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Psychological Stress Increases Pituitary Cyclic AMP[1,2]

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BUNNELL, B. N., J. L. MEYERHOFF AND G. J. KANT. *Psychological stress increases pituitary cyclic AMP.* PHARMACOL BIOCHEM BEHAV **29**(1) 151–155, 1988.—Exposure to physical stressors has been shown to produce increases in pituitary cyclic AMP in laboratory rats. In this experiment, the generality of these findings has been extended to include a psychological stressor, defined as returning the animals to a situation in which they had been exposed to footshock for four days. Rats in the psychological stress group exhibited increases in pituitary cyclic AMP and in plasma corticosterone and prolactin which were similar to those seen in animals that received the physical stressor on the test day. At present it is not known whether the effects are due to associative conditioning or to sensitization of the neuroendocrine system following repeated presentations of the physical stressor.

Psychological stress Pituitary Cyclic AMP Prolactin Corticosterone

LABORATORY rats subjected to either physical or psychological stressors respond with increases in plasma immunoreactivity of a number of anterior pituitary hormones, including adrenocorticotropic hormone, β-endorphin, β-lipotropin, and prolactin [1, 3, 4, 8, 14, 18]. The molecular mechanisms by which hormones, releasing factors and neurotransmitters regulate the secretion of anterior pituitary hormones are still unknown, although it has been suggested that cyclic AMP (adenosine 3′, 5′-monophosphate) may be involved [28]. Cyclic AMP functions as a second messenger in both the central nervous system and in the periphery to mediate the effects of many neurotransmitters and hormones at receptor sites [7]. Pituitary cyclic AMP is probably involved in the synthesis and/or release of anterior pituitary hormones. *In vitro* studies have shown that neurotransmitters and releasing factors (including corticotropin releasing factor) increase levels of pituitary cyclic AMP [2, 5, 15, 16] and that the incubation of pituitaries with cyclic AMP and its analogues increases the release of hormones into the medium [27].

Exposure to physical stressors, such as forced running, immobilization, or electric footshock produces a significant elevation of pituitary cyclic AMP in rats [9–12, 21]. These changes in cyclic AMP in response to stress do not occur in any of the brain regions we have examined, including the hypothalamus [9]. If pituitary cyclic AMP is involved in mediating the organism's general response to stress, then levels should increase in animals subjected to psychological as well as physical stressors. Indeed, a related experiment with rats has shown that β-endorphin, β-lipotropin, and prolactin increase in response to psychological stress and analogous increases in these hormones have been reported in humans undergoing interview stress [20,23]. In the present study, the psychological stressor consisted of exposing rats to environmental stimuli that previously had been paired with an aversive stimulus known to produce increases in pituitary cyclic AMP.

METHOD

Animals

Male albino rats (275–350 g), either WRC stock from Walter Reed Army Institute of Research production colony or commerically purchased Sprague Dawley animals were used. (There were no differences in the data obtained from the two strains.) The rats were individually housed in light and temperature controlled rooms with lights on between 0600-1800 hours. Food and water were available at all times except during training and testing sessions.

[1]In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

[2]This material has been reviewed by the Walter Reed Army Institute of Research, and there is no objection to its presentation or publication. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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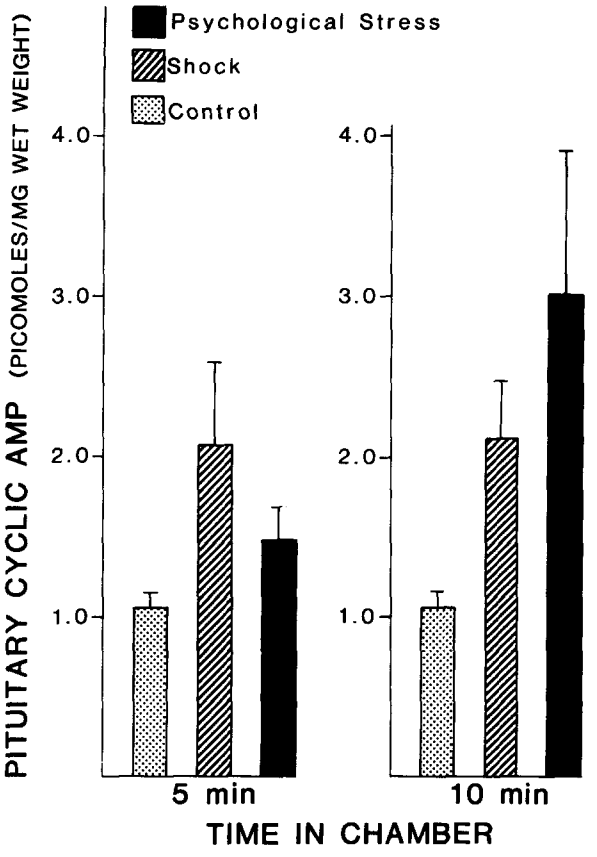

