippocampus, hypothalamus, septum and amygdala as sites of binding for corticosterone in the rat.

[1] Supported by MRC Project Grant G.972/189/B and a grant-in-aid from the Royal Society.
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Interestingly, the last three named areas of the brain have frequently been implicated in the modification of various kinds of fighting in a variety of species (e.g. [18,29]).

One may speculate that hormones may modulate fighting behavior following neural concentration in brain loci where they alter the regional concentrations of neurotransmitters and, subsequently, the pattern of neuronal firing. Strand *et al.* [33] have even suggested that ACTH may have a direct action on neuromuscular junctions. This makes differentiation of ACTH/glucocorticoid action difficult. Moyer and Leshner [28] have suggested, on the basis of studies employing hypophysectomized/ACTH-maintained mice, that corticosterone influences on fighting behavior are distinct from those of ACTH.

Because of these possible relationships, a series of experiments are described in which the acute effects of a number of preparations of ACTH and ACTH-related peptides on isolation-induced intermale fighting are assessed. The likely involvement of glucocorticoids from the adrenal cortex is also investigated. In addition, an attempt is made to determine whether any effect on fighting is mediated by variations in gonadal function or can be accounted for by altered motor activity.

EXPERIMENT 1

ACUTE EFFECTS OF PHARMACOLOGICAL DOSES OF GELATIN PREPARATIONS OF ACTH 1–24 ON FIGHTING BEHAVIOR IN INTACT MALE MICE

Because injection stress stimulates endogenous production of ACTH, relatively high (pharmacological) doses of peptide were employed in our earlier chronic studies. An initial experiment investigated the effects of the two doses of ACTH 1–24 (Organon Labs. Ltd) employed in these earlier studies [7] as well as employing a number of durations between injection of material and behavioral testing. The time course between injection and behavioral testing has been shown to be important in many drug studies, including the effects of peptide preparations on CAR acquisition and extinction.

METHOD

Animals

All studies reported here were carried out on male Tuck TO strain albino mice bred and housed under highly controlled conditions in the Animal House facilities at Swansea. Mice were the products of primiparous females, mated individually at 9 weeks of age, with identically-aged males. Litters were reduced to 6 on the day of birth and fathers remained with litters until 10–15 days after parturition. Throughout this period and until 18–22 days of age, the mice remained unhandled. At this time, they were sexed and randomly allocated to single-sex groups of six until experimentation or mating at nine weeks of age. All mice were housed in white, opaque Makrolon cages (North Kent Plastics) measuring 30 × 12 × 11 cm with wire mesh tops. Food and water were provided ad lib. The Animal House, in which extraneous noise was minimized, was maintained on a reversed lighting schedule (white lights on 22:00–10:00 hr). The building was kept at 18–22°C and only mice of this strain were housed in the building.

Seventy-eight adult male mice were individually housed for 4 weeks before being utilized in this initial experiment.

Behavioral Testing

The aggression tests employed were modifications of the standard opponent test described by Brain and Poole [6]. Mice received a 7 min encounter on three consecutive days with standard opponents which were adult, group-housed (6's) male mice with a history of repeated defeat. Such mice rarely attacked experimental animals. The tests were conducted in a black-painted neutral arena measuring 68.5 × 34 × 20 cm with a perspex side and a double partition to encourage initial spatial separation of the test mouse and his standard opponent. The tests were carried out under dim red light at the same time each (10.00–12.00 hr) in an attempt to avoid complications due to possible circadian factors. The measures employed are fully described elsewhere [7] and included the numbers of animals in the different categories fighting in at least two out of three tests, the latency to attack (in seconds) and the number of attacks directed towards the standard opponent.

Injection Schedule

After 4 weeks individual housing, categories of mice received an intra-muscular (IM) injection on 3 consecutive days of 0.1 ml of one of the following solutions: (1) a control injection containing 40 mg/ml gelatin control ≡ 4 mg/day; (2) a gelatin solution containing 200 μg/ml ACTH 1–24 ≡ 20 μg/day; and (3) a gelatin solution containing 400 μg/ml ACTH 1–24 ≡ 40 μg/day. Mice were given an aggression test, as described above, 3, 12 or 18 hr after each injection. Categories of control injected or 20 μg ACTH treated mice were behaviorally tested at all 3 time intervals but only one 40 μg ACTH category was employed (tested 12 hr after injection).

RESULTS

The percentages of individuals fighting in at least two out of three tests, together with the mean latencies of attack, mean accumulated attacking times (AAT) and mean numbers of attacks in each category are given in Table 1. Statistical comparisons between appropriate control and treatment categories employed the Fisher exact probability test [30] and the two tailed Mann-Whitney 'U' test [30], probabilities being indicated on the table.

