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RESPONSE OF THE ADRENAL CORTEX AND MEDULLA OF UNILATERALLY SPLANCHNICOTOMIZED RATS TO SHORT IMMOBILIZATION STRESS

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SUMMARY

The early response of the adrenal cortex and medulla to short immobilization stress of 5 min was studied in adult male rats after unilateral splanchnicotomy. Plasma and adrenal corticosterone as well as adrenal catecholamines were measured fluorometrically 5, 15, 30 and 120 min after the onset of the stress. Denervation induced a slight increase in adrenal corticosterone levels and a decrease in adrenal adrenaline content without change in noradrenaline level. The short immobilization stress provoked the same type of increase in corticosterone in both intact and denervated gland. Adrenaline pattern was also similar in both glands but the levels remained significantly lower in the denervated gland. On the other hand, the early increase after 15 min of stress interval in noradrenaline levels of intact glands was abolished by denervation. The results suggest that intact nervous supply does not seem to be necessary for adrenocortical response to short immobilization stress. On the other hand adrenaline and noradrenaline responses depend to some extent on intact nervous supply.

INTRODUCTION

The variations in adrenal and blood content of neurotransmitters and certain steroid hormones during emotional and mechanical stress are well known. The effects of prolonged immobilization stress on the enzymes of catecholamine biosynthesis and degradation have been studied extensively [1–3]. Neuronal regulation of the adrenal medullary enzymes could be evaluated after denervation of the left adrenal gland by severing the left splanchnic nerve [2]. These authors concluded that for an increase of catecholamine regulating enzymes to be induced by immobilization stress an intact nerve supply was necessary. The response of the adrenal cortex to immobilization stress has been extensively studied [4, 5]. It has been established that a short period of immobilization is sufficient to provoke a quick and a high increase in the adrenal as well as blood corticosterone levels [6]. This effect is mediated by ACTH [7]. However, the adrenal cortex of hypophysectomized rats has been shown to have increased levels of corticosterone after short immobilization stress [8]. It has been speculated that the nervous system by itself or through the media of the adrenal medulla may be implicated for this response [6]. Most of the past experiments have employed prolonged immobilization stress to study interrelations between adrenal cortical and medullary function. The present experiments were designed to investigate the effects of short-term immobilization stress on adrenal cortical and medullary function in the presence or absence of nerve supply by estimating corticosterone in blood and adrenals as well as adrenal catecholamines.

MATERIALS AND METHODS

Male Sherman rats were used in all the experiments. They weighed between 180 and 200 g. The left adrenal gland was denervated by cutting the left splanchnic nerve just under the diaphragm. All the surgical procedures were carried out under ether anaesthesia. The animals were sacrificed 3 weeks after the operation and the validity of the denervation was verified by microscopic examination.

Immobilization stress. The rats were held in a prone position with their four limbs fixed to a wooden board for 5 min. The stress period commenced from the moment the animals were removed from the cage of their normal habitation. The immobilized rats were killed after different specified intervals which ranged between 5 to 120 min. The animals were sacrificed by breaking the cervical vertebra. Blood was collected by aortic puncture and the adrenals were rapidly removed. These manipulations were performed in a room adjacent to the animal house to minimize uncontrolled stress.

Determination of corticosterone. Corticosterone was determined in plasma as well as the adrenals. The adrenals were homogenized in 3 ml of 4% trichloroacetic acid. This homogenization in trichloroacetic acid did not interfere with the fluorescence intensity of corticosterone as compared to homogenates in distilled water of KCl. Fluorometric measurements of corticosterone were carried out according to the method of De Moor [9]. The experimental procedure consisted of delipidation in petroleum ether (60–80°C), extraction with methylene chloride and washing with NaOH 0.1 N. The original method was

slightly modified since the readings were taken 30 min after the addition of sulphuric acid + ethanol reagent instead of 5 min. The precision of the method (coefficient of variation) was 7.3%. All the measurements for fluorescence intensity were made in an Aminco-Bowman spectrofluorometer (excitation at 465 and emission at 525 nm).

