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Mouse Strain Differences in Plasma Corticosterone Following Uncontrollable Footshock

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SHANKS, N., J. GRIFFITHS, S. ZALCMAN, R. M. ZACHARKO AND H. ANISMAN. *Mouse strain differences in plasma corticosterone following uncontrollable footshock.* PHARMACOL BIOCHEM BEHAV **36**(3) 515–519, 1990.—Exposure to acute inescapable footshock provoked marked increases of plasma corticosterone concentrations in six strains of mice (A/J, Balb/cByJ, C57BL/6J, C3H/HeJ, DBA/2J and CD-1). However, the magnitude of the increase, as well as the time required for corticosterone to return to control values, varied appreciably across strains. Moreover, it appeared that the strain-specific corticoid increases ordinarily observed after acute shock were also evident following a chronic stressor regimen. The data were related to previously observed strain differences in stressor-induced alterations of brain norepinephrine, dopamine and serotonin, as well as variations in performance in several behavioral paradigms.

Strain difference Stress Corticosterone

STRESSORS have been shown to induce a variety of behavioral, neurochemical and hormonal alterations (29,30). These alterations may be influenced by organismic variables, such as the age of the organism (13,17) and experiential factors, including previous stressor experience and chronicity of the stressor (2, 12, 14). However, considerable interindividual and interstrain variability exists in response to stressors which cannot be ascribed to these variables. Indeed, it has become increasingly evident that genetic factors may contribute significantly to the expression of the behavioral and neurochemical alterations elicited by stressors (19,22).

The behavioral effects of stressors in animals may be reminiscent of the behavioral symptoms which characterize clinical depression in humans (29). Moreover, stressors may influence the neurochemical processes thought to subserve depression, and repeated treatment with antidepressants have, in fact, been shown to ameliorate the behavioral disturbances ordinarily elicited by uncontrollable stressors in animals (3). One of the problems inherent in modelling depression is that the symptoms of the illness vary considerably across individuals, and interindividual variability likewise exists with respect to the therapeutic efficacy of drug treatments. Indeed, it has been posited that depression may be a biochemically heterogeneous disorder (26).

As in the case of human depression, where the symptom profile varies appreciably across individuals, exposure to a stressor may induce profound behavioral disturbances in some animals, while in other animals these behaviors seem hardly affected by the stressor (3). In an attempt to assess the interindividual responses to stressors, we have assessed the behavioral and neurochemical consequences of aversive stimulation in different strains of mice. It was observed that the "symptom profile" associated with a stressor (i.e., the behaviors disrupted by stressors) may vary considerably across strains (20,31). In a like fashion, we have observed that marked strain differences exist with respect to norepinephrine (NE), dopamine (DA) and serotonin (5-HT) alterations induced by a stressor, as well as the brain regions in which these occur (22,23). Furthermore, the effectiveness of antidepressants in ameliorating the behavioral distrubances may vary across strains of mice (21).

In addition to the contribution of central transmitters, there is reason to suspect that ACTH and corticoids may be related to depression in humans. For instance, it has been demonstrated that depression may be associated with increased basal cortisol levels, alterations in the diurnal variations of cortisol secretion or early escape from dexamethasone-induced suppression of cortisol secretion (25). Inasmuch as the magnitude of the stressor-provoked increases of plasma corticosterone may be influenced by genetic factors (28), it was of interest to determine whether stressor-induced corticosterone increases would parallel the behavioral or neurochemical changes we previously observed in different strains of mice. The present experiments assessed the magnitude and the decay rate of plasma corticosterone following an acute stressor, and also determined whether the corticoid response varied with stressor chronicity.

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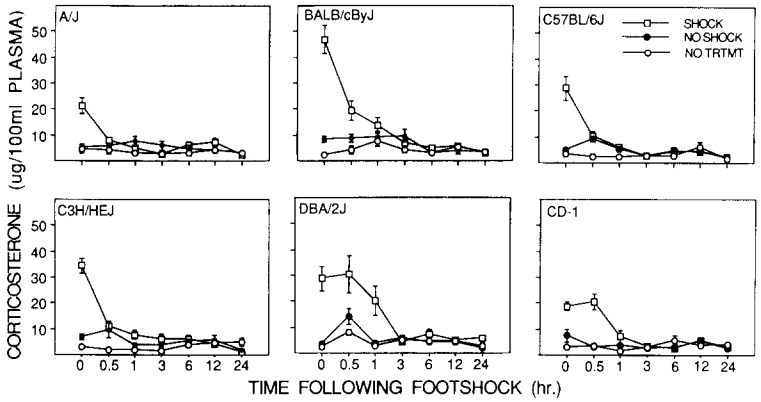

FIG. 1. Mean (±S.E.M.) plasma corticosterone concentrations (μg/100 ml) in six strains of mice at various intervals following shock treatment, no shock but exposure to the test apparatus (no shock) or neither shock nor apparatus exposure (no treatment).