Numbers fighting in 2/3 tests were increased using both doses of ACTH, with respect to gelatin controls, 12 hr after injection ($p<0.05$ and $p<0.025$ on the Fisher exact probability test for animals receiving respectively 20 and 40 μg ACTH 1–24 each day). Injection with both doses of ACTH 1–24 also resulted, at 12 hr, in significant declines in attack latency with respect to controls ($p<0.002$ on the Mann-Whitney U test) and significant increases in accumulated attacking times and the number of attacks ($p<0.025$ on the Mann-Whitney U test). Although neither of the other 2 time intervals employed resulted in changes in the incidence of fighting, significant augmentation with respect to controls of fighting intensity was apparent when the corticotrophin was injected 3 hr before testing. Such mice had reduced attack latency and an increased accumulated attacking time and an enhanced number of attacks (all $p<0.025$ on the Mann Whitney U test). However, when the time interval between injection and testing was lengthened to 18 hr there were no significant differences between ACTH treatment and controls.

TABLE 1

ACUTE EFFECTS OF GELATIN SOLUTION OR AN ACTH PREPARATION ON MEAN AGGRESSION SCORES ASSESSED AT DIFFERENT TIMES AFTER INJECTION OVER THREE STANDARD OPPONENT TESTS

Daily Treatment	Time Preceding Testing	N	% Fighting in at Least 2/3 Tests	Mean Attack Latency (sec)	Mean AAT(sec)	Mean No. of Attacks
Gelatin Control	3 hr	12	25	359.2	2.4	1.3
Gelatin Control	12 hr	10	20	341.2	10.8	2.2
Gelatin Control	18 hr	12	37	314.5	7.4	3.1
20μg ACTH	3 hr	12	58	281.0†	8.5†	3.6†
20μg ACTH	12 hr	10	78*	239.3‡	16.9†	5.2†
20μg ACTH	18 hr	12	42	318.8	10.6	3.4
40μg ACTH	12 hr	10	80†	239.2‡	18.4†	5.9†

*differs from appropriate control $p<0.05$
†differs from appropriate control $p<0.025$
‡differs from appropriate control $p<0.002$

DISCUSSION

The basic conclusion to be drawn from these results is that a single pharmacological dose of ACTH 1–24 in gelatin administered IM into intact isolated male mice increases the incidence and intensity of fighting behavior, as assessed 12 hr after injection. This acute influence of ACTH is in general agreement with the preliminary study reported by Brain and Evans [4], which employed a long-acting preparation Cortrophin/Zn (Organon Labs. Ltd). In the present experiment there was little difference at 12 hr between the 20 and 40 μg/day doses of ACTH 1–24, i.e. dose dependency was not evident. Consequently, in most of the subsequent experiments in this study, the lower dose was routinely employed.

The trends with time suggest that a maximal augmentation by corticotrophin of fighting behavior, is evident when the 20 μg dose precedes testing by 12 hr. This time influence is suggestive, as carboxymethyl cellulose- and gelatin-based preparations of ACTH are said to be effective only up to 12–16 hr after injection [21]. The relationship between the ACTH-generated corticosterone and these behavioral influences are, however, necessarily tentative.

EXPERIMENT 2

RELATIONSHIP BETWEEN TIME AFTER INJECTION OF TWO PREPARATIONS OF ACTH AND PLASMA CORTICOSTERONE VALUES IN MICE

It seemed important to follow the time course of corticosterone production, following single IM injections of 20 μg of ACTH 1–24 in gelatin, in order to attempt to relate the recorded behavioral changes to alterations in circulating plasma glucocorticoids. However, the effects of the gelatin preparations are reported to be transient. Also the majority of chronic, as well as the earlier cited acute behavioral influences of ACTH and ACTH-related peptides, involved long-acting preparations of material chelated with zinc hydroxide to reduce enzymatic degradation. Consequently, for comparative purposes, a similar response/duration curve was generated using Cortrophin/Zn (the long-acting ACTH preparation available from Organon Labs, Ltd).

METHOD

One hundred and twenty-eight adult male TO strain mice, similar to those employed in Experiment 1, were randomly assigned to categories of 16 individuals. Eight individuals in five categories received a single 0.1 ml IM injection of 20 μg ACTH 1–24 in gelatin and the other eight a gelatin control injection. ACTH 1–24 categories were injected 1, 3, 12, 18 and 24 hr before plasma sampling. Mice in the remaining three categories, of 16 animals, received 0.1 ml IM of a control zinc hydroxide solution (pH 8) or 2 IU of Cortrophin/Zn. Mice were given a single injection 1, 12 or 24 hr before plasma sampling.

Plasma samples were obtained by rapidly decapitating the animal over a heparinized beaker. The resultant plasmas were stored in a deep-freeze until assay. Total plasma corticosterone concentrations were determined by a modification of the fluorometric method of Glick *et al.* [15]. Samples were determined in duplicate using 100 μl aliquots of plasma. Plasma from adrenalectomized males was used in order to subtract non-specific fluorescence.

RESULTS

The meaned data, at the different post-injection time intervals are graphed in Fig. 1. Two-tailed Mann-Whitney *U* test comparisons were carried out between ACTH treatment and controls at the different time intervals. A non-parametric test was utilized because the two populations of results did not overlap, nor were they normally distributed. Use of the Student *t*-test is thus inappropriate.

