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BRIEF COMMUNICATION

Central Norepinephrine and Plasma Corticosterone Following Acute and Chronic Stressors: Influence of Social Isolation and Handling[1]

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IRWIN, J , P AHLUWALIA, R M ZACHARKO AND H ANISMAN. *Central norepinephrine and plasma corticosterone following acute and chronic stressors Influence of social isolation and handling* PHARMACOL BIOCHEM BEHAV 24(4) 1151–1154, 1986.—Exposure to acute inescapable shock resulted in a decline of hypothalamic norepinephrine (NE), and an increase of plasma corticosterone concentrations With repeated application of the stressor over 15 successive days the reduction of NE was eliminated and concentrations of the amine actually exceeded those of control animals In contrast to the NE variations, plasma corticosterone concentrations were elevated irrespective of whether mice received a single or repeated sessions of inescapable footshock Moreover, unlike NE concentrations, handling mice on successive days in the absence of the shock treatment was sufficient to provoke a modest, but reliable increase of corticosterone concentrations It is suggested that the hypothalamic NE and plasma corticosterone changes may be reflective of different attributes of the stressor or are subserved by different mechanisms It is suggested that variations in both these systems represent adaptive changes to meet environmental demands

Stress Isolation Corticosterone Norepinephrine.

STRESSOR application has been shown to increase the utilization and synthesis of norepinephrine (NE) in several brain regions. If the stressor is uncontrollable and sufficiently severe, then the rate of utilization may exceed synthesis, resulting in a net reduction of NE concentrations [3, 28, 29]. It is thought that the reduced amine concentrations may render the organism less well prepared to deal with subsequent environmental demands With repeated application of a stressor the activity of the synthetic enzymes, tyrosine hydroxylase and dopamine-β-hydroxylase increases, and hence the NE reduction is prevented [13,27]. Indeed, it seems that in animals repeatedly exposed to a stressor NE concentrations may come to exceed those of nonstressed animals [10,22] It was hypothesized that the increased NE activity and levels may leave the organism better prepared to deal with environmental stressors, such that performance deficits typically engendered by acute shock may be absent following a chronic shock regimen [27].

Paralleling the central NE alterations, secretion of adrenal corticoids may also be influenced by stressor controllability and chronicity. For instance, it was demonstrated that increases in plasma corticosterone concentrations were more profound after an uncontrollable stressor than after a controllable stressor [5]. Moreover, it has been reported that following chronic stressor application adaptation occurs such that plasma corticosterone levels are lower than after a single session of aversive stimulation [4,12].

Central NE and plasma corticosterone variations have been implicated in subserving the behavioral and physical pathologies associated with stressors [1, 21, 25]. However,

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because NE and corticoid alterations are affected similarly by aversive stimuli, it is often difficult to distinguish their relative contributions Indeed, it has been assumed that the congruency between the hypothalamic NE and plasma corticosterone variations associated with stressors are related to one another That is, the reduced availability of hypothalamic NE following stressor application may limit the inhibitory effect on CRF release ordinarily exerted by this amine, thereby increasing ACTH secretion and the consequent increase of plasma corticoid concentrations Indeed, it was suggested that evaluation of endocrine activity may provide an effective method of indirectly assessing brain functioning [23] Yet, there is considerable evidence indicating that hypothalamic transmitters other than NE, including acetylcholine, dopamine and serotonin, which are influenced by stressors [1] also contribute to CRF secretion [20] Moreover, it has been demonstrated that stressors that do not appreciably influence NE concentrations may have very profound effects on plasma corticosterone concentrations [28,29], and manipulations which greatly influence NE may not affect corticosterone in a like manner (cf [1, 5, 28]) Furthermore, it is entirely possible that corticoid and amine variations may be influenced by different attributes of the stressor (e g , arousal, anxiety, pain, previous stress history), and, thus may not be similarly affected by all stressors

In view of the proposition that stressors may contribute to the provocation or exacerbation of psychological and physical pathology, and that such effects may be related to alterations of corticoids and central amines, it was of interest to determine whether variations of these endogenous substances would occur concomitantly Inasmuch as the effects of acute and chronic stressors on NE and corticosterone have been examined independently, it was the purpose of the present investigation to establish concurrently whether the effects of these treatments on NE concentrations would be paralleled by variations of plasma corticosterone In addition, it has been demonstrated that background conditions (e g , social housing conditions, handling, shipping and maintenance of animals) upon which a stressor is applied may influence both plasma corticosterone [7, 21, 24] and central NE concentrations and turnover [2, 17, 26] and may also affect the development of physical pathologies [25] Accordingly, in the present investigation the contribution of handling and housing conditions on stressor provoked NE and corticosterone variations were also evaluated

