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# Effects of Social Conflict on POMC-Derived Peptides and Glucocorticoids in Male Golden Hamsters[1,2]

KIM L. HUHMAN, BRADFORD N. BUNNELL,* EDWARD H. MOUGEY AND JAMES L. MEYERHOFF

*Department of Medical Neurosciences, Division of Neuropsychiatry*
*Walter Reed Army Institute of Research, Washington, D.C. 20307-5100*
**Department of Psychology, University of Georgia, Athens, GA 30602*

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Aggression Agonistic behavior Hamsters Adrenocorticotropin Cortisol Beta-endorphin Stress

AGGRESSION is thought to be modulated by various hormones such as the endogenous opioid peptides and the hormones of the hypothalamic-pituitary-adrenocortical (HPA) axis. However, the data have been contradictory in part because so many different approaches have been used [for review, see (35,47)]. Brain (8) pointed out the need for systematic study of the hormonal correlates of aggression in a large number of species in order to establish a generalizable profile of the neuroendocrine concomitants of aggressive behavior.

Hamsters are a useful and interesting species with which to examine the neuroendocrine concomitants of aggression or, more broadly, agonistic behavior for several reasons. In the laboratory, both male and female hamsters are highly aggressive spontaneously (43). Hamsters do not require artificial stimulation or complex paradigms to elicit agonistic behavior. This is in contrast to laboratory rats, which usually require footshock or labor-intensive paradigms such as the colony-intruder model (4) to induce the desired behaviors. Males of some strains of mice are highly aggressive, but they are too small to allow the collection of enough blood to complete multiple hormone assays. In addition to the fact that both sexes are aggressive, hamsters differ from rats and mice in that the usual sexual dimorphism of adrenal gland size is reversed. In hamsters, males exhibit larger adrenals, whereas the reverse is true in rats and mice (17,50).

Early studies indicated that the gonadal hormones had both organizational and activational effects on aggressiveness in rodents (7–9). The hormones of the HPA axis were subsequently shown to have important effects as well. For example, Brain (6) found that isolation increased adrenal weights and aggressiveness in golden hamsters. Louch and Higginbotham (28) and Bronson and Eleftheriou (11) showed that dominant mice had lower plasma corticosterone than subordinates. Acute adrenocorticotropin (ACTH) treatment increased fighting in mice, apparently through an increased release of glucocorticoids, while chronic treatment decreased aggression presumably via a direct mechanism in the CNS (8,9). Brain and Evans (10), however, reported that ACTH treatment had no effect on fighting behavior in hamsters.

The use of paradigms involving social conflict between conspecifics has also been advocated as a way to explore the actions of endogenous opiate systems (49). Beta-endorphin, for example, increases in plasma in rats following exposure to a stressor such as footshock (20), but little is known about biologically relevant

[1]The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (par 4-3, AR 360-5).
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situations in which endorphins are released. Miczek *et al.* (36) found that following defeat, submissive mice showed delayed tailflick responses to nociceptive challenge and that this analgesia was blocked by the opioid antagonists naloxone and naltrexone. The quaternary form of naltrexone, which does not pass the blood-brain barrier, failed to block the defeat-induced analgesia indicating that the effect was due to a central mechanism. Rodgers and Hendrie (48) tested pain responsiveness in both Sprague-Dawley and Listar rats following social conflict, but found no analgesia. The rats displayed much less aggressive behavior than did the mice in the Miczek *et al.* study, and the authors felt that the milder aggression might account for the lack of opiate activation. The response of the endogenous opioid peptides in fighting should be examined directly following exposure to social conflict in a species that normally exhibits more fighting than do rats.

The present experiments were designed to examine changes in plasma ACTH, cortisol, corticosterone, beta-endorphin, and beta-lipotropin in golden hamsters following an agonistic encounter with a novel conspecific. We were interested in whether these hormones respond to the stimulation of fighting in golden hamsters and if the time course or pattern of this response is different from that following exposure to a known stressor—footshock. In addition, Leshner (26), among others, has suggested that the hormonal response should be greater in animals that "lose" than in those that "win." The analgesia displayed by submissive mice (36) is consistent with this hypothesis. Thus, we were interested in determining whether there was a discernable hormonal activation in submissive hamsters (Experiment 1) and, if so, whether there was a difference between the response of the submissive hamsters and that of the dominant hamsters (Experiment 2).