Significant differences ($p<0.001$) were only evident between treatment and control values, 1 and 3 hr after injection in the case of the gelatin ACTH 1–24. There

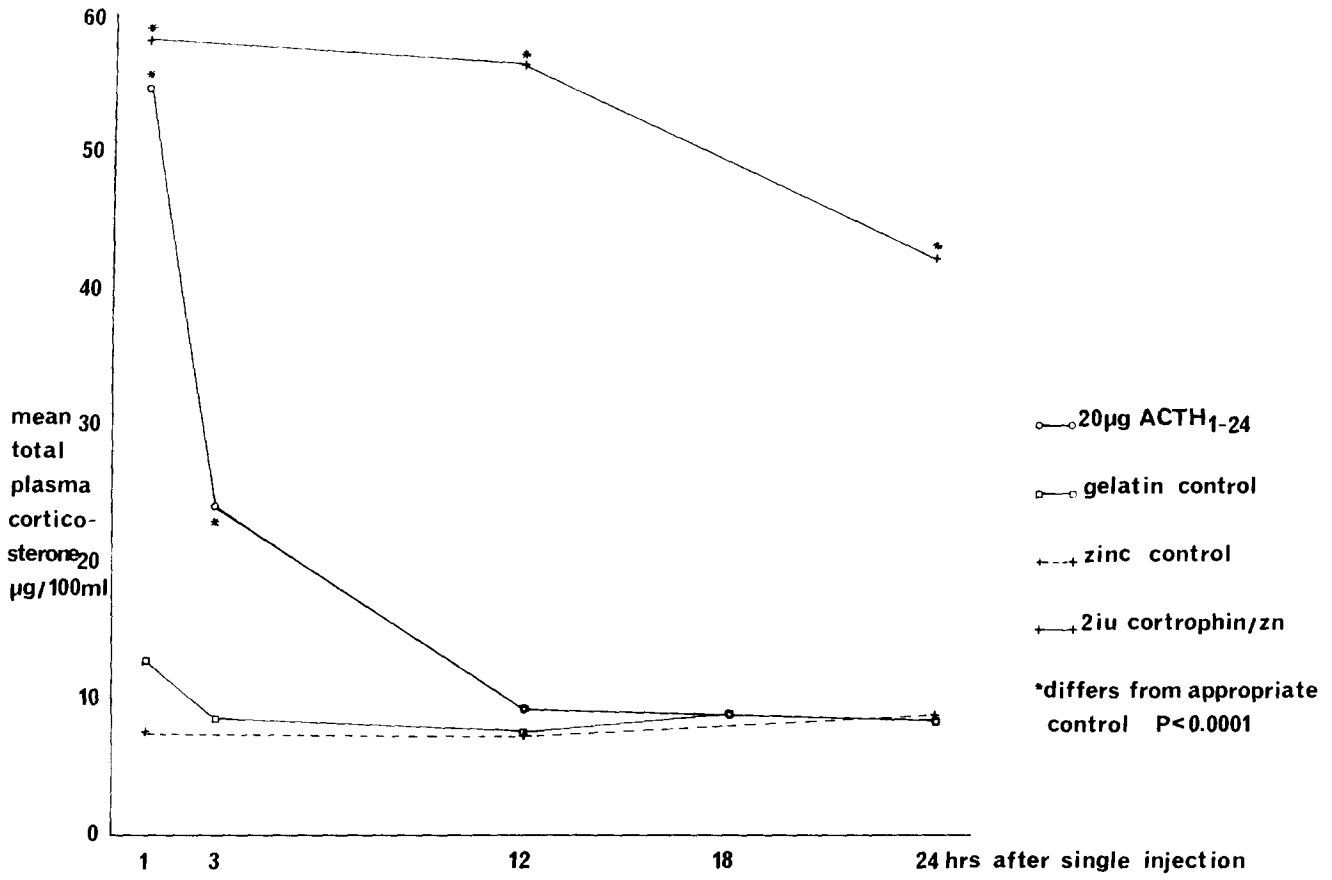

FIG. 1. Time course of adrenocortical response to cortrophin/Zn and a gelatin preparation of ACTH 1–24 (with appropriate controls) in isolated adult male 'to' strain mice (N = 8).

were, however, highly significant ($p<0.001$) elevations of plasma corticosterone titers (compared to appropriate controls) in Cortrophin/Zn treated mice at all time intervals employed. The maximal stimulation of total plasma corticosterone was, as in the case of the gelatin preparation, evident 1 hr after injection but there was a very slow rate of decline using this material (c.f. [21]).

DISCUSSION

It appears that the gelatin preparation of ACTH 1–24 produces its maximal effect on plasma corticosterone titer in 1 hr or less after injection. After this time, the response is diminished, although an effect is still apparent 3 hr after injection. Subsequently the effect is negligible.