METHOD

Subjects

Experiment 1 involved a total of 639 male mice comprising 5 inbred strains of mice (A/J, BALB/cByJ, C57BL/6J, DBA/2J and C3H/HeJ) and one noninbred strain (CD-1). In Experiment 2, a total of 247 male mice of these strains was employed. The inbred strains (Jackson Laboratory, Bar Harbor, ME) and the CD-1 mice (Charles River Inc., St. Constance, Quebec) were obtained at 5 weeks of age and permitted to acclimatize to the laboratory for approximately three weeks before serving as experimental subjects. Mice were housed, by strain, in groups of 5 in polypropylene cages and permitted free access to food and water. Mice were maintained on a 12-hr light-dark cycle (light: 0700–1900 hr), and shock treatments were applied between 0800 and 1000 hr.

Apparatus

Inescapable footshock was administered in five black Plexiglas chambers (30.0 × 14.0 × 15.0 cm) covered by red translucent lids to reduce illumination. The floor of each chamber consisted of 0.32 cm stainless steel rods spaced 1.0 cm apart, connected in series by neon bulbs. The end walls of each chamber were lined with stainless steel plates and were connected in series with the grid floor. Shock could be delivered to the grid floor through a 3000-V source.

Procedure

Mice of each strain were randomly assigned to three treatment conditions. Mice of one condition were individually placed in the shock chambers and exposed to a series of 360 footshocks (300 μA) of 2 sec duration, at intervals of 9 sec. In the second condition mice were placed in the chambers, but the footshock was withheld (apparatus control), while mice of the third group were left in their home cages. Following the initial treatment mice were housed individually rather than being returned to their home cages. This procedure was adopted since the interaction with cage mates may have influenced the corticoid response. At various intervals after the initial treatment (immediate, 0.5, 1, 3, 6, 12 or 24 hr) mice (n = 4–6/group) were decapitated and trunk blood was collected. Plasma was frozen and stored at −60°C for subsequent corticosterone determinations using the method of Givner and Rochefort (10). A between-groups procedure was employed to avoid potential contamination that might have been induced by the stress associated with repeated blood sampling and handling.

In Experiment 2 mice of each strain were subdivided and exposed to either a chronic or an acute stressor regimen. In the chronic condition mice were individually placed in the shock chambers and exposed to either 360 shocks of 2 sec duration (300 μA) on each of 14 consecutive days, or were placed in the apparatus but not shocked (n = 6–8/group). In the acute condition mice received only a single exposure to the shock treatment (360 shocks, 2 sec duration, 300 μA) or exposure to the apparatus (n = 12–15/group). Immediately after the last session mice were decapitated and trunk blood collected for subsequent corticoid determination.

RESULTS

The mean plasma corticosterone concentrations as a function of the treatment condition are shown in Fig. 1. It is clear from the figure that while the stressor markedly increased corticosterone concentrations in each of the strains, pronounced differences were apparent with respect to both the magnitude of the increase and the decay rate of corticosterone. The analysis of variance confirmed the presence of a Strain × Stress Treatment × Time interaction, $F(60,513) = 2.94$, $p < 0.01$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) of the simple effects comprising this interaction indicated that unlike the diurnal rhythms seen in humans and in rats, in the nontreated mice corticosterone concentrations remained fairly stable across the various time periods. Basal corticosterone levels did not differ between strains at the various intervals, although comparisons of the strains collapsed over time period indicated higher basal corticosterone values in DBA/2J and BALB/cByJ mice than in C57BL/6J or C3H/HeJ mice. The handling procedure was found to produce a modest, but significant, increase of corticosterone concentrations 0.5 hr after handling in DBA/2J, C3H/HeJ and C57BL/6J mice. In BALB/cByJ mice an increase was likewise noted immediately after apparatus exposure.