METHOD

Subjects

A total of 95 male, CD-1 mice, 55–60 days of age, were obtained from Charles River (Canada) Ltd Mice were housed in groups of 5 in standard polypropylene cages for 14 days prior to being used as experimental subjects Throughout the experiment mice were maintained on a 12 hr light/dark cycle, and were permitted free access to food and water

Apparatus

Footshock was delivered in four identical black Plexiglas chambers which measured 30×14×15 cm, and were covered by red Plexiglas lids to reduce illumination. The floor of each chamber was made up of 0 32 cm stainless steel rods spaced 1 0 cm apart (center to center) The grid rods were connected in series with neon bulbs, and shock (150 μA, 60 Hz, AC) was delivered to the grid through a 3,000 V source The end walls of each chamber were lined with stainless steel, connected in series with the grid floor

Procedure

Mice were assigned to either an isolated or grouped housing condition (5/cage) and exposed to one of 5 treatments (n=8–10/group) Mice in three of these groups were placed in the apparatus for a 1 1 hr period on each of 15 days These animals were exposed to footshock on either all 15 days, on the last 3 days, or only on the last day of the experiment (360 shocks, 2 sec duration, 9 sec intertrial interval) Mice of the remaining two groups were not shocked Animals of one group were, however, placed in the apparatus on each of 15 days, whereas mice of the other group were not removed from their home cages and were left undisturbed throughout the 15 day period (except to change cage bedding on Day 7) Immediately following the last shock session mice were decapitated, brains removed, dissected and frozen in liquid nitrogen Additionally, trunk blood was collected and centrifuged, and plasma samples frozen Samples were stored at −70°C until concentrations of brain NE were determined using a modification of the hydroxyindole method [15,16], and plasma corticosterone concentrations were assessed fluorometrically [6]

RESULTS

Analysis of variance revealed that hypothalamic NE concentrations were not influenced appreciably by the housing condition Concentrations of the amine were, however, found to vary as a function of the shock treatment mice received, $F(4,87)=3.00$, $p<0\ 05$ Newman-Keuls multiple comparisons ($\alpha=0.05$) of the shock main effect indicated that a single shock session provoked a modest reduction of NE relative to nonhandled animals ($p<0\ 10$) Consistent with our earlier observations [10] NE levels following 3 sessions of shock did not differ from those of nonshocked animals, while concentrations of the amine following 15 shock sessions significantly exceeded those of both nonshocked groups

It was previously reported that reductions of hippocampal NE are less readily provoked than are the hypothalamic NE reductions, although a reduction of hippocampal NE can be achieved provided that the stressor is relatively intense or of sufficient duration [10,19] In the present investigation acute shock did not result in a reduction of hippocampal NE concentrations This was the case even when the shock was applied over 3 consecutive days Together with the earlier observations, it appears that in order for hippocampal NE reductions to be provoked a stressor of sufficient intensity or duration must be applied during a single session

Paralleling the variations of hypothalamic NE, analysis of the plasma corticosterone concentrations revealed that the housing manipulation was without effect, while the shock treatment significantly influenced steroid concentrations, $F(4,85)=42\ 28$, $p<0\ 01$ Newman-Keuls multiple comparisons ($\alpha=0\ 05$) indicated that corticosterone concentrations were elevated irrespective of whether mice received 1, 3 or 15 shock sessions This finding is clearly distinguishable from the central NE variations where 1 and 15 shock sessions had opposite effects The Newman-Keuls multiple comparisons also confirmed that in nonshocked mice that had been handled throughout the chronic phase of the study

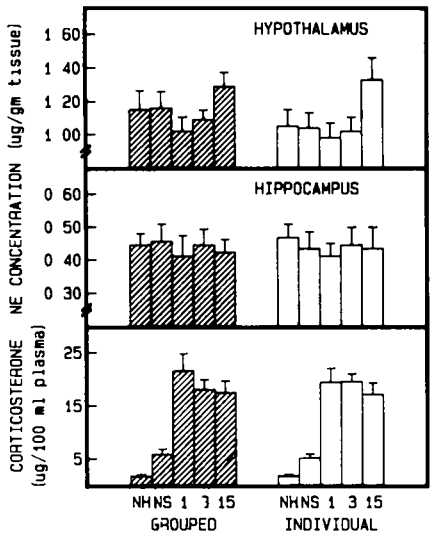

FIG 1 Concentrations of norepinephrine (±S E M) in hypothalamus and hippocampus, and the concentrations of plasma corticosterone as a function of housing condition and stress treatment NH=nonhandled-nonshocked, NS=handled-nonshocked Numbers (1, 3, 15) denote the number of shock sessions mice received

