Pharmacology Biochemistry & Behavior, Vol. 22, pp. 631–634, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Habituation to Repeated Stress Is Stressor Specific[1,2]

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Received 25 June 1984

KANT, G. J., T. EGGLESTON, L. LANDMAN-ROBERTS, C. C. KENION, G. C. DRIVER AND J. L. MEYERHOFF. *Habituation to repeated stress is stressor specific.* PHARMACOL BIOCHEM BEHAV **22**(4) 631–634, 1985.—Rats were exposed to 15 min of restraint or footshock or forced running in an activity wheel once a day for 10 days. Control groups were handled only. On the 11th day, rats from each stressor group and controls were exposed to 15 min of one stressor in a crossed design such that all combinations of one chronic stressor and one acute stressor were performed. Rats were sacrificed immediately following removal from their home cage or after 15 min stressor exposure on the 11th day and plasma corticosterone and prolactin and pituitary cyclic AMP levels were determined. There were no measured differences in these stress indices among groups of rats sacrificed immediately upon removal from their home cage on day 11 regardless of previous history on days 1 through 10. Plasma corticosterone and plasma prolactin and pituitary cyclic AMP levels were elevated in all rats exposed to any of the three stressors immediately prior to sacrifice as compared to all rats not exposed to stress immediately before sacrifice. However, plasma prolactin and pituitary cyclic AMP responses to each of the 3 stressors were attenuated in rats which had previous exposure to that specific stressor as compared to rats which had previous experience with a different or no stressor. We conclude that habituation results from behavioral experience with a particular stressor rather than biochemical adaptation resulting from repeated challenge to hormonal and neurochemical systems responsive to stress.

Habituation Stress Pituitary Cyclic AMP Prolactin Corticosterone

ACUTE stress increases the release of ACTH, β-endorphin and prolactin from the pituitary gland, the release of corticosterone from the adrenal cortex and the release of catecholamines from the adrenal medulla [6–10, 18, 19]. We have found that acute stress also increases the level of pituitary cyclic AMP *in vivo* and have suggested that this increase is related to the regulation of pituitary hormone release or synthesis in response to stress [1, 4–8, 15, 16].

We recently reported that repeated exposure (one 15 min exposure/day for 10 days) to a stressor resulted in a diminished stress response following subsequent exposure to the same stressor as compared to the stress response observed following initial exposure to the stressor [6]. Stress response was assessed by plasma corticosterone and prolactin and pituitary cyclic AMP elevations immediately following a 15 min stressor exposure.

Habituation in these experiments could be viewed as a desensitization to the stressor stimulus such that the perceived stress was lessened, i.e., a behavioral adaptation. Alternatively, repeated stress could cause changes in biochemical systems, e.g., changes in pituitary adenylate cyclase activity or prolactin synthesis, that might affect the amount of stress-released hormones.

The following experiment was designed to determine whether the habituation we observed in our previous study was primarily the result of daily challenge and adaptive changes in biochemical systems or primarily the result of behavioral familiarization to a specific stressor. We chose stressors that were very dissimilar from a behavioral perspective but that evoked similar plasma hormone and pituitary cyclic AMP responses. Stressors that evoked dissimilar biochemical responses would not be useful discriminators between the two posed alternative habituation mechanisms.

Animals were exposed to one stressor for 10 days and then challenged by the same or a different stressor immediately prior to sacrifice. If the habituation we observed in our previous experiments was primarily the result of behavioral familiarization, then no attenuation of stress response should be seen in animals exposed to a different stressor.

METHOD

Animals

Male Sprague-Dawley rats (225–275 g) were individually housed in a light and temperature-controlled room with food and water freely available. Lights were on from 0600 to 1800.