In contrast to the small effects elicited by apparatus exposure, the shock treatment markedly increased corticosterone in all of the strains. The increase was particularly marked in BALB/cByJ mice, and immediately after shock corticosterone levels in this strain were significantly higher than in the remaining strains. The increase of corticosterone in the C3H/HeJ, C57BL/6J and DBA/2J mice was intermediate, significantly exceeding that of the A/J and CD-1 mice. Interestingly, the magnitude of the increase appeared to be unrelated to the time for corticoid concentrations to return to control levels. In DBA/2J mice, for instance, control values were

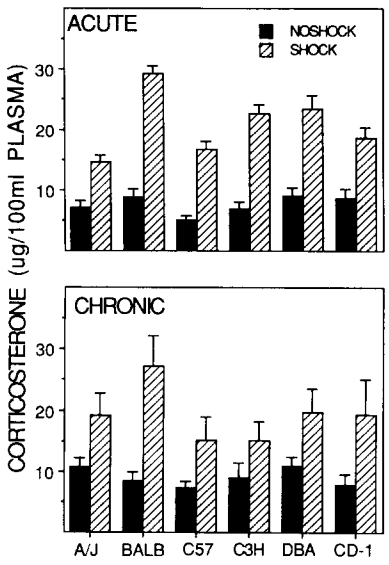

FIG. 2. Mean (+S.E.M.) plasma corticosterone concentrations (μg/100 ml) in six strains of mice immediately after a single session of inescapable shock or no shock (upper panel) or exposure to a chronic shock regimen (14 sessions of shock) or no shock (lower panel).

exceeded as long as 1 hr after stressor application, and at the 0.5 and 1 hr intervals corticosterone values exceeded those of the remaining strains. In both the BALB/cByJ and CD-1 mice, corticosterone levels were still elevated above control values at the 0.5 hr interval, while in remaining strains, the steroid concentrations had returned to the levels of either the nontreated or apparatus exposed animals by this time.

Corticosterone concentrations in Experiment 2 were increased by shock exposure, and the magnitude of this effect varied across strains of mice (see Fig. 2). Chronicity of the stressor did not influence the magnitude of the corticosterone increase ($F<1$). The analysis of variance revealed a significant Shock × Strain interaction, $F(5,223)=3.83$, $p<0.01$. Newman-Keuls multiple comparisons ($\alpha=0.05$) of the simple effects comprising the interaction confirmed that in the absence of shock treatment corticosterone concentrations varied marginally across strains, but these differences were not statistically significant. Exposure to shock increased corticosterone concentrations in all strains, but the extent of the increase was more pronounced in BALB/cByJ mice than in the remaining strains. As in Experiment 1, the corticosterone increase in DBA/2J and C3H/HeJ was marked as well, with corticosterone levels exceeding those of C57BL/6J mice. There was no indication of the chronic stressor treatment having effects different from those of the acute treatment.

DISCUSSION

The results of the present investigation demonstrated that exposure to an acute stressor increased corticosterone secretion; however, the extent of the corticoid increase, as well as the time for corticoid levels to return to control values, varied appreciably across strains of mice. The magnitude of the corticoid increase appeared to be independent of its decay rate. For instance, the greatest stressor-provoked corticosterone increase was evident in BALB/cByJ mice, but within 1 hr of stressor termination the steroid values approached those of nonstressed animals. In contrast, a less marked corticoid increase was apparent in DBA/2J mice, but 1 hr after stressor termination corticosterone concentrations were still five-fold higher than those of nonstressed animals. The mechanisms subserving the strain differences in the stressor-induced corticoid response remain to be determined. Inasmuch as the magnitude of the corticoid release and the time course for this effect were unrelated, it is likely that different mechanisms may be operative in determining these effects. Moreover, it should be considered that the regulation of corticoid release across these strains may differ across several dimensions, including corticosterone clearance rate, feedback inhibition, stimulation of CRF release, pituitary sensitivity to CRF, as well as ACTH release.

Consistent with earlier reports (4,15) there was no indication of the corticosterone response being diminished following a chronic stressor regimen. This was the case in strains which exhibited a large corticosterone response to an acute stressor, as well as in strains that exhibited a relatively small response to a single session of shock. In the present investigation the stressor employed was relatively intense (360 shocks of 2 sec duration, 300 μA, applied over a 1.1 hr period) and it is conceivable that adaptation might have been evident had a less severe stressor been used. Moreover, there is reason to believe that the schedule of stressor presentation may influence the emergence of an adaptation. Several investigators have reported adaptation of the corticoid response, particularly when the stressors were applied at relatively close temporal intervals (5,8). It was suggested that negative feedback of corticosterone (11), presumably through specific receptors at the pituitary, hypothalamic and hippocampal level may account for the adaptation, although there is reason to believe that mechanisms other than negative feedback of corticoids on hypothalamic-pituitary activity may contribute to such an effect (7,9). It remains to be determined whether strain differences exist with respect to the adaptation under conditions where stressor sessions are administered at close intervals; however, given the strain differences in the rate of decay of the stress-induced corticosterone increase, a strain-dependent adaptation of the corticosterone response would not be unexpected. It might be noted at this juncture that the duration of the stressor session might also come to influence the development of an adaptation effect. By using a relatively protracted stressor session, such as that of the present investigation, the corticosterone secretion engendered by the acute stressor may have resulted in feedback inhibition of further CRF release, hence precluding the detection of possible differences between the acute and chronic treatments.