Assay of adrenal catecholamines. Adrenal catecholamines were determined fluorometrically [10]. One adrenal gland was homogenized in 3 ml of trichloroacetic acid (4%). After centrifugation 50 μl of supernatant were processed for direct assay of adrenaline and noradrenaline [10]. Trihydroxindole reaction was employed for differential estimation of catecholamines in mixed samples [11, 12]. The coefficient of variation for adrenaline as well as for noradrenaline was 2%.

Protein determination. Adrenal proteins were determined using folin reagent [13].

Statistical analysis. The experiments were designed in a completely randomized manner. Single (blood samples) or two-way (adrenal samples) analysis of the variance (ANOVA) model I were carried out after logarithmic transformation of the data, if required, to avoid heteroscedasticity (F-max. test). When the overall ANOVA was significant an SNK posteriori test was applied to study the differences among the means [14].

RESULTS

Figure 1 illustrates the effects of sympathetic denervation upon adreno-cortical response to short immobilization stress. After 5, 15 and 30 min of the beginning of immobilization stress, a progressive and significant increase in corticosterone content of the intact gland was observed. The response of the denervated gland followed the same pattern of evolution. After 120 min the mean value in both glands returned to the value of pre-stress levels. The results followed the same pattern whether expressed in μg/adrenal or μg/mg or adrenal protein.

Figure 2 shows variations in blood corticosterone content at different intervals after short immobilization stress of 5 min. Similar to adrenals, constant increases in blood corticosterone occurred at 5, 15 and 30 min. After 120 min no significant variation in blood corticosterone was observable.

The changes in adrenaline content of intact and denervated gland of rats subjected to short immobilization stress are given in Fig. 3. The intact gland showed slight but insignificant decreases in adrenaline content (μg/adrenal as well as μg/mg of adrenal protein) up to 15 min after the beginning of the stress. The denervated gland showed a non-significant rise at 5 min. After 30 and 120 min progressive increases in adrenal content of adrenaline took place in both intact and denervated glands. The results again followed a similar pattern when expressed in μg/adrenal or μg/mg of adrenal protein. The pattern of evolution in intact and denervated glands was similar, but the

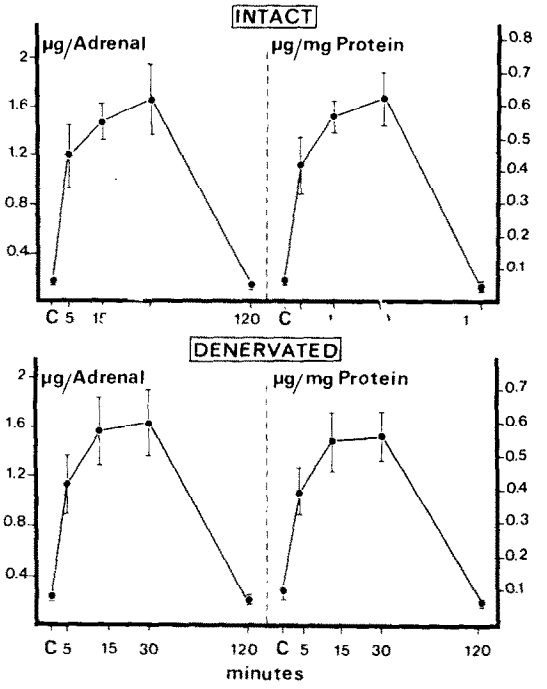

Fig. 1. Time course for adrenal corticosterone elevation following 5 min immobilization stress in intact and denervated glands. Mean corticosterone values ± S.E.M. ($n = 8$) are plotted against time of survival since the beginning of stress. Two way analysis of the variance (ANOVA) was significant ($P < 0.001$) due to differences in time factor ($P < 0.001$) for both ways of expression of results (μg/adrenal and μg/mg adrenal protein). No significance was found for denervation factor and interaction. The SNK a posteriori test gave significant differences at level $\alpha = 0.01$ between: C (control) vs. 5 min and 30 vs. 120 min.