## GENERAL METHOD

### *Subjects*

The experimental animals were male golden hamsters that weighed 120–190 g and were 90–120 days old at the beginning of each experiment. The animals in Experiment 1 were obtained from the University of Georgia breeding colony (outbred Lakeview hamsters crossed with hamsters from Charles River Laboratories). The hamsters in Experiment 2 were obtained from Charles River Laboratories. All hamsters were individually housed for at least three weeks (range three through five weeks) in a temperature-controlled colony room on a 14:10-hr light:dark cycle with lights off at 1100 hr. Individual housing has been shown to increase aggressive behavior in hamsters and is a commonly employed methodology in studies examining aggressive behavior (6, 23, 25). Food and water were available ad lib except during testing. In Experiment 1 the resident hamsters were ovariectomized females (182–215 g) approximately 300 days old. All animals were handled for at least two weeks before the beginning of each experiment to allow them to habituate to the experimenter.

### *Surgery*

Chronic, indwelling catheters were placed in the right jugular vein of all male hamsters used in Experiment 1. Surgery was performed using sodium pentobarbital anaesthesia (90 mg/kg). Catheters consisted of 3 cm of Silastic tubing overlapping 1.5 cm with Intramedic Polyethylene Tubing (PE-50). The catheter was inserted 3 cm into the right jugular vein and tied into place with 3 small loops of 5.0 surgical silk. The PE-50 tubing was then passed subcutaneously, externalized in the midscapular region and secured to the animal's back with a small piece of Velcro (3.0 × 1.5 cm). The catheters were filled with heparinized saline and were flushed daily to maintain patency. Behavioral testing began 48 hr after surgery. We have found this recovery period to be sufficient to enable neuroendocrine measures to approach baseline values (unpublished data).

Ovariectomy of the opponent females used in Experiment 1 was completed at least four weeks before testing.

### *Agonistic Encounters and Behavioral Scoring*

Hamsters were grouped by weight and randomly assigned to experimental or control groups. All behavioral testing occurred during the first two hr of the dark phase of the daily cycle in order to minimize the effects of circadian variation of the neuroendocrine measures (2,23). This is also the time when hamsters normally exhibit most of their fighting behavior (23). Agonistic encounters took place in the home cage of one of the hamsters (the ovariectomized female in Experiment 1) using a resident-intruder paradigm (48). All agonistic encounters were 15 min in duration unless otherwise noted as in Experiment 2. In addition, any encounter in which a hamster was bitten hard enough to bleed was immediately terminated in order to minimize possible painful stimulation. The intruder was returned to its home cage until the time preset for blood collection (encounters had to be stopped only twice during this study). All encounters took place under normal room illumination. Landau (23) demonstrated that illumination conditions do not affect the behavior during a social encounter if that encounter occurs during the first few hours of the dark phase of the daily cycle. Encounters were scored by two observers (one of which was blind as to which animal was the intruder—interobserver reliability >95%) using a behavioral inventory (see Table 1) derived from several existing inventories (12, 18, 25). Animals producing behaviors such as flee, tooth chatter, tail lift, and full submissive posture were rated submissive while animals producing behaviors such as offensive postures, chase, and attack were labelled dominant. Lerwill and Makings (25), among others, found that dominance in hamsters is usually established quite rapidly during the first encounter between a particular pair. Once submissive behavior, especially the full submissive posture, is elicited from one of the animals, the dominance relationship for that pair is decided. If an encounter did not lead to an appreciable level of agonistic behavior, however, and a dominant-subordinate relationship was not established, the hamsters were rated as "neither" and their hormonal data were not used in the study. In all cases in Experiment 1 and in ambiguous cases (uncertainty about the rating by one or both observers or a rating of "neither") in Experiment 2, the behavioral assessments were verified using a videotape of the encounter. At the time of videotape review, the observers were blind to the results of the hormonal assays.

### *Controls*

The two control conditions were a) novel cage controls and b) home cage controls. The novel cage condition consisted of placing a hamster in an empty cage recently occupied by an opponent hamster. This group was included to control for the effects of handling and being placed in a strange cage as well as being exposed to the resident's odor. Home cage controls remained undisturbed in their home cages until they were removed for collection of blood samples.