Several attempts have been made to relate corticosterone release to individual differences in emotionality (or reactivity). While open-field activity, poststressor locomotor activity, and conditioned emotional response might all provide some indication of emotionality, the conclusions derived concerning differences between strains of mice are not consistent across these paradigms [cf. (1, 6, 19, 20, 24)]. In their review of the literature Walker, Aubert, Meaney and Driscoll (27) indicated that basal corticosterone concentrations were not consistently correlated with degree of emotionality in several lines of selectively bred rats (i.e., Maudsley reactive vs. nonreactive: Roman High vs. Roman Low Avoidance; Syracuse Low and Syracuse High Avoidance). Moreover, it was reported that while the Roman High and Low Avoidance lines exhibited limited differences in basal corticosterone concentrations, following challenge with the stress of being placed in an open field the corticosterone rise was considerably more marked in the more emotional Roman Low Avoidance (RLA) line than in the Roman High Avoidance (RHA) line (28). It is particularly interesting that although the RLA rats exhibited lower basal ACTH levels, corticosterone secretion was greater in RLA than in RHA rats. After CRF administration pituitary ACTH output was greater in RLA rats, thus it was suggested that in these rats hypothalamic CRF discharge may be impaired, possibly owing to reduced negative glucocorticoid feedback potency or

altered corticosterone clearance rate.

The strain-dependent corticoid changes induced by the stressor in the present investigation were unrelated to a variety of behavioral alterations which were previously shown to occur in these strains following exposure to uncontrollable footshock (i.e., locomotor activity and exploration in a Y-maze, forced swim performance, shuttle escape, self stimulation from the nucleus accumbens). In particular, while uncontrollable shock provoked strain-dependent disturbances of these behaviors (20,31), these impairments could be distinguished from the strain profile of corticosterone secretion observed in the present investigation. Although genetic differences were evident with respect to stressor-induced catecholamine and serotonin changes in different brain regions (22,23), none of these paralleled the corticoid variations. For instance, the NE variations in the hypothalamus were as marked in low corticoid secreting A/J mice as they were in the higher secreting BALB/cByJ strain, or in the DBA/2J mice where corticoid concentrations remained elevated for a relatively protracted period. In locus coeruleus the stressor-induced NE reductions were particularly marked in BALB/cByJ, DBA/2J and C57BL/6J mice relative to the remaining strains, but it will be recalled that the corticoid secretion in C57BL/6J mice was less pronounced than in the former two strains. Likewise, in hippocampus turnover of NE among BALB/cByJ, DBA/2J and CD-1 mice exceeded that of the remaining strains, but were indistinguishable from one another. Hippocampal 5-HT concentrations and turnover in the BALB/cByJ mice were actually more pronounced after stressor exposure than in the remaining strains, but the 5-HT alterations in DBA/2J which is also a high corticoid secreting strain was comparable to that of the remaining strains. These caveats notwithstanding, it is significant that the stressor-induced NE and 5-HT alterations were consistently high in the BALB/cByJ mice.

Although genetic variations contribute to the diversity of behavioural, neurochemical and hormonal alterations elicited by stressors, the relationship between steroid changes and alterations in central neurochemical activity across strains of mice is not entirely clear. This should not be misconstrued as implying that the amine changes induced by stressors are unrelated to either the behavioral variations or to the steroid changes that are observed. It is likely that several stressor-induced neurochemical alterations contribute to the behavioral disturbances and hence it is not surprising that a one-to-one correspondence was not observed with respect to any single neurochemical and behavioral change elicited by a stressor. Likewise, inasmuch as several transmitters may contribute to corticoid secretion, coupled with the possibility that the magnitude and the time course for the stressor-induced corticosteroid variations may involve several mechanisms (e.g., clearance rates, negative feedback, CRF secretion), it would be expected that the relationship between the steroid variations, neurochemical alterations, and behavioral impairments across strains of mice would be complex. Indeed, in addition to assessing the contribution of corticosterone release and decay times, behavioral analyses should consider that the distribution of type I and II corticosteroid receptors, which are differentially sensitive to adrenocorticoids, varies across brain regions (e.g., hippocampus, lateral septum, brain stem nuclei and ventral tegmentum) (8,16) and may contribute to the provocation of central neurochemical changes (18). Furthermore, just as strains may differ with respect to the secretion or clearance of corticoids, the possibility should be entertained that the distribution and density of corticoid receptors may vary across strains of mice.

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