FIG. 1. Mean (±SEM) pituitary cyclic AMP in the Psychological Stress, Shock Control, and Unshocked Control Groups after 5 or 10 min in the test chambers.

Footshock

Footshock was delivered to the grid floor of Plexiglas boxes contained within ventilated, sound attenuating chambers. The source was a constant power shocker which was set to provide 0.017 watts of scrambled footshock on a variable time (VT-30 sec) schedule such that the animals received 5 sec of shock approximately once every 30 sec.

Assays

At the completion of testing, the rats were killed by microwave irradiation to prevent postmortem degradation of cyclic AMP [17,19]. After irradiation, the animals were decapitated to obtain trunk blood for plasma hormone measurements. The heads were cooled and the pituitaries were dissected, weighed, and sonicated in 1 ml of 50 mM sodium acetate buffer, pH 6.2. Sonicates were centrifuged and the supernatants stored at −70°C until assayed for cyclic AMP. Cyclic AMP was determined by radioimmunoassay using antibodies developed and characterized in our laboratory [18]. Trunk blood was centrifuged and the plasma assayed for prolactin and corticosterone as described elsewhere [18,24].

Training and Testing

Training sessions were conducted between 0800-1600 hours. On the critical test days, trials were restricted to 0830–1230 hours to reduce circadian effects on plasma hormone levels.

A preliminary investigation was conducted to determine the time course of the rise and fall of pituitary cyclic AMP levels in response to varying numbers of footshocks. In this pilot experiment, different groups of rats (n=6 per group) were given different numbers of shocks on the VT-30 sec schedule and then killed at varying time intervals following their removal from the test chambers. Rats given either 1 or 5 shocks and then killed after delays of 0, 2.5, or 5 min showed no increase in pituitary cyclic AMP when compared with animals taken directly from their home cages and killed. The baseline levels of cyclic AMP in all of these groups were slightly less than 1 picomole/mg wet weight. Animals receiving 10 shocks (approximately 5 min in the test chamber) had a threefold increase in cyclic AMP which persisted for 5 min after removal from the chamber. Those given 30 shocks (over approximately 15 min) exhibited a fourteenfold rise immediately after removal from the chamber and significant elevations of pituitary cyclic AMP were still present 15 min after removal. (The rats were returned to their home cages during the delay intervals.) The data from this experiment were used to help establish the parameters for administering footshock during the psychological stress experiment.

In the psychological stress experiment, there were three groups of 12 animals: a Psychological Stress Group, a Shock Group and a Control Group. Groups were matched for body weight. After one week of adaptation to handling and the plastic tubes used with the microwave apparatus, all animals were placed individually in the test chambers for 20 min a day on four consecutive days. On these four days, animals in both the Psychological Stress Group and the Shock Group were shocked on the VT-30 sec schedule described above. Rats in the Control Group were placed in the chambers for 20 min but were never shocked. On the fifth, or test day, the rats in the Psychological Stress Group were returned to the test chambers, but no shock was administered. Six animals from this group were removed from the chambers after 5 min and killed immediately; the remaining 6 animals in the Psychological Stress Group were left in the chambers for 10 min before being killed. Rats in the Shock Control Group received shock on the test day just as they had on the preceding four days. Half of the rats in this group were killed after 5 min (approximately 10 shocks) and half after 10 min (approximately 20 shocks). The Control Group received no shocks and the animals were killed after either 5 or 10 min in the chambers.