There was also some tentative indication of an injection stress in gelatin treated animals at the 1 hr sampling time, an effect which would be expected to confound the behavioral effects (c.f. [3]). However, in terms of plasma corticosterone elevation, the effect was slight. Uninjected male mice of the same strain, age and duration of housing generate a mean basal plasma corticosterone value of 9.2 ± 1.0 µg/100 ml (N = 8), when sampled at the same time of day. However, the stress effects of injection could be more marked, in behavioral terms, than suggested by the modest elevation of glucocorticoids.

Because the maximal change in glucocorticoid titer (after 1 hr) precedes the maximal behavioral influence (12 hr) it seems likely that, if the two are related, there is an incubation period for the neural accumulation and behavioral effects of this steroid (see Introduction).

It appears likely that the zinc preparations of ACTH, whose use was originally discontinued because it was suspected that this metal suppressed fighting behavior [7], could be expected to have more persistent effects on fighting as well as on plasma glucocorticoid titers. This would seem to make such preparations more suitable for studies of chronic effects of stress and also studies on the stress of defeat (which produces a prolonged elevation in plasma glucocorticoids) whereas the pulsile nature of minor environmental stresses is more likely to be mimicked by the gelatin preparations.

There was no indication of an injection stress (in terms of glucocorticoid elevation) when using the zinc control injection. As it was less viscous, it was easier to inject [11].

EXPERIMENT 3

ACUTE EFFECTS OF GELATIN PREPARATIONS OF SOME SHORT ACTH-RELATED PEPTIDES AND AN OILY SOLUTION OF CORTICOSTERONE ON FIGHTING BEHAVIOR IN INTACT MALE MICE

Short amino-acid sequences of the ACTH polypeptide

have been shown to exert behavioral effects comparable with the parent molecule in a number of studies [7, 9, 19] without apparently influencing adrenocortical activity. ACTH 4–10 (Org. 01 63) has been reported to chronically suppress fighting behavior in intact male mice of this strain [7]. It was consequently thought to be useful to determine whether some of these preparations acutely influenced fighting behavior in the same direction as ACTH 1–24. If they augmented fighting, it could be argued that the action of ACTH was extra-adrenal and did not involve the generation of glucocorticoids. In addition, it was thought desirable to attempt to confirm that an injection of corticosterone stimulated fighting.

METHOD

Seventy-two male mice which had been isolated for 4 weeks were allocated to categories of 10–12 individuals. Mice received single 0.1 ml IM daily injections of one of the following materials over 3 days: (1) 20 μg ACTH 1–10; (b) 20 μg ACTH 11–24; (c) 20 μg ACTH 4–10; (d) gelatin control for a–c; (e) 250 μg corticosterone (Sigma Ltd.); and (f) oil control (for e).

The injections were given 12 hr before 7 min standard opponent tests which were conducted on three consecutive days (as in Experiment 1). A new gelatin control injection was employed because this study was carried out at a different time of year from the ACTH 1–24 investigation.

RESULTS

The percentages of individuals fighting in at least two out of three tests, together with the meaned aggression test data (over 3 days) are presented in Table 2. The 20 μg dose of ACTH 1–10 produced significant augmentations on all measures of fighting with respect to the gelatin control. No such effects were apparent using ACTH 11–24 or ACTH 4–10. Confirmation was also obtained that a 250 μg dose of corticosterone augmented fighting (significantly on all measures) with respect to the oil control.

DISCUSSION

The results confirm that corticosterone, the naturally occurring glucocorticoid of the mouse, can stimulate fighting in this species (c.f. [20]). Taken in conjunction with the results using the peptide fragments, one could tentatively suggest that the acute augmentation of fighting behavior produced by ACTH 1–24 may be partially a consequence of the glucocorticoids which they generate. Interestingly, however, at the dose employed ACTH 1–10 also increased fighting behavior in intact male mice. As ACTH 1–10 is said to be without an action on steroidogenesis, the possibility exists that ACTH may also have an extra adrenal acute influence on fighting. If this is the case. it seems likely that a different peptide sequence is effective to that evident in CAR studies (ACTH 4–10 activates). Obviously, many more doses and materials would have to be looked at before this result could be completely validated.

EXPERIMENT 4

EFFECTS OF GELATIN PREPARATIONS OF SOME SHORT ACTH-RELATED PEPTIDES ON PLASMA CORTICOSTERONE LEVELS IN INTACT MALE MICE

Because gelatin preparations of ACTH 1–10 but not ACTH 4–10 or ACTH 11–24 influenced fighting behavior in intact male mice, it was regarded important to confirm that they did not influence plasma corticosterone titers 1 or 12 hr after injection (the maximal adrenocortical and behavioral influences of ACTH 1–24 being found at these times in Experiments 1 and 2).