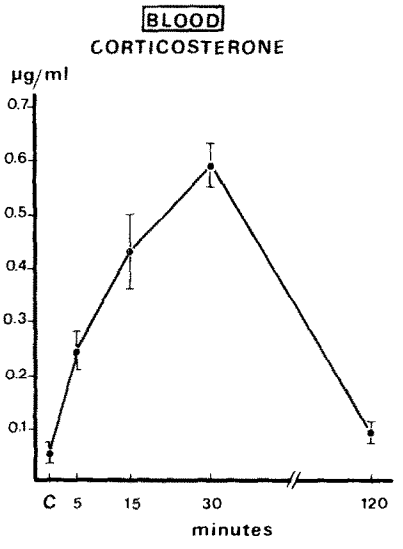

Fig. 2. Time course for blood corticosterone elevation following 5 min immobilization stress in unilaterally splanchnicotomized rats. Mean corticosterone values ± S.E.M. ($n = 8$) are plotted against time of survival since the beginning of stress (5, 15, 30 and 120), C (controls). One-way analysis of the variance (ANOVA) $P < 0.001$ SNK test: C vs. 5 S($\alpha = 0.01$); 5 vs. 15 S($\alpha = 0.05$); 15 vs. 30 S($\alpha = 0.05$); 30 vs. 120 S($\alpha = 0.01$); C vs. 120 not significant.

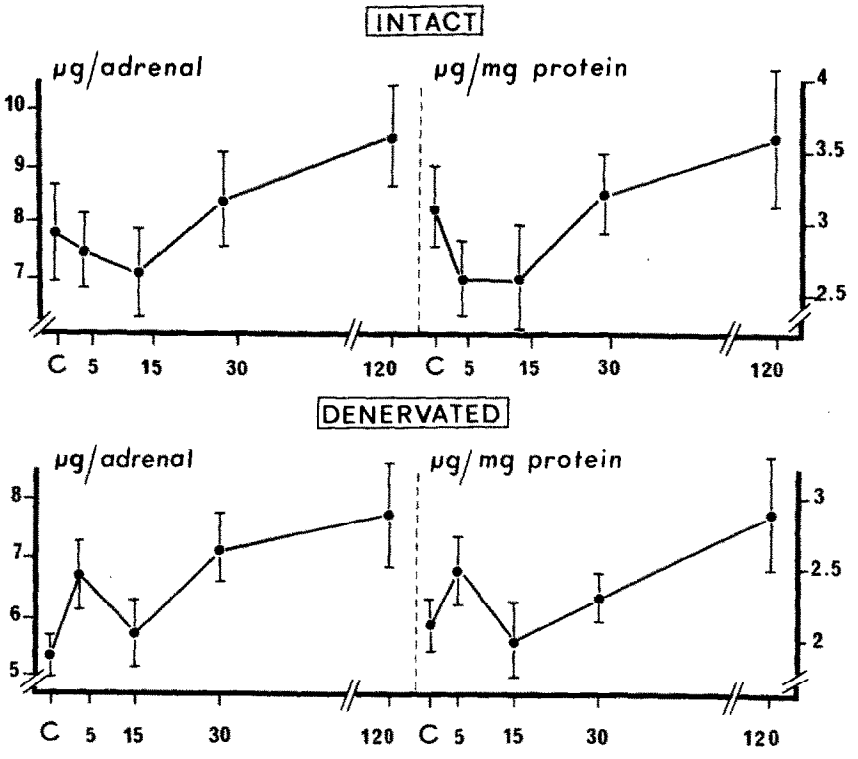

Fig. 3. Variations in adrenal content of adrenaline following 5 minutes of immobilization stress in intact and denervated glands. See Fig. 1 for further details. Overall analysis of the variance (ANOVA) $P < 0.05$ (μg/mg adrenal protein), $P < 0.01$ (μg/adrenal). Denervation factor, $P < 0.001$. Time factor and denervation, non significant. No SNK test was performed due to the non-significant values for time factor and interaction.