### *Blood Collection, Tissue Preparation, and Biochemical Assays*

Immediately following each trial, the "intruder" hamsters were returned to their home cages and either 2 cc of venous blood was collected via the jugular catheter or trunk blood was collected

TABLE 1

HAMSTER BEHAVIORAL CATEGORIES AND OPERATIONAL DEFINITIONS*

| |
|---|
| Nonsocial |
| Locomotor exploratory (walking about cage) |
| Self grooming |
| Nesting (picking up or pouching nesting material) |
| Feeding (picking up or pouching chow) |
| Sleeping (characteristic crouched position with eyes closed) |
| Social Orientating-Investigating |
| Attend |
| Approach |
| Investigate |
| Nose |
| Defensive |
| Defensive posture (side and upright) |
| Boxing (standing on hind legs with mutual pushing with forepaws) |
| Aggressive |
| Offensive posture (upright and side) |
| Chase |
| Bite |
| Attack (combination of chase and bite, i.e., rapid pursuit with biting) |
| Submissive |
| Tail lift (with or without hindlimb adduction) |
| Flee (includes those behaviors defined as flight, retreat, or escape—rapid movement away from opponent or attempt to escape from cage) |
| Full submissive posture (lies unmoving on back with limbs spread sometimes even after the opponent has moved away) |
| Fight |
| Fight (also called rolling or locked fighting—usually includes mutual biting) |

*This behavioral inventory was adapted from (12, 18, 25). Operational definitions are given when the present usage is different from the earlier papers.

after decapitation (blood collection occurred within five minutes of the end of each trial). Residents and intruders were sampled in a counterbalanced order. (We have found that the hormones measured in these experiments are not affected by a single 2 cc withdrawal, and that hematocrit and hormonal values return to baseline values within seven days after initial sampling.) Blood was collected in heparinized beakers or syringes containing 0.05 ml of aprotinin, a protease inhibitor. Blood was spun in a refrigerated centrifuge and plasma was stored at −70°C until measured by radioimmunoassay. ACTH, β-EP, and β-LPH were all measured in this study because, even though they are colocalized in the same secretory granules in anterior pituitary corticotroph cells (54) and are released concomitantly upon exposure to a stressor (18), there is a possibility of β-EP release from the intermediate lobe of the pituitary (5,44) or of differential post-release processing of the endorphins. In addition, we have found that the β-EP/β-LPH ratio in hamster plasma is different from that seen in humans or rats (38).

ACTH was assayed using an RIA kit for human ACTH supplied by INCSTAR Corporation (Stillwater, MN; Cat. No. 24130). The ACTH antiserum was made in rabbits against human $ACTH_{1-24}$. This antiserum has been shown to cross-react with many other species' ACTH (INCSTAR Corp., unpublished data). Since we do not have a purified sample of hamster ACTH, we cannot state with certainty that it cross-reacts quantitatively with this antiserum, however, most of the species differences in ACTH occur in the portion of the molecule beyond that with which the antiserum reacts (the species differences occur in the $ACTH_{25-39}$ portion). The standard used in the assay was human $ACTH_{1-39}$. Human $ACTH_{1-24}$ cross reacts 100% as does porcine $ACTH_{1-39}$. There is virtually no cross reaction with alpha-melanocyte stimulating hormone, endorphins, enkephalins, follicle stimulating hormone, vasopressin, or oxytocin. Assay sensitivity was 6 pg/ml. The intraassay coefficient of variation was 3%, and the interassay coefficient of variation was 8%. The values will be reported as ACTH-like immunoreactivity (ACTH-LI).

Plasma was assayed for cortisol and corticosterone using antibodies developed at Walter Reed Army Institute of Research (37). Recovery of cortisol and corticosterone were 93% and 88%, respectively. Within assay variation was 3%, and between assay variation was less than 12%.