[1]In conducting the research described in this report, the investigator(s) adhere 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]The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

TABLE 1
EFFECT OF STRESSORS ON PITUITARY CYCLIC AMP AND PLASMA HORMONES

	Acute Stressor Day 11			
	Control	Restraint	Running	Shock
Days 1–10				
	Pituitary Cyclic AMP (picomoles/mg wet weight)			
Control	1.8 ± 0.2	3.8 ± 1.3	30.2 ± 4.6	9.4 ± 4.9
Restraint	1.4 ± 0.1	2.4 ± 0.6	25.1 ± 6.2	10.6 ± 4.6
Running	1.4 ± 0.1	10.5 ± 7.6	6.6 ± 2.7	6.1 ± 2.5
Shock	1.4 ± 0.1	6.7 ± 3.8	21.1 ± 4.5	6.6 ± 3.7
	Plasma Prolactin (ng/ml plasma)			
Control	10 ± 1.5	148 ± 32	158 ± 39	136 ± 30
Restraint	46 ± 26	107 ± 34	226 ± 46	158 ± 36
Running	16 ± 11	218 ± 22	108 ± 34	110 ± 40
Shock	22 ± 6.6	146 ± 26	203 ± 12	111 ± 43
	Plasma Corticosterone (μg/100 ml plasma)			
Control	6.9 ± 1.0	20.8 ± 1.4	23.6 ± 1.9	25.9 ± 3.0
Restraint	10.2 ± 2.4	21.3 ± 2.4	30.3 ± 2.4	21.5 ± 2.0
Running	9.2 ± 2.4	27.5 ± 3.6	24.7 ± 1.7	22.8 ± 4.0
Shock	7.8 ± 1.4	25.4 ± 2.9	26.3 ± 2.4	21.2 ± 2.9

All animal experimental procedures (stressor exposures and sacrifice) were performed between 8:30 a.m. and 12:30 p.m. to minimize circadian variations in baseline and stress-induced hormonal and cyclic AMP responses. In addition, different experimental groups were stressed and sacrificed throughout each morning to randomize the effects of circadian rhythms on the collected data. Groups were staggered such that the entire experiment was conducted over 3 mornings.

Experimental Procedures

Initially, rats were divided into 4 treatment groups. One group was removed from their home cages daily and handled before replacement into their home cages. Rats in a second treatment group were individually exposed to 15 min a day of forced running in motorized activity wheels (38 cm diameter, 8 rpm). A third group of rats was subjected to footshock for 15 min daily (1.6 mA, variable interval schedule with an average intershock interval of 30 sec and shock duration of 5 sec). The fourth group of rats was restrained by placement into the plastic tube (5.7 cm diameter) used to immobilize rats for the microwave device described below. Rats were placed into the tube for 15 min daily and then returned to their home cage. All treatments were performed for 10 consecutive days.

On the 11th day, six rats from each of the four treatment group were sacrificed immediately upon removal from the home cage; six rats from each of the four groups were exposed to 15 min of footshock and then immediately sacrificed. Six rats from each of the four treatment groups were subjected to 15 min of restraint and then immediately sacrificed and six rats from each group were sacrificed immediately following 15 min of forced running. Thus a complete cross-stress design was employed.

Sacrifice and Assay Procedures

Animals were sacrificed by a 5 sec exposure to high power microwave irradiation, a technique which has been shown to prevent post-mortem increases in cyclic AMP and other metabolites [3, 12–14]. The microwave power generator was a modified Varian PPS-2.5, with a power output of 2.5 KW at a frequency of 2450 MHz [11]. Rats were placed in the 5.7 cm diameter plastic cylinder used to restrain rats above. However, the brief immobilization (<1 min) required is not sufficient to elevate indices of stress.

After sacrifice, the rats were decapitated and the trunk blood was collected in heparinized beakers and then centrifuged. Plasma was assayed for prolactin and corticosterone by radioimmunoassay as previously described [10,17]. For the corticosterone assay, the within assay coefficient of variation was <5% and the between assay variation was <12%. For prolactin, within assay coefficient of variation was <8% and between assay variation was <12%.

Pituitaries were dissected free, weighed and sonicated in 1 ml of 0.05 M sodium acetate buffer, pH 6.2. After centrifugation, the supernatants were stored at −70°C until assayed for cyclic AMP using antibodies characterized in our laboratory as previously described [10,12]. For cyclic AMP, the within assay coefficient of variation was 7% and the between assay coefficient of variation was 18%.

