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BRIEF COMMUNICATION

Time Course for Plasma 11-Hydroxycorticosteroid Elevation in Rats during Stress[1]

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BASSETT, J. R. AND K. D. CAIRNCROSS. *Time course for plasma 11-hydroxycorticosteroid elevation in rats during stress.* PHARMAC. BIOCHEM. BEHAV. 3(1) 139–142, 1975. – The time course of plasma 11-hydroxysteroid elevation was studied in two stress situations: regular unsignalled foot shock which produces an intermediate steroid elevation and irregular signalled foot shock with the possibility of escape, which produces an extreme steroid elevation. The initial time course for steroid elevation followed a similar pattern for both treatment groups with the exception that in the irregular signalled group the plasma steroid elevation was more pronounced and there was an indication of biphasic response. The results are discussed in terms of possible inhibitory feedback pathways.

Stress Corticosteroid elevation Inhibitory feedback

BASSETT *et al.* [1] in a parametric study on the corticosterone release in the rat indicated two degrees of corticosteroid response to stress, an intermediate steroid elevation (50–60 μg/100 ml plasma) and extreme steroid elevation (90–100 μg/100 ml plasma). In order to explain these results, it was suggested there exists a two component system acting centrally on the hypothalamic-pituitary-adrenocortical system. In normal circumstances corticosterone released in response to a stressor acts through an inhibitory feed-back mechanism on the hypothalamus thus preventing further release of ACTH from the anterior pituitary. However, in situations involving a high degree of psychological stress it was suggested that the inhibitory feedback mechanism could be modified or by-passed through involvement of higher centres such as the hippocampus.

The possibility exists however, that the two levels of steroid response reported by Bassett *et al.* [1] do not reflect absolute differences in steroid levels. The intermediate steroid response may reflect a slower time course for steroid elevation such that similar steroid levels would be obtained eventually with all stressors. Conversely the intermediate steroid response may reflect a high steroid response which, due to the differing stress parameters, is not maintained over the treatment period. In order to clarify this point the time course for plasma steroid elevation was studied in two stress situations. The treatments selected were regular unsignalled foot-shock (Reg.-unsig.) since this produced intermediate steroid elevation, and irregular signalled foot-shock with the possibility of escape (Irreg.-sig. Escape) as this produced extreme plasma steroid elevation [1].

METHOD

Animals

Male CSF rats 87–93 days old were used in all experiments. The animals were housed in groups of 3 under conditions of constant temperature and humidity (21 ± 0.5°C, 46% humidity) and subjected to a 12 hr night–day routine (light 8 a.m.–8 p.m.) beginning at least 14 days prior to commencement of experimentation and continuing until its conclusion. Food and water were provided ad lib. Both control and stressed rats were housed under identical conditions.

Apparatus and Procedure

The apparatus and stress parameters for the Reg.-unsig. and Irreg.-sig. Escape groups were the same as that described by Bassett *et al.* [1]. In the case of the Reg.-unsig. group naive animals were placed in a white Plexiglas box of

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internal dimensions 34 x 23 x 33 cm high with a grid floor of stainless steel 0.65 cm dia. rods set at 1.9 cm centers. The unconditioned stimulus (UCS) was 2 sec of scrambled footshock repeated every 88 sec and delivered through the grid floor as a 2 mA, 50 pulses/sec d.c. square wave. The animals remained in the stress box for either 2, 5, 10, 20, 25 or 35 min and upon removal were immediately sacrificed. Nine animals were stressed for each of the stress periods mentioned above. For the Irreg.-sig. Escape group the animals were placed in automated 1-way avoidance boxes (Lafayette Model No. 85200) described in detail by Bassett *et al.* [1]. An escape platform was made available to the animal by an automated movable partition. A light conditioned stimulus of 2 W was located on the wall of the grid chamber opposite to the escape platform. The UCS was delivered by a generator-scrambler through the grids as a 2 mA, 50 pulses/sec square wave. Each rat was placed on the escape platform at the commencement of the treatment session. Treatment consisted of 7CS-UCS exposures randomly placed in the 35 min session. On each trial the CS onset 4 sec before the animal was pushed by the movable partition from the platform onto the grid which was simultaneous with the onset of the UCS. At this the movable partition immediately retracted and the animal was able to jump from the grid to the re-exposed platform with a minimum latency of 0.3 sec. The UCS was terminated by the return of the animal to the platform. Irreg.-sig. Escape animals were stressed daily, one session/day, for 4 days in order to obtain a minimum stable escape latency thus ensuring that all animals would receive approximately the same duration for footshock in the final stress session [1]. Such a prolonged exposure to Irreg.-sig. Escape stress does not affect the extreme steroid elevation produced by this stressor [1]. During the final stress session animals were removed after 2, 5, 10, 15, 20, 25 and 35 min exposure and immediately killed. The duration of exposure in the final stress session and their relation to the sequence of intermittent footshock is shown in Fig. 2. As with the Reg.-unsig. group 9 animals were stressed for each stress period. Both Reg.-unsig. and Irreg.-sig. Escape stress procedures were carried out between the hours 9 a.m.–12 noon.