The antiserum used to assay the endorphins was generated in rabbits against human β-EP. This antibody exhibited a high degree of cross-reactivity with camel β-EP (93%). Consequently, camel β-EP standard (Peninsula Labs.) and iodinated marker were used for assaying small animal plasmas since it more closely approximates rodent β-EP in molecular structure. Using the camel β-EP system of standard and iodinated marker, rat β-EP (Peninsula Labs.) exhibited 89% cross-reaction, porcine β-EP 71%, human β-EP 86%, human β-LPH 21%, and human $\beta\text{-EP}_{1-27}$ 35% cross-reaction. There was virtually no cross-reaction with alpha-endorphin, the enkephalins, or ACTH. The antibody, therefore appears to be directed toward the C-terminal half of the molecule. Values will be reported as β-EP-like immunoreactivity (β-EP-LI). Hamster plasma and distilled water were added to 150 mg of bulk $C_{18}$ material (Waters Associates, Cat. No. 51150) and mixed in 12×75 mm tubes. Following centrifugation, the supernatant and three subsequent 1-ml water rinses were discarded. β-LPH was eluted with two 1-ml rinses of acetone:water (1:1) and β-EP was eluted from the adsorbent with two 1-ml rinses of acetone: water:trifluoroacetic acid (80:19:1). All eluates were evaporated to dryness under nitrogen and assayed. The β-LPH values represent β-EP-LI equivalents and will be reported as β-LPH-LI. To prove separation of the two components, we assayed hamster plasma pool before and after the addition of camel β-EP standard or highly purified rat β-LPH (isolated from rat pituitaries). This was done in each assay. Approximately 5% of the β-EP-LI added appeared in the β-LPH-LI fraction, and vice versa. The separation for the solvent system reported here was confirmed using iodinated forms of these peptides. Recoveries of camel β-EP and rat β-LPH from hamster plasma were 74% and 68%, respectively, and all values were corrected for recovery. Assay sensitivity was 3 pg/tube. The intraassay and interassay coefficients of variation were 6% and 12%, respectively.

## SPECIFIC EXPERIMENTAL PROCEDURES

### *Experiment 1*

We were interested initially in determining whether submission was associated with a significant neuroendocrine response in hamsters. Thus, this experiment was a pilot study designed to maximize the chances that the experimental hamsters would become submissive. Accordingly, we examined the effect of a 15-min agonistic encounter with an ovariectomized female on plasma hormone levels in catheterized male golden hamsters. Ovariectomized females were used as opponents because they are likely to be aggressive and to be dominant over males (53), especially when the female's body weight is greater (30). During the encounter the hamster's behavior was scored by an observer and a rating of dominant, submissive, or neither was assigned to each hamster. The encounters were also videotaped and all of the behavioral assessments were later verified by an observer that was blind to the results of the hormonal assays. At the end of each encounter, the males were returned to their home cages and 2 cc of blood was collected within 5 min via the jugular catheter. Control animals were placed in novel cages for 15 minutes as described above. Blood was collected after each control hamster was returned to its home cage. Twenty-three (11 experimental and 12 control) hamsters were used in this experiment. ACTH, cortisol,

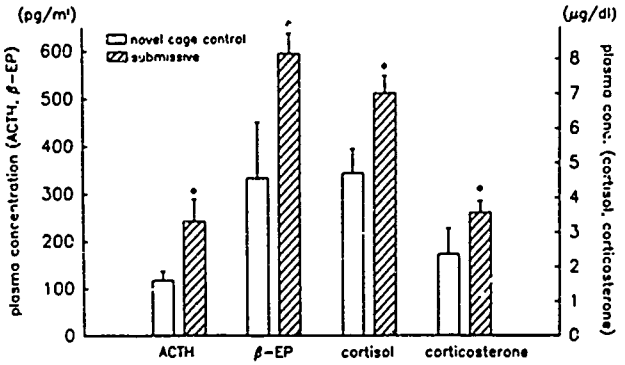


FIG. 1. Effect of defeat on plasma adrenocorticotropin-like immunoreactivity (ACTH-LI), beta-endorphin-like immunoreactivity (β-EP-LI), cortisol and corticosterone. Each bar represents the mean and standard error of the mean of eight observations with the exception of β-EP-LI (four observations each) *Indicates that the submissive hamsters exhibited significantly higher (one-way analysis of variance, $p<0.05$) hormonal values than did the hamsters that were placed in the empty cage of a resident ovariectomized female (novel cage controls).

and corticosterone were assayed in all eight submissive animals and eight controls. Because the β-EP/β LPH assay required the largest amount of plasma, we were only able to measure these hormones in four experimental and four control animals.

*Results and Discussion*

The paradigm used in this experiment was effective in producing submissive behavior in the experimental males. ACTH-LI, cortisol, corticosterone, and β-EP-LI rose in plasma, as expected, following exposure of the experimental male to an aggressive female. The apparent rise in plasma β-LPH-LI was not significant.