METHOD

Sixty-four male mice which had been isolated for 4 weeks were allocated to categories of 8 individuals. Mice received a single IM gelatin control injection or 0.1 ml of a gelatin preparation containing 20 μg of ACTH 1–10, ACTH 11–24 or ACTH 4–10 one or 12 hr before rapid

TABLE 2

ACUTE EFFECTS OF A 20 μg DOSE OF A GELATIN PREPARATION OF ONE OF 3 ACTH ANALOGUES OR A 250 μg CORTICOSTERONE PREPARATION IN OIL GIVEN 12 HR PREVIOUSLY ON MEAN AGGRESSION TEST SCORES OVER THREE STANDARD OPPONENT TESTS

Treatment	N	% Fighting in at least 2/3 Tests	Attack latency (sec)	AAT (sec)	Number of attacks
Gelatin Control	10	40	331	3.6	6.2
ACTH 1–10	12	83*	169§	10.5§	14.6§
ACTH 11–24	12	50	298	4.0	6.1
ACTH 4–10	11	36	368	1.7	3.6
Oil Control	10	10	403	0.4	1.0
Corticosterone (250 μg)	12	75†	196‡	11.4‡	11.9‡

*differs from gelatin control $p<0.05$ on Fisher's test.
†differs from oil control $p<0.01$ on Fisher's test.
‡differs from oil control $p<0.001$ on Mann-Whitney U test (2 tails).
§differs from gelatin control $p<0.003$ on Mann-Whitney U test (2 tails).

TABLE 3

EFFECTS OF A SINGLE DOSE OF 20 μg OF GELATIN PREPARATIONS OF SOME ACTH RELATED PEPTIDES GIVEN 1 HR OR 12 HR BEFORE SAMPLING ON MEAN ± SE PLASMA CORTICOSTERONE TITERS (μg/100 ML) OF ISOLATED MALE MICE (N = 8 USUALLY)

	1 hr previously	12 hr previously
Injected Material	Mean plasma corticosterone	Mean plasma corticosterone
Gelatin Control	6.6 ± 0.81	7.84 ± 0.86
ACTH 1–24	54.63 ± 2.92*	9.31 ± 0.95
ACTH 1–10	17.90 ± 3.91*	8.09 ± 0.49
ACTH 4–10	6.9 ± 0.62	7.34 ± 1.24
ACTH 11–24	12.93 ± 1.00*	14.02 ± 1.66

*differs from control $p<0.0001$ on 2-tailed Mann-Whitney U test.

decapitation and plasma sampling. The time of injection was adjusted so that the plasma samples were obtained at the same time of day. Total corticosterone titer was determined as described previously.

RESULTS

The mean plasma concentrations of corticosterone ± SE for each experimental category is given in Table 3.

All the gelatin preparations showed a slight but significant augmentation from their appropriate gelatin control with the exception of the 20 μg dose of ACTH 4–10, 1 hr after injection ($p<0.0001$ Mann Whitney U test).

DISCUSSION

It seems that ACTH 4–10 and ACTH 11–24, which appear to have little acute influence on behavior, do not have marked influences on plasma glucocorticoid levels. This could be taken to suggest that their lack of effectiveness is a consequence of their relative inability to stimulate production of glucocorticoids from the adrenal cortex. However, as ACTH 1–10 did augment fighting, the possibility of an extra adrenal influence requiring these 10 amino acids exists. The significant influence at 1 hr of some ACTH-related peptides is worthy of additional investigation and suggests that these short peptides are not always without effect on steroidogenesis in all species. Although the results with ACTH 4–10 were disappointing, the present data does extend observations on the effects of this compound on fighting behavior [3]. Furthermore, ACTH 4–10 is the peptide which is commonly reported to be the active principle in ACTH-mediated effects on conditioned avoidance in rats. Consequently, the negative results in the present tests suggest that peptide-mediated changes in fighting are not simply a consequence of alterations in CAR as was previously suggested [2].

EXPERIMENT 5

AN INVESTIGATION ON THE EFFECTS OF A GELATIN PREPARATION OF ACTH 1–24 ON MOTOR ACTIVITY IN INTACT MALE MICE

It could be argued that the acute augmentation of fighting behavior in ACTH treated mice is a non-specific consequence of changes in motor activity. Greater activity would be expected to result in shorter latencies of attack and more fighting behavior. An experiment to determine the likelihood of this being the case was undertaken.

METHOD

Twenty male TO strain mice which had been individually housed for 4 weeks were allocated to two categories of ten individuals. One category received an 0.1 ml IM injection of gelatin control and the other a gelatin preparation equivalent to 20 μg ACTH 1–24.

Twelve hr after injection, the activity of each mouse was assessed for 7 min in a cage (similar to its own) which was placed on top of an Animex (LKB Ltd) activity recorder set at a sensitivity of 40. Additionally, using automated recording devices, the total time and events for freezing (immobilization), rearing and grooming behaviors which have been related to emotional reactivity were recorded.

RESULTS

The mean Animex activity reading, and mean total durations and events in the three behaviors for the two treatment categories are presented in Table 4. There were no significant differences between these two categories on any of the measures.

DISCUSSION

In spite of the fact that no significant differences between the treatment and control categories could be detected, there was a slight indication, that activity levels were higher in the control treatment category. It consequently seems most unlikely that the acute influence of ACTH 1–24 on fighting behavior can be explained by reference to a non-specific increase in motor activity. A similar lack of influence of two ACTH 4–10's on motor activity has been described in the rat [36].