corticosterone concentrations significantly exceeded those of mice which had neither been handled nor exposed to shock. Handling itself has been shown to be a moderate stressor which ordinarily produces an increase of plasma corticosterone [23] It was expected that by prehandling animals adaptation to this procedure would be established, and consequently corticosterone concentrations would be reduced relative to animals that had not been handled previously The fact that corticosterone concentrations were elevated in repeatedly handled mice provisionally suggests that the use of this procedure over the 15 day period had, contrary to expectation, served to increase the stressfulness of the situation It might have been expected that the effects of handling on corticosterone secretion would vary as a function of the background stimulation that animals received However, the effects of handling were comparable in grouped (that received stimulation from conspecifics) and individually housed animals.

DISCUSSION

Consistent with previous reports [4, 23, 24], exposure to footshock in the present investigation resulted in a marked increase of plasma corticosterone This was the case regardless of whether mice received 1, 3 or 15 shock sessions These groups did not differ from one another, and there was little indication of adaptation with repeated shock exposure Although it has been reported that the increased corticosterone concentrations ordinarily associated with acute stressors are diminished following repeated stressor application [4], such an outcome was not evident after repeated handling or footshock in the present investigation Other investigators have likewise failed to detect an adaptation effect with a wide variety of stressors, including placement in a novel environment [7,9], saline injection, cold exposure, forced running, immobilization and footshock [9, 11, 12] The source for the divergent results seen across laboratories is not immediately evident; however, given the large number of social, organismic and experiential variables that influence corticosterone secretion [23], the differential outcomes are not particularly surprising.

The effects of handling and repeated shock exposure on central NE concentrations were distinct from those of plasma corticosterone. Whereas handling increased plasma corticosterone levels, this manipulation did not influence hypothalamic NE concentrations. Furthermore, while corticosterone concentrations remained elevated after both acute and repeated shock, the variations of NE concentrations associated with acute shock were eliminated in chronically shocked animals. In fact, the NE concentrations associated with chronic shock were diametrically opposed to those seen after acute shock. Clearly, the variations of central NE associated with stressor application are incongruent with the corticoid alterations engendered by these treatments As such, these data suggest that variations of NE and corticosterone may reflect different attributes of the stressor (e g , arousal, defense styles) or are subserved by different underlying mechanisms.

The amine and steroid alterations following acute and chronic stressor application may be reflective of efficient adaptive responses to environmental demands, Earlier work in this and in other laboratories [10, 14, 26, 28, 29] revealed that following acute stressor application NE release exceeds utilization, resulting in a net reduction of amine concentrations [1,29] As a result the organism's ability to contend with environmental stressors may be impaired. Following chronic stressor application NE release proceeds at an accelerated rate, but a compensatory increase of amine synthesis may be provoked, thus resulting in increased NE concentrations [1,10]. As in the present report, it was observed that concentrations of NE in chronically shocked animals may actually come to exceed those of nonshocked animals [1,22] Moreover, the increased synthesis and concentrations may persist for some time after stressor termination, thereby assuring adequate amine stores in the event that the stressor is again encountered [26]. It is thought that the increased amine synthesis and concentrations may be essential in order for the animal to meet immediate environmental demands, and would augment the preparedness of the organism to deal with impending stressors.

It has long been maintained that corticosterone secretion in response to stressors would ensure that the organism would be optimally prepared to deal with environmental demands (e.g , through increased availability of blood glucose, anti-inflammation) Under some conditions adaptation in the form of reduced corticosterone secretion may not be evident, and in fact, enhanced corticosterone secretion may be apparent after a repeated stressor regimen [8,9] The factors which determine whether adaptation or sensitization of glucocorticoid activity would occur have not yet been determined. However, in considering the adaptive value of pituitary-adrenal activation, it is significant that glucocorticoids actually suppress some physiological processes (e.g., immune functioning) Thus, it was suggested that one of the primary functions of the stressor-provoked glucocorticoid secretion is to protect the organism from potential physiological overshoots, which could have adverse consequences, e g , autoimmune disorders [18] Following this line of reasoning, it might be expected that the adrenal cortex would be capable of generating a rapid response to acute stressors. This system, however, would be relatively resistant to adaptation, and would occur only after homeostasis had been achieved in other systems.

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