*Behavioral.* Eight of 11 experimental males were classified as submissive. In two cases there was no appreciable agonistic behavior and, in one encounter, the male was dominant. Data from these three animals were discarded. Pearson's correlation coefficients were calculated to examine the relationship between the frequency of submissive behaviors produced and the hormonal levels measured. The frequency of submissive behaviors (such as flee, tail lift, and full submissive posture) shown by the eight submissive males was correlated only with plasma levels of ACTH-LI ($r=.752$, $p<0.01$).

*Hormonal.* The hormonal values obtained in Experiment 1 are shown in Fig. 1. Analysis of variance indicated that ACTH-LI was higher in the submissive hamsters, $F(1,14)=6.28$, $p<0.05$, as was β-EP-LI, $F(1,6)=6.56$, $p<0.05$. Cortisol, $F(1,14)=8.13$, $p<0.025$, and corticosterone, $F(1,14)=7.82$, $p<0.025$, were also elevated in the experimental males. Although β-LPH-LI tended to be higher in submissive hamsters [novel cage control $=48.3\pm11.3$ and submissive $=67.4\pm17.4$ pg/ml; $F(1,6)=0.671$, $p>0.05$], this trend was not statistically significant.

In general, the increase in the plasma hormones was in accordance with Leshner's (26) hypothesis that "losing" should be associated with an activation of the HPA axis and a release of endogenous opiates. The increase in ACTH and the glucocorticoids also indicated that social conflict between conspecifics could be viewed as a biologically relevant stressor in hamsters as well as in mice (34).

β-LPH-LI did not respond as strongly to losing as did ACTH-LI and β-EP-LI. Again, these hormones are derived from a common precursor, proopiomelanocortin (POMC) (29,45), and are stored together in anterior pituitary corticotroph cells (54). Further, the POMC-derived peptides are released concomitantly upon exposure to a stressor (19). However, other investigators have reported differences in the responsiveness of β-LPH relative to the other POMC peptides (13). It may be that there is important postrelease processing of β-LPH. An additional pool of β-EP neurons in the intermediate lobe of the hamster pituitary could also account for the dissociation (44). A more likely explanation is that we did not assay enough samples for β-LPH to be able to detect a significant change. We reexamined the β-LPH response to fighting in Experiment 2.

The responses of cortisol and corticosterone were comparable in magnitude. As expected, plasma concentrations of both hormones increased following defeat. Ottenweller *et al.* (41) reported that cortisol is the most responsive glucocorticoid in hamsters following exposure to a stressor, although corticosterone exhibited a significant increase as well. No additional information was obtained by measuring both glucocorticoids. Therefore, it was decided that only cortisol would be measured in Experiment 2 in order to conserve plasma for the other radioimmunoassays.

*Experiment 2*

The purpose of this experiment was to compare directly the hormonal response to fighting in dominant and submissive hamsters in order to assess any differences in the neuroendocrine reactivity in the two groups. In addition, the magnitude of the hormonal response to social encounters was compared to the response in a separate group exposed to a footshock stressor. We were interested in determining if the magnitude and time course of hormonal stimulation following fighting was the same as that following footshock which would suggest that fighting might be viewed simply as a nonspecific stressor.

The subjects were 77 male hamsters that were naive to behavioral testing. These animals had been individually housed for at least three weeks before testing. On the day before the beginning of the experiment, all of the animals were grouped by weight and randomly assigned to one of 10 groups. The control groups consisted of a home cage group ($N=8$) and three novel cage groups (each with $N=6$). The latter remained in the novel cages for either 5, 15, or 30 minutes after which they were immediately decapitated. Nine hamsters were divided into three footshock groups in which the animals were placed in sound-attenuated shock chambers for 5, 15, or 30 minutes. These animals were subjected to intermittent 5-second, 1.5-mA scrambled footshocks delivered on a 30-second variable time schedule. The remaining 42 hamsters were evenly divided into 6 groups. These subjects were residents and intruders that were paired for either 5, 15, or 30 minutes. The animals were observed and assigned the label of dominant or submissive as in Experiment 1. If the animals did not establish a clear dominance relationship during the encounter, then that pair was not included in the experiment. The encounters were videotaped and the behavioral ratings confirmed at a subsequent viewing as described above. At the end of each agonistic encounter, the intruder was returned to its home cage and the animals were sacrificed (the order of sacrifice was counterbalanced in the agonistic groups). To insure that the testing and blood collection was completed within a narrow circadian window, the experiment was carried out over six days. The experimental schedule was counterbalanced over the days to prevent any difference between the groups due to time or day of sacrifice.