Behavior ratings of the rats' responses to shock were made during the 1st, 10th, and 20th minutes of the training trials and during the 1st, 5th, and, for those in the 10 min groups, the 10th min on the test day. A 4-point rating scale was used. A score of 0=no response, 1=flinching, 2=rapid raising of alternate paws, and 3=jumping and running. Ratings were made of the Control Group as well as the Psychological Stress and Shock Groups.

RESULTS

Pituitary cyclic AMP was elevated in the Psychological Stress and Shock Groups (Fig. 1). A 3×2 analysis of variance (Stress conditions and Time in chamber) yielded an $F(2,30)=3.82$, $p<0.05$ for the Stress condition.

Although the figure suggests that pituitary cyclic AMP rose more slowly in the Psychological Stress Group than in the Shock Group, neither the Stress × Time interaction nor

TABLE 1

PLASMA CORTICOSTERONE, PROLACTIN AND PITUITARY CYCLIC AMP FOR THE STRESS CONDITIONS

Group	Corticosterone (μg/100 ml) 5 min	Corticosterone (μg/100 ml) 10 min	Prolactin (ng/ml) 5 min	Prolactin (ng/ml) 10 min	Pituitary cAMP (pmoles/mg wet wt.) 5 min	Pituitary cAMP (pmoles/mg wet wt.) 10 min
Control	10.1 (1.1)	18.4 (2.6)	17.0 (6.3)	30.6 (8.3)	1.1 (0.1)	1.1 (0.1)
Psychological Stress	18.6 (2.6)	23.1 (1.9)	72.4 (7.1)	104.3 (6.6)	1.5 (0.2)	3.0 (0.9)
Shock	19.0 (2.0)	25.9 (4.1)	93.7 (13.5)	140.2 (19.9)	2.1 (0.5)	2.1 (0.4)

Values represent the Mean ± S.E.M.; n=6 per group.

the Time in chamber condition were significant. *A posteriori* comparisons of the group means for the stress condition (Duncan's new multiple range test) indicated that both the Shock and Psychological Stress means differed from the Control Group mean, $p<0.05$ ($q_2=0.92$ for the Shock vs. Control comparison and $q_3=0.97$ for the Psychological Stress vs. Control comparison).

Means and standard errors of plasma prolactin and plasma corticosterone, together with those for pituitary cyclic AMP (from Fig. 1) are given in Table 1. Analysis of variance of the prolactin data produced significant F's for both treatments, Stress condition, $F(2,30)=35.00$, $p<0.01$, and Time in chamber, $F(1,30)=10.85$, $p<0.01$. The interaction was not significant. Both the Psychological Stress and Shock Groups had elevated prolactin in comparison to the Control Group, $p<0.01$ ($q_2=31.4$ for Psychological Stress vs. Unshocked controls and $q_3=32.8$ for Shock vs. Control). Also, prolactin was higher in the Shock Group than in the Psychological Stress Group, $p<0.05$ ($q_2=23.3$). Similar results were obtained from the corticosterone analysis; Stress, $F(2,30)=5.92$, $p<0.01$, and Time, $F(1,30)=9.97$, $p<0.01$, were significant while the interaction was not. Mean differences between the Control Group and both the Psychological Stress and Shock Groups were significant, $p<0.01$ ($q_2=5.72$ for the Psychological Stress vs. Control comparison and $q_3=5.96$ for the Shock vs. Control comparison). Psychological Stress and Shock Groups did not differ from each other in corticosterone levels.

The increased amounts of both hormones at 10 min indicate that the plasma hormone levels were still rising after 5 min in the chambers. Corticosterone levels in the 5 min unshocked Control Group were higher than we normally see in home cage controls and rose substantially after 10 min in the chambers.