EXPERIMENT 6

ATTEMPTS TO ASSESS ACUTE ACTIONS OF ACTH 1–24 ON FIGHTING INDEPENDENTLY OF INFLUENCES ON GONADAL FUNCTION OR PHEROMONE PRODUCTION

It is well known that chronic administration of gluco-

TABLE 4

EFFECTS OF ACTH 1–24 OR PLACEBO GIVEN 12 HR PREVIOUSLY ON MEAN ACTIVITY MEASURES IN MALE TO STRAIN MICE (N = 10)

Treatment	ANIMEX Reading	Freeze		Rear		Groom	
		Time (Sec)	Events	Time (Sec)	Events	Time (Sec)	Events
Gelatin Control	526	5.7	2.8	183	91	33.0	6.1
20 μg ACTH 1–24	492	6.1	3.1	177	86	46.3	5.4

N.B. None of the differences reached statistical significance.

corticoids can suppress gonadal function. However, there are also reports that the stress of defeat stimulates a surge of luteinizing hormone (LH) in mice [13, 14] which could acutely augment sex steroids. It is also known [5] that differences in attack in this strain of mouse may be related to androgen-mediated alterations in aggression-promoting pheromone. These possibilities could represent alternative explanations for the apparent acute stimulation of fighting behavior by ACTH 1–24 preparations rather than the behavior being a direct consequence of actions of ACTH glucocorticoids on the CNS.

It was thought to be of utility to show that these possibilities are unlikely by employing a study similar to that reported by Leshner [22] utilizing the recent observation [8] that estradiol benzoate (EB) is at least as effective as testosterone (T) in maintaining fighting behavior in castrated aggressive males. EB, far from stimulating production of aggression-promoting pheromone, has the effect of reducing the attack to which an animal is subjected in the mouse [17].

METHOD

Forty adult males were removed from cohabitation with female mice. These mice were of proved fertility and have been shown in previous studies [8] to be (a) highly aggressive, (b) extremely sensitive to steroid treatment. The mice were housed in individual cages, for a further 14 days, before being bilaterally castrated under ether anaesthesia. The mice were allowed a 3 day recovery period before being allocated to two equal categories receiving replacement with ether 50 μg T per day (an oily solution obtained from Evans Medical Ltd) or 10 μg EB per day (Evans Medical Ltd). These injections were given daily IM over the subsequent 17 days. On the 15, 16 and 17th day of oil-based injections, equal numbers of mice in each category were given an 0.1 ml IM injection containing gelatin control or 20 μg ACTH 1–24 in gelatin. Aggressiveness was assessed in a 7 min standard opponent test 12 hr after these latter injections, the tests taking place in the cleaned home cages of the test animals. Otherwise the conditions and measures obtained were identical with those described in the previous experiments.

RESULTS

The percentages of individuals showing fighting in all 3 tests, together with the mean scores for attack latency, AAT and numbers of attacks in all 4 categories are given in Table 5. Twenty μg of ACTH 1–24 produced a highly significant augmentation of fighting behavior with respect to appropriate control injections in both testosterone and EB maintained animals.

DISCUSSION

These results suggest that the ACTH 1–24-induced augmentation of fighting is not dependent on a suppressive or stimulatory action of the generated glucocorticoids on either production of gonadal hormones or the postulated aggression-promoting pheromone. It is interesting to note that the behavioral influence of ACTH was particularly pronounced in these mice. The results obtained are in contrast to the recently described effects of pituitary-adrenocortical factors on fear-of-being attacked [23, 24, 28]. Moyer and Leshner [28], for example, claim that corticosterone largely maintains avoidance-of-attack levels in mice. It could be argued that either fear-of-being-attacked [24], in the short term, increases intermale attack as a defensive reaction or that the changes are not related to 'fear'. Certainly, the mice used in the present study were behaviorally naive but likely to prove dominant in the encounter with the standard opponent. They may have showed a different response to being attacked. Another suggestion is that the acute and chronic influence of stress hormones on fighting behavior are in opposite directions because they serve different purposes (see below).

GENERAL DISCUSSION

Acute elevation of ACTH, which may be a concomitant of the initial stages of fighting in rodents, augments intermale fighting behavior, an effect which was also obtained by corticosterone administration (250 μg given 12 hr before testing). It seems likely that this influence, which may be a physiological mechanism, is partially a consequence of generated glucocorticoids and is not related to (a) an effect on motor activity; (b) an effect on sex steroid production; and (c) an effect on pheromone production.