*Results and Discussion*

Both submission and footshock were associated with clear

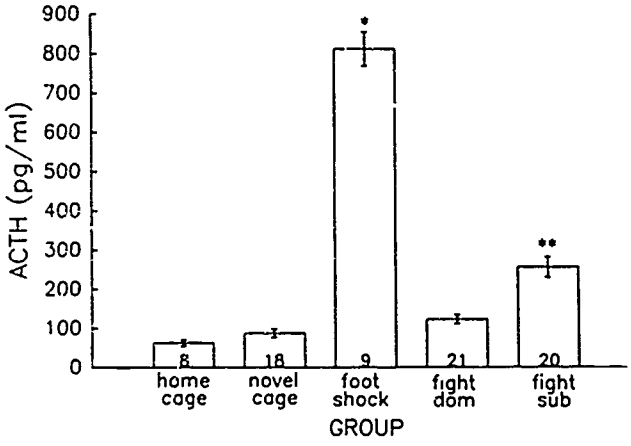


FIG. 2. Effect of footshock, dominance (DOM), and submission (SUB) on plasma adrenocorticotropin-like immunoreactivity (ACTH-LI). Each bar represents the mean and standard error of the mean. Group N's are given in each bar (5, 15, and 30 min groups were pooled because there were no differences between them). *Greater than all other groups (Tukey's, $p<0.05$). **Greater than dominant and home cage and novel cage controls (Tukey's, $p<0.05$).

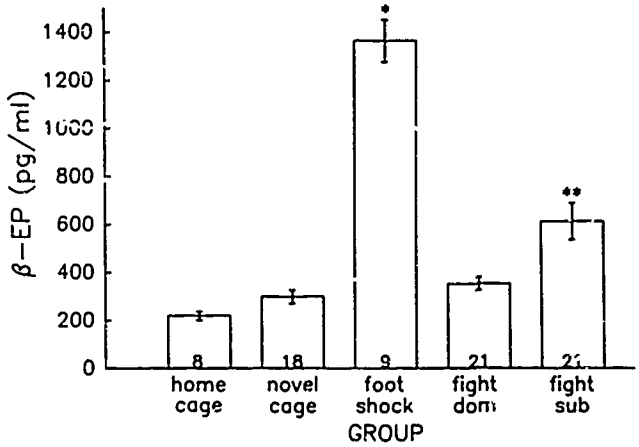


FIG. 3. Effect of footshock, dominance (DOM), and submission (SUB) on plasma beta-endorphin-like immunoreactivity (β-EP-LI). Each bar represents the mean and standard error of the mean. Group N's are shown in each bar (pooled 5, 15, and 30 min groups). *Greater than all other groups (Tukey's, $p<0.05$). **Greater than dominant and home cage and novel cage controls (Tukey's, $p<0.05$).

increases in the circulating hormones. Fighting did not appear to be a nonspecific stressor; the cues (either psychological, physiological or a combination of both) associated with losing seemed to be crucial for the activation of the POMC peptides and cortisol. These data support Leshner's (26) hypothesis that "winning" a fight should not be particularly stressful in terms of pituitary-adrenocortical and opioid activation. There were no significant differences within treatments in any of the hormonal values at the three time points (even cortisol was already elevated in the 5-min submissive and shock groups), so the three groups were collapsed over time for further statistical analysis.

The hormonal values observed in Experiment 2 were similar to those obtained in Experiment 1 in terms of baseline values and magnitude of change following social conflict. This similarity occurred despite the fact that blood was sampled via jugular catheters in Experiment 1 and via decapitation in Experiment 2, indicating that the two methods produced comparable results in terms of plasma POMC-derived peptide and cortisol levels.