Correlations were computed between the cyclic AMP (cAMP), prolactin (PRL), and corticosterone (CS) data for all subjects. The correlations between cAMP and the two hormones were low and positive: $r(34)_{cAMP\text{-}PRL}=0.33$, $p<0.03$; $r(34)_{cAMP\text{-}CS}=0.32$, $p<0.02$ (one-tailed). The correlation between PRL and CS was higher: $r(34)_{PRL\text{-}CS}=0.58$, $p<0.001$.

All of the rats which received shock had high scores on the behavior rating scale throughout the first 4 days of the experiment. The mean score during the first minute of shock was 2.71±0.07; during the tenth minute it was 2.57±0.25. The 12 Control Group rats always scored zero. On the last day, the mean score of the rats in the Shock Group was 2.75±0.19 during the first minute; this dropped to 0.83±0.34 at 5 min and to 0.50±0.22 at 10 min. Eleven of 12 rats in the Psychological Stress Group received ratings of zero in the first minute on the last day; the mean rating at 10 min in the Psychological Stress Group was 1.17±0.44.

DISCUSSION

Exposure to a psychological stressor, in this case simply returning the rats of the Psychological Stress Group to the environment where they had received shock on four previous days, produced an increase in pituitary cyclic AMP and plasma corticosterone and prolactin similar to that seen in rats that received shock on the test day.

Absolute levels of pituitary cyclic AMP in both the Psychological Stress and the Shock Group were lower than those seen after acute (1 day) exposure to footshock of the same intensity in the preliminary experiment. In a related experiment [10] we found similar decreases in comparing 10 days of exposure to 15 min footshock with a single exposure. It appears that there was habituation of the pituitary cyclic AMP response across days in the present study, but the nature of the habituation process remains uncertain. Both behavioral and physiological adaptations may have been operating. Rats are capable of altering the amount of footshock received on a trail by jumping, tensing their muscles, straddling the bars of the shock grid, etc. Although behavioral ratings of response to shock during the first minute were high across all five days of the experiment, they declined sharply over 10 min on the last day, suggesting that some behavioral adaptation had taken place. Perhaps the use of tailshock in place of footshock to minimize the occurrence of behavioral adaptations to the stressor might produce more easily interpretable data on the operation of habituation in studies of psychological stress.

The low, positive correlations between pituitary cyclic AMP and the two plasma hormone measures were similar to what we had found in an earlier study [11]. At that time we noted that there did not appear to be any cause and effect relationships between pituitary cyclic AMP and these hormones. However, more recent data suggest that the pituitary

cyclic AMP response to stress is related to the release of the proopiomelanocortin derived hormones ACTH, β-endorphin and β-lipotropin from the anterior pituitary [13,22]. Since corticosterone reaches maximum levels following relatively mild stressors [9,10], the failure to obtain a stronger correlation between corticosterone and pituitary cyclic AMP may simply reflect a lack of adrenal cortical sensitivity to the higher levels of ACTH presumably generated in response to the increases in pituitary cyclic AMP.

The design of this experiment does not allow us to distinguish between two possible causes of the increases in pituitary cyclic AMP and plasma hormones seen in the Psychological Stress Group. The results might be due to the operation of a learning process such that the environmental cues of the shock chambers became associated with shock so that placing the animals in the boxes where they had previously been shocked caused a rearousal of the neuroendocrine events produced by shock [25,26]. The alternative is that repeated exposure to the shock stressor sensitized the neuroendocrine system to environmental stimuli without the operation of an associative learning process. (See [6], footnote 1, for a discussion of differences between associative conditioning and nonassociative sensitization effects with regard to the role of contextual cues in reinstating responses.) This distinction could have considerable impact on the selection of strategies that might be utilized to modify or reduce physiological responses to psychological stress. Use of a classical conditioning paradigm, in which specific experimental controls for sensitization are employed, could resolve this issue.

In conclusion, psychological stress causes increases in levels of pituitary cyclic AMP. These data provide additional support for the hypothesis that pituitary cyclic AMP is involved in the regulation of neuroendocrine responses to stress.

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