RESULTS

As shown in Table 1, levels of pituitary cyclic AMP and plasma corticosterone and prolactin were similar in all animals not stressed immediately prior to sacrifice (acute controls, column 1 in Table 1) regardless of treatment received on previous days (pituitary cyclic AMP, $F(3,28)=1.5$, $p=0.24$; prolactin, $F(3,28)=1.2$, $p=0.34$; corticosterone,

$F(3,28)=0.35$, $p=0.79$). Thus no "carryover" effect was observed; all rats started from approximately the same baseline on the 11th day. In rats never exposed to stressors prior to the stress challenge on the 11th day (chronic controls, row 1 in Table 1), all three stressors produced significant increases in pituitary cyclic AMP, plasma corticosterone and plasma prolactin (pituitary cyclic AMP, $F(3,115)=15.6$, $p<0.0001$; prolactin, $F(3,115)=17.6$, $p<0.0001$; corticosterone, $F(3,115)=42.8$, $p<0.0001$). The increases in plasma prolactin and corticosterone were similar following all three stressors (corticosterone, $F(2,19)=1.3$, $p=0.29$; prolactin, $F(2,19)=0.10$, $p=0.90$). The pituitary cyclic AMP increase following stress varied depending upon the stressor, $F(2,19)=12.0$, $p<0.001$. Forced running caused a larger increase in pituitary cyclic AMP than either restraint or footshock (Student's t-test, two-tailed, $p<0.05$). The effects of footshock and restraint were not significantly different from each other.

Prior exposure to stressors *per se* did not attenuate pituitary cyclic AMP or plasma prolactin or corticosterone responses to an acute stressor applied before sacrifice. If all acutely stressed rat data (all data except column 1 in Table 1) are compared with respect to previous stress history (comparison among rows in Table 1, including chronic controls), no significant differences are seen (pituitary cyclic AMP, $F(3,86)=0.7$, $p=0.56$; prolactin, $F(3,86)=0.1$, $p=0.96$; corticosterone, $F(3,86)=0.1$, $p=0.96$).

However, if the data are compared with respect to similar chronic and acute stressor exposure (control-control, restraint-restraint, etc.) vs. dissimilar acute and chronic stressor exposure (control-shock, running-restraint, etc.), then significant habituation of pituitary cyclic AMP and plasma prolactin responses but not corticosterone response to a previously encountered stressor is seen (pituitary cyclic AMP, $F(1,115)=5.0$, $p=0.027$; prolactin, $F(1,115)=4.0$, $p=0.048$; corticosterone, $F(1,115)=1.3$, $p=0.25$).

DISCUSSION

We have previously reported that pituitary cyclic AMP and plasma prolactin are two indices of stress that respond to a variety of stressors and increase in a graded manner to different intensities of footshock [1, 5, 7, 8]. In our experiments, we have found that plasma corticosterone is a very sensitive indicator of stress or arousal that reaches maximum levels following relatively mild stressors. Because of this sensitivity, we have not been able to demonstrate habituation of the plasma corticosterone response to the stressors employed in these experiments. Pituitary cyclic AMP and prolactin, on the other hand, do habituate to repeated presentation of forced running, restraint and footshock [6].

The experiment described in the present report was designed to choose between two possibilities as the primary mechanism of the observed stress response habituation. The first possibility was that repeated presentation of the same stressor reduced the perceived aversiveness of the stressor and lessened the subjective severity of the stressor. This behavioral adaption could have included both mental and physical coping strategies, e.g., "relaxing" during restraint or improved pacing on the motorized wheel. The second possibility was that repeated activation of stress-sensitive neurochemical systems resulted in biochemical adaption. An example of biochemical adaption following repeated challenge is the increased ability of liver enzymes to metabolize drugs following repeated drug administration. Stimulation by one drug results in increased capability to metabolize other drugs that share detoxification enzymes resulting in a cross-tolerance for several different drugs [2].

In the present study, habituation only occurred following exposure to the same stressor although all stressors activated a biochemical stress response. We conclude that under these conditions it is the increased familiarity with these stressors that diminishes the evoked stress response rather than an adaptive change at the biochemical level.

ACKNOWLEDGEMENTS

We wish to thank Edward Mougey, David Collins, Lee Pennington, Clint Wormley, and Willie Gamble for assistance with the cyclic AMP and hormone assays.

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