Glucocorticoid Assay

Immediately following the last stress episode the animals were sacrificed by cervical dislocation and exsanguinated. The blood was collected in heparinized tubes and centrifuged in order to obtain cell free plasma which was then frozen. Corticosterone levels in plasma were determined subsequently by the fluorometric method of Mattingley [8], which is specific for free 11-hydroxycorticosteroids.

RESULTS

A plot of mean plasma corticosterone levels (± S.E.) versus duration of stress for the Reg.-unsig. group is shown in Fig. 1. The critical value for a significant difference between any two means, as determined by the Tukey test, was 9.7 at the 5% level and 11.4 at the 1% level of confidence (MS_{error} = 42.8, df = 64). The plasma corticosterone level rose rapidly during the 10 min immediately following commencement of stress, and began to plateau after some 15 min. No further significant elevation occurred between 20–35 min of stress, the peak steroid elevation being 51 ± 2.3 μg/100 ml plasma after 35 min.

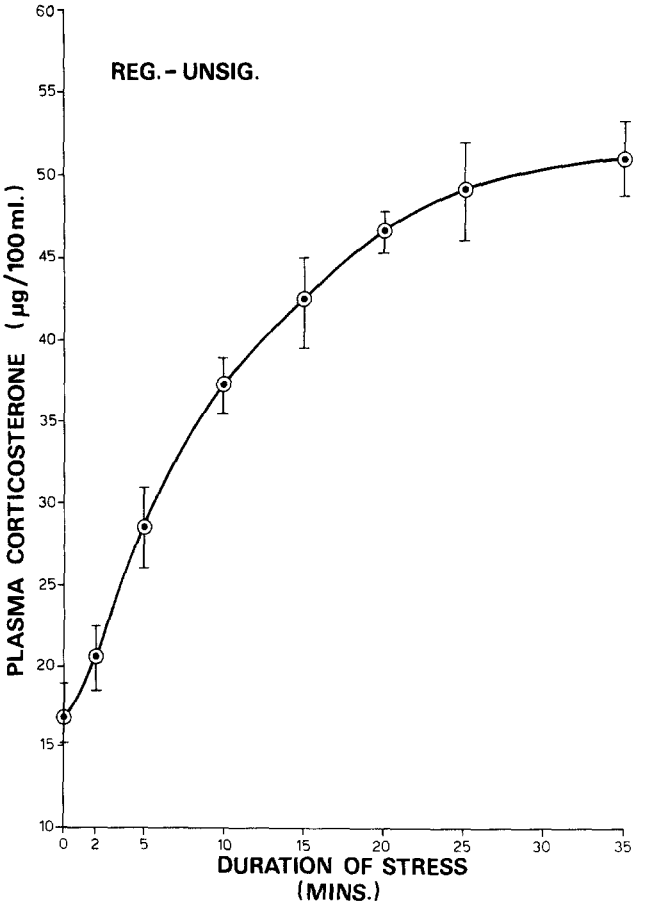

FIG. 1. Time course of plasma corticosterone elevation following regular unsignalled foot-shock. Each point represents the the mean level of plasma steroid (±S.E.) derived from nine animals.

These results are in close agreement with those reported by Bassett *et al.* [1] where the plasma steroid elevation resulting from the same stress parameters was found to be 49 ± 3.8 μg/100 ml plasma after 45 min of stress. It would appear, therefore, that the maximum steroid elevation resulting from regular unsignalled footshock is maintained without change during continued exposure to the stressor for a 45 min period.

A plot of mean plasma corticosterone levels (± S.E.) versus duration of stress for the Irreg.-sig. Escape group is shown in Fig. 3. In this case the maximum steroid elevation after 35 min of stress was 90 ± 6.4 μg/100 ml plasma. The critical value for a significant difference between any two means was 23.0 at the 5% level and 27.2 at the 1% level of confidence (MS_{error} = 221.4, df = 56). These figures again correlate well with those reported in our earlier paper [1] where 35 min of Irreg.-sig. Escape stress resulted in a steroid elevation of 89 ± 4.9 μg/100 ml plasma.

DISCUSSION

It can be seen from Figs. 1 and 3 that the initial time course for elevation of plasma corticosterone follows a similar pattern for both treatment groups with the excep-