*Hormonal.* Plasma ACTH-LI levels from Experiment 2 are shown in Fig. 2. One-way analysis of variance indicated a significant effect of treatment on circulating levels of ACTH-LI, $F(4,72)=144.72$, $p<0.01$. Pairwise comparisons indicated that ACTH-LI levels were greater following footshock than they were after any other treatment, and that ACTH-LI levels in submissive hamsters were significantly higher than in dominant animals, novel cage controls, and home cage controls (Tukey's, $p<0.05$). Plasma β-EP-LI levels are shown in Fig. 3. There were, again, significant differences between the groups, $F(4,75)=53.59$, $p<0.01$, with the footshock group exhibiting higher β-EP-LI levels than all other groups and the submissive animals having higher β-EP-LI than the dominant and control animals ($p<0.05$). Plasma β-LPH-LI values are shown in Fig. 4, $F(4,75)=93.17$, $p<0.01$. Although the changes in β-LPH-LI levels are similar to those of ACTH-LI and β-EP-LI in that there is a large increase in β-LPH-LI in the footshock animals and a smaller increase in the submissive animals, there was not a significant difference between the response of the dominant and subordinant hamsters. The additional finding of a difference in the responsiveness of β-LPH-LI from that of ACTH-LI and β-EP-LI indicates that there may be some important differences in the release or processing of the POMC peptides.

There was also a significant effect of treatment on cortisol levels, $F(4,75)=28.31$, $p<0.01$. As shown in Fig. 5, plasma cortisol was significantly elevated in the footshock animals. The submissive hamsters also exhibited cortisol levels that were higher than controls, but there was not a significant difference between the dominant and submissive hamsters. This result may not be surprising because the glucocorticoids are thought to respond in a fairly nonspecific way to challenges faced by an organism (51). It has been suggested that glucocorticoids are not sensitive indicators of the intensity of arousal because the adrenal gland is most sensitive to ACTH at basal concentrations (39). Furthermore, there could be a ceiling effect on glucocorticoid responsiveness (24). The latter is clearly not the case in this experiment

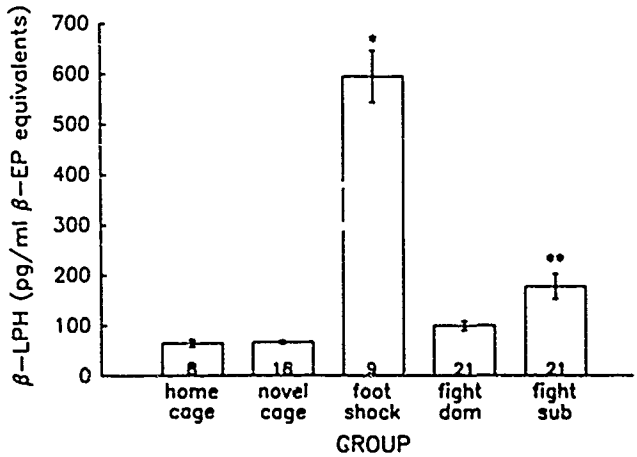


FIG. 4. Effect of footshock, dominance (DOM), and submission (SUB) on plasma beta-lipotropin-like immunoreactivity (β-LPH-LI). Each bar represents the mean and standard error of the mean. Group N's are given in each bar (pooled 5, 15, and 30 min groups). *Greater than all other groups (Tukey's, $p<0.05$). **Greater than home cage and novel cage controls (Tukey's, $p<0.05$).

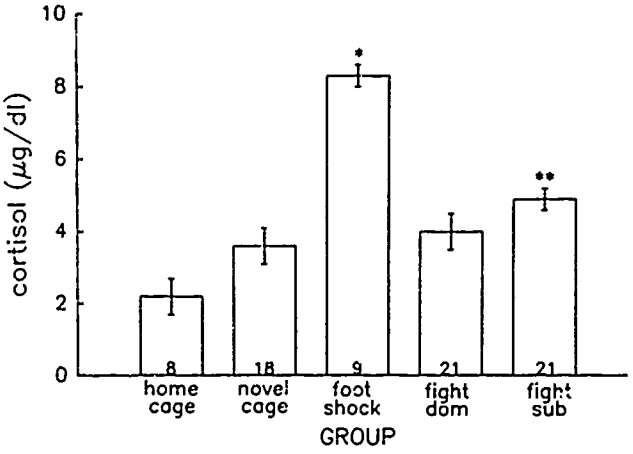


FIG. 5. Effect of footshock, dominance (DOM), and submission (SUB) on plasma cortisol. Each bar represents the mean and standard error of the mean. Group N's are given in each bar (pooled 5, 15, and 30 min groups). *Greater than all other groups (Tukey's, $p<0.05$). **Greater than home cage and novel cage controls (Tukey's, $p<0.05$).