The possibility also exists that ACTH 1–10 may exert an extra-adrenal action on this behavior. The effects are most obvious in animals taken from cohabitation with females. One scheme in which both the chronic and the present acute findings with ACTH/glucocorticoids on intermale fighting behavior, both appear logical, involves

TABLE 5

ACUTE EFFECTS OF A DOSE OF ACTH 1-24 ON AGGRESSION SCORES OVER THREE STANDARD OPPONENT AGGRESSION TESTS IN CASTRATED/SEX STEROID MAINTAINED AGGRESSIVE MICE (N = 10)

Daily Dose of sex steroid used	Daily Treatment	% of mice showing fighting in at least 2/3 tests	Mean attack latency (sec)	Mean AAT (sec)	Mean numbers of attacks
50 μg Testosterone	Gelatin Control	30	285.0	6.4	3.5
50 μg Testosterone	20 μg ACTH 1–24	78†	172.5†	22.0†	10.4†
10 μg Estradiol Benzoate	Gelatin Control	0	379.8	1.1	0.8
10 μg Estradiol Benzoate	20 μg ACTH 1–24	78‡	168.5‡	13.3‡	8.3

*differs from appropriate control $p<0.05$ on 2-tailed Mann-Whitney U Test or Fisher's Exact Probability.

†differs from appropriate control $p<0.005$ on 2-tailed Mann-Whitney U Test of Fisher's Exact Probability.

‡differs from appropriate control $p<0.001$ on 2-tailed Mann-Whitney U Test of Fisher's Exact Probability.

distinguishing between single encounters (e.g. by a territorial dominant on his boundary) and repeated encounters (e.g. by a subordinate animal within a group). In the former case it seems of survival value to respond to attack (by a conspecific) by fighting back. In an animal repeatedly subjected to defeat (the latter case), it could be that survival is best served if the animal shows a lowered tendency to fight and reacts to approach by fleeing or showing submissive postures. As this form of attack is generally stressful [7], ACTH and/or glucocorticoids would be generated and could serve the functions described above. Dominant mice generally show a much lower adrenocortical response to fighting than comparable subordinate animals. Consequently dominants may show a transient increase in adrenocortical activity and subordinates a much more prolonged response. These findings are yet another indication that stress hormones can exert potent actions on rodent behaviors. It is likely that the hypothalamic-pituitary-adrenocortical axis will be seen ultimately to be of considerable importance in the modulation of a number of behaviors. These hormones may also prove to be a source of treatments for a variety of behavioral disorders in man and laboratory animals.

ACKNOWLEDGEMENTS

The authors thank Professor E.W. Knight-Jones for facilities in the Department of Zoology, the University College of Swansea. Mr. R. Guntrip is thanked for the care of animals. The ACTH analogues employed in this study were kind gifts of Dr. H.M. Greven of Organon N.V., Oss, The Netherlands. Miss J.F. Goldsmith and Mr. N.J. Bowden are gratefully thanked for technical assistance.

REFERENCES

1. Banerjee, U. The influence of some hormones and drugs on isolation-induced aggression in male mice. *Communs Behav. Biol.* **6**: 163–170, 1971.
2. Brain, P. F. The possible role of the pituitary-adreno-cortical axis in aggressive behavior. *Nature* **233**: 489, 1971.
3. Brain, P. F. Mammalian behavior and the adrenal cortex – a review. *Behav. Biol.* **7**: 453–477, 1972.
4. Brain, P. F. and C. M. Evans. Some recent studies on the effects of ACTH on agonistic behavior in the house mouse and the golden hamster. *J. Endocr.* **57**: xxix–xl, 1972.
5. Brain, P. F. and C. M. Evans. Effects of androgens on the attackability of gonadectomized mice by TO trained fighter individuals: confirmatory experiments. I.R.C.S. *(Endocr. Neurobiol. Physiol. Psychol.)* **2**: 1730, 1974.
6. Brain, P. F. and A. E. Poole. Some studies on the use of 'standard opponents' in intermale aggression testing in TT albino mice. *Behavior* **50**: 100–110, 1974.
7. Brain, P. F. and A. E. Poole. The role of endocrines in isolation-induced intermale fighting in albino laboratory mice 1. Pituitary-adrenocortical influences. *Aggress. Behav.* **1**: 39–69, 1974.
8. Brain, P. F. and A. E. Poole. The role of endocrines in isolation-induced intermale fighting in albino laboratory mice II. Sex steroid influences in aggressive mice. *Aggress. Behav.* **2**: 55–76, 1976.
9. DeWied, D. Inhibitory effects of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. *Proc. Soc. exp. Biol. Med.* **122**: 28–32, 1966.
10. DeWied, D. Opposite effects of ACTH and glucocorticoids on extinction of conditioned avoidance behavior. In: *Proc. 2nd Int. Congress on Hormonal steroids,* edited by L. Martini, F. Fraschini and M. Motta. Netherlands: Mouton, 1967.