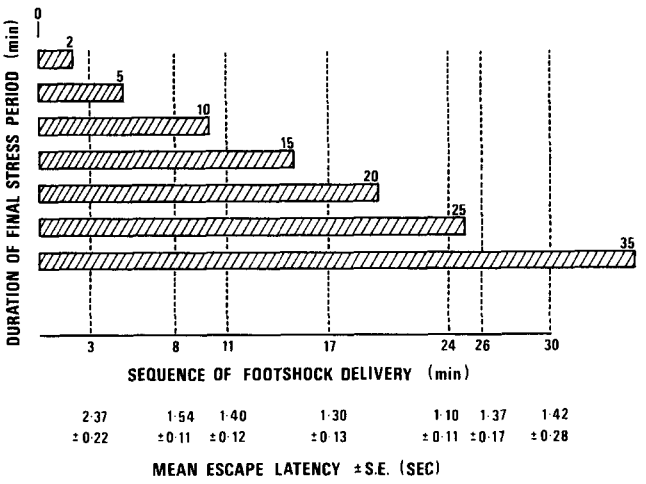

FIG. 2. Irregular signalled footshock where escape is possible. The sequence of stress episodes in the last stress period, the mean escape latency for each stress episode (±S.E.) and the time of killing are shown.

tion that in the Irreg.-sig. Escape group the plasma steroid elevation is more pronounced and there is an indication of a biphasic response. With both treatments the steroid level initially rose rapidly than plateaued to a level which was maintained for a period of at least 45 min in the Reg.-unsig. group and 35 min in the Irreg.-sig. Escape group. It would appear therefore, that the results presented in our previous study which indicated two different levels of steroid response cannot be explained on the basis of differences in the time course of plasma corticosterone elevation.

The possibility of the existence of a two component system acting centrally on the hypothalamic-pituitary-adrenocortical system can now be re-examined. Such a two component system need not involve solely neural connection to the hypothalamus. Stressful stimuli have been classified as humoral or neural according to whether they trigger the release of ACTH in the absence or presence of a central nervous input to the hypothalamic-pituitary complex [2, 3, 5, 6]. Makara *et al.* [7] suggested that it may be the severity of the stress which determines the extent of the humoral or neural component. More severe trauma may rapidly activate not only neural but also humoral pathways triggering further ACTH release. Such a suggestion would explain the intermediate and extreme steroid levels seen in these experiments as well as the biphasic appearance of the extreme steroid response since the humoral component is slower in onset [7].

Differences in the rate of rise of plasma steroid levels may also determine the level of steroid response. Jones *et al.* [4] found that the inhibitory action of corticosterone on further release of ACTH was rate sensitive. There was a critical rate of rise of plasma corticosterone in excess of which the stress response was inhibited. Rates of rise less than the critical values did not affect the stress response. Jones *et al.* [4] found, however, that the inhibitory feedback control could be saturated at high plasma corti-

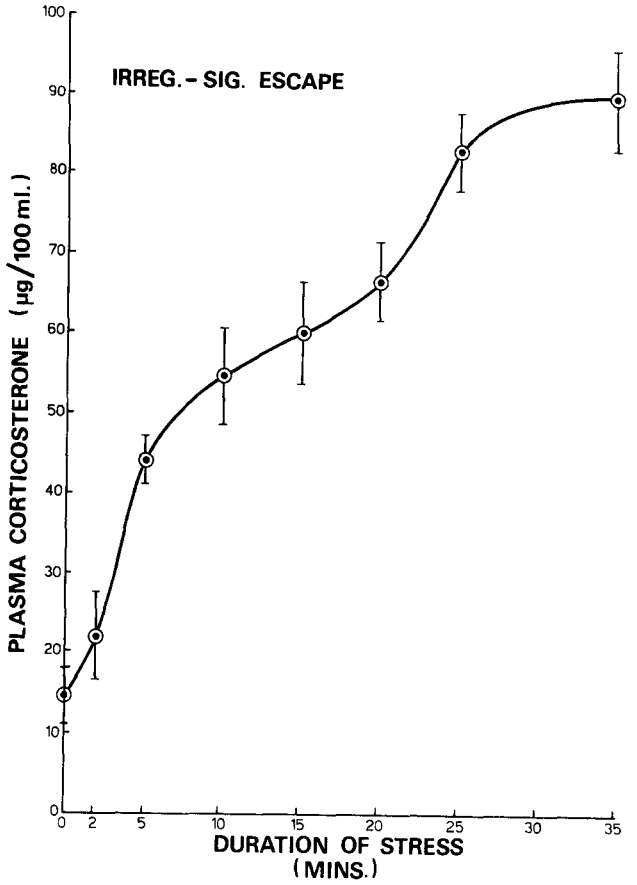

FIG. 3. Time course of plasma corticosterone elevation following irregular signalled footshock where escape is possible. Each point represents the mean level of plasma steroid (±S.E.) derived from nine animals.

costeroid concentrations and when this occurred the steroid response to stress was no longer inhibited. In the present series of experiments the rate of rise of plasma steroid in both the Reg.-unsig. and Irreg.-sig. Escape groups is in excess of the critical value proposed in the Jones study. As a result of this the degree of steroid release would be limited by the inhibitory feedback control producing an intermediate elevation. However, in the case of Irreg.-sig. Escape treatment which produces a high degree of psychological stress, the plasma steroid level is greater. In this situation saturation of the inhibitory feedback mechanism could occur. The biphasic response and extreme steroid levels demonstrated in Fig. 3 could therefore reflect a receptor saturation of the feedback control systems and consequent lifting of the inhibition of ACTH release.

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