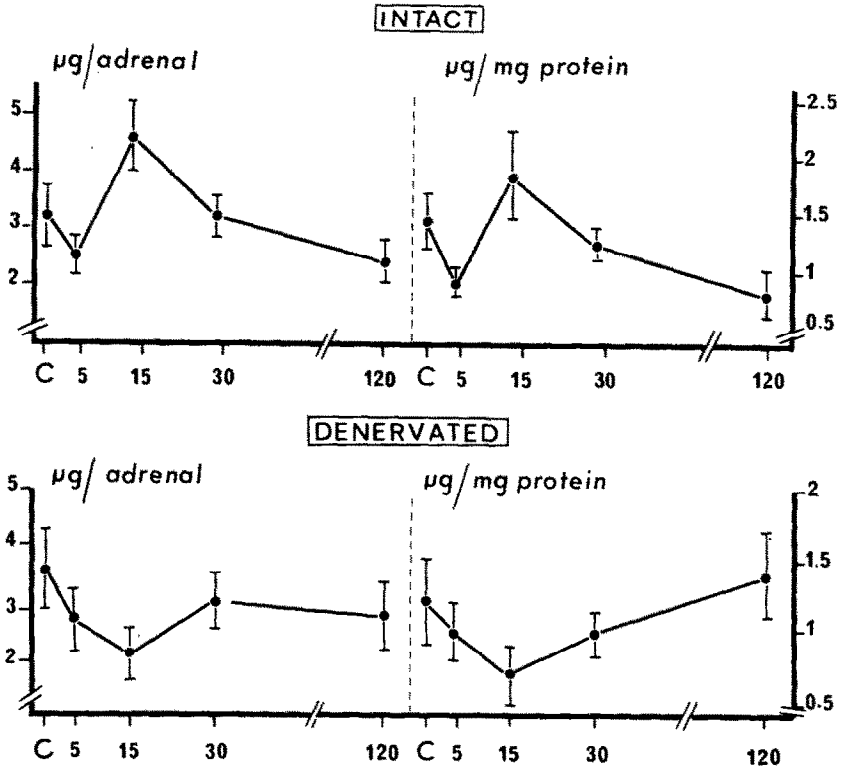

Fig. 4. Noradrenaline content of adrenal gland during short term immobilization stress. For details see Fig. 3. Overall two-way analysis of the variance $P < 0.01$ (μg/adrenal), $P < 0.05$ (μg/mg of protein). Time factor, not significant; denervation factor, not significant; interaction, $P < 0.05$. SNK test: No difference among denervated gland values. For intact glands: 5 vs. 15 S($\alpha = 0.01$), 15 vs. 120 S($\alpha = 0.01$).

Table 1. Total catecholamine (adrenaline + noradrenaline) content of intact and denervated adrenals of rats killed before (control) or at several intervals (5, 15, 30, 120 minutes) after the onset of 5 min immobilization stress

Treatment	Control	Total catecholamines (μg/adrenal gland) 5 min	15 min	30 min	120 min
Intact gland	10.1 ± 1.1	9.4 ± 1.0	12.0 ± 0.8	11.7 ± 0.9	11.4 ± 0.7
Denervated gland	9.3 ± 1.1	8.6 ± 0.7	7.5 ± 0.9	9.9 ± 0.9	10.4 ± 0.6
		(μg/mg of adrenal-protein)			
Intact gland	4.2 ± 0.5	3.5 ± 0.4	4.8 ± 0.5	4.4 ± 0.3	4.3 ± 0.5
Denervated gland	3.6 ± 0.4	3.3 ± 0.3	2.9 ± 0.4	3.2 ± 0.2	4.0 ± 0.4

Number of cases = 8. Overall two-way analysis of variance (ANOVA) – $P < 0.05$, denervation factor, $P < 0.01$, time factor and interaction, not significant. No SNK test was performed due to non-significant values for time factor and interaction.

content of adrenaline in denervated gland was significantly lower than intact gland for all the intervals studied.

Figure 4 provides noradrenaline content of intact and denervated adrenal gland in rats subjected to short immobilization stress lasting 5 min. After 5 min of stress adrenal noradrenaline in intact as well as in denervated gland showed a slight but insignificant decrease. The effects were inversed after 15 min between