11. DeWied, D. Effects of peptide hormones on behavior. In: *Frontiers in Neuroendocrinology*, edited by W. F. Ganong and L. Martini. London: Oxford University Press, 1969, pp. 97–140.
12. Dessi-Fulgheri, F., C. Lupo Di Prisco and P. Valeri. Uptake of corticosterone by the brain of rats reared under different conditions. First European Neurosciences Meetings (Munich), 1975, p. 448.
13. Eleftheriou, B. E. and R. L. Church. Effects of repeated exposure to aggression and defeat on plasma and pituitary levels of luteinizing hormone in C57BL/6J mice. *Gen. comp. Endocr.* **9**: 263–266, 1967.
14. Eleftheriou, B. E. and R. L. Church. Levels of hypothalamic luteinizing hormone-releasing factor after exposure to aggression (defeat) in C57BL/6J mice. *J. Endocr.* **42**: 347–348, 1969.
15. Glick, D., D. Von Redlich and S. Levine. Fluorometric determination of corticosterone and cortisol in 0.02–0.05 milliliters of plasma and submilligram samples of adrenal tissue. *Endocrinology* **74**: 653–655, 1964.
16. Goldsmith, J. F., P. F. Brain and D. Benton. Effects of age at differential housing and the duration of individual housing/grouping on intermale fighting behavior and adrenocortical activity in TO strain mice. *Aggress. Behav.* **2**: 307–323, 1976.
17. Haag, C., B. Jerhoff and J. K. Kirkpatrick. Ovarian hormones and their role in aggression inhibition among mice. *Physiol. Behav.* **13**: 175–177, 1974.
18. Kaada, B. Brain mechanisms related to aggressive behavior. In: *Aggression and Defense: Neural Mechanisms and Social Patterns*, edited by C. D. Clemente and D. B. Lindsley. Berkeley: University of California Press, pp. 95–133.
19. Klein, S. B. ACTH-induced reactivation of prior active avoidance training after intermediate intervals in hypophysectomized, adrenalectomized and sham-operated rats. *Physiol. Psychol.* **3**: 395–399, 1975.
20. Kostowski, W., W. Rewerski and T. Piechocki. Effects of some steroids on aggressive behavior in mice and rats. *Neuroendocrinology* **6**: 311–318, 1970.
21. Kraicer, J. and R. G. Conrad. Duration of action of two 'long-acting' adrenocorticotrophin preparations in the rat. *Can. J. Physiol. Pharmac.* **49**: 774–776, 1971.
22. Leshner, A. I. The adrenals and testes: two separate systems affecting aggressiveness. *Hormones* **5**: 272–273, 1972.
23. Leshner, A. I. A model of hormones and agonistic behavior. *Physiol. Behav.* **15**: 225–235, 1975.
24. Leshner, A. I., J. A. Moyer and W. A. Walker. Pituitary adrenocortical activity and avoidance-of-attack in mice. *Physiol. Behav.* **15**: 689–693, 1975.
25. McEwen, B. S., J. M. Weiss and L. S. Schwartz. Uptake of corticosterone by rat brain and its concentration by certain limbic structures. *Brain Res.* **16**: 227–241, 1969.
26. McEwen, B. S., J. M. Weiss and L. S. Schwartz. Retention of corticosterone by cell nuclei from brain regions of adrenalectomized rats. *Brain Res.* **17**: 471–482, 1970.
27. McEwen, B. S., V. N. Luine, L. Plapinger and E. R. De Kloet. Putative estrogen and glucocorticoid receptors in the limbic brain. *J. Steroid Biochem.* **6**: 971–977, 1975.
28. Moyer, J. A. and A. I. Leshner. Pituitary-adrenal effects on avoidance-of-attack in mice: separation of the effects of ACTH and corticosterone. *Physiol. Behav.* **17**: 297–301, 1976.
29. Pradhan, S. N. Aggression and central neurotransmitters. In: *International Review of Neurobiology*, edited by C. C. Preiffer and J. R. Smythies. New York: Academic Press, 1975, pp. 213–262.
30. Seigel, S. *Non-Parametric Statistics.* New York: McGraw-Hill, 1956.
31. Steiner, F. A. Local effects of adrenal steroids on cerebral neurons. In: *Steroid Hormones and Brain Function*, edited by C. H. Sawyer and R. A. Gorski. Berkeley: University of California Press, 1971, pp. 43–49.
32. Steiner, F. A., K. Ruf and K. Akert. Steroid-sensitive neurones in rat brain: anatomical localization and responses to neurohumours and ACTH. *Brain Res.* **12**: 74–85, 1969.
33. Strand, F. L., H. Stoboy and A. Cayer. A possible direct action of ACTH on nerve and muscle. *Neuroendocrology* **13**: 1–20, 1973/74.
34. Taylor, A. N., G. K. Matheson and N. Dafny. Modification of the responsiveness of components of the limbic-midbrain circuit by corticosteroids and ACTH. In: *Steroid Hormones and Brain Function*, edited by C. H. Sawyer and R. A. Gorski. Berkeley: University of California Press, 1971, pp. 67–78.
35. Van Delft, A. M. L. and J. I. Kitay. Effect of ACTH on single unit activity in the diencephalon of intact and hypophysectomized rats. *Neuroendocrology* **9**: 188–196, 1972.
36. Wolthuis, O. L. and D. De Wied. The effect of ACTH-analogues on motor behavior and visual evoked responses in rats. *Pharmac. Biochem. Behav.* **4**: 273–278, 1976.