because the footshock values are almost twice as high as the values seen in the animals that fought. The hormonal response of golden hamsters to physical and psychological stressors has not received much attention to date. Ottenweller *et al.* (41) reported that cortisol and corticosterone increase in plasma following exposure to a restraint stressor. The cortisol values measured in this experiment were slightly higher than in the above study. In the Ottenweller study, however, blood was collected during the light phase of the daily cycle when circulating glucocorticoids are naturally lower. Our baseline values are in accordance with the early dark phase values reported by Albers *et al.* (2) who charted the circadian rhythm of cortisol and corticosterone in hamster plasma. We have also shown that ACTH-LI, cortisol, β-EP-LI and β-LPH-LI exhibit large increases in hamster plasma following footshock. There are no reports in the literature with which to compare these values.

## GENERAL DISCUSSION

The hamster model used in this study appears to be valuable for the investigation of the hormonal correlates of agonistic behavior. With this paradigm, little preparation is needed before the animals are paired, and no artificial stimulation is required to stimulate fighting in hamsters. Fighting, in itself, does not appear to be a nonspecific stressor in terms of significant hormonal release. As hypothesized, the particular experience of defeat appears to be associated with opiate and pituitary-adrenocortical activation. This finding supports Leshner's (26) hypothesis that "losing" should be associated with activation of these axes, and is consistent with Bronson and Eleftheriou's (11) report that corticosterone is elevated in submissive mice. In addition, our findings are consistent with those of Miczek *et al.* (36) who reported an opioid analgesia in defeated mice.

One surprising detail about our use of the resident-intruder paradigm is the fact that, in our study, residence did not necessarily confer dominance. Residents were dominant in about 50% of the trials. Miczek (34) reported that resident mice are almost always dominant over intruders, and Payne (42) reported the same for golden hamsters. The animals in the Payne study, however, were individually housed much longer than in our experiments, and Payne's hamsters were at least 300 days old at the time of pairing whereas ours were about 120 days. In addition, our animals were matched for weight. Any of these three factors (age, time of individual housing, or weight) might account for the difference in the studies. Each of these factors could be examined in subsequent experiments.

This paradigm may also be valuable in exploring biologically relevant situations in which the endogenous opioid peptides are activated. The opioid peptides are thought to play a role in the organism's global response to a stressor possibly by influencing neural systems that mediate sensory, motivational, and affective processes (3). Similarly, LaMotte *et al.* (22) suggested that endorphins influence emotional tone following exposure to a stressor and allow the animal to adjust to the stressful conditions. Finally, an endogenous pain-inhibitory system might have adaptive significance in situations in which responding to the pain might disrupt behavioral performance (1). Our study did not evaluate these contentions directly, but the fact that submission does lead to increases in β-EP is consistent with these hypotheses.

Increases in plasma levels of POMC-derived peptides have been reported in response to psychological stressors in both humans (33) and rats (32). Physical exertion is associated with increases in plasma β-EP and ACTH in humans (40). In the present study, although both dominant and submissive hamsters displayed exertion (chase in dominant animals, flee in submissives, and rolling fights with mutual biting) during the behavioral test, β-EP-LI and ACTH-LI were elevated only in the submissive animals. This selective activation in submissive animals reinforces the notion that the hormonal response to some stressors may vary depending on environmental cues and is not always a reflection of nonspecific activation or arousal.

In addition to regulating behavioral responses in stressful situations (21), the hormones of the HPA axis may influence learning (14,15). Corticotropin-releasing factor is thought to be involved in coordinating processes involved in survival (52), while ACTH seems to play a role in learning possibly through an increase in the motivational significance of environmental cues (16). Intraventricular administration of corticotropin-releasing factor decreases aggressive behavior and increases defensive behavior in mice (31). Further, postdefeat injections of both ACTH and corticosterone increase measures of submissiveness 24 and 48 hours after defeat (27,46). The hormonal response of the submissive hamsters presented in this study is consistent with the above, and further investigation of the role of the HPA hormones in the modulation of agonistic behavior is planned.

There are many ways in which the present paradigm could be used to explore further the influence of hormones and neurotransmitters on agonistic behavior. We are currently examining in more detail the neuroendocrine and immune responses of male golden hamsters to both acute and chronic exposure to social conflict. The paradigm reported here is both robust and versatile, and warrants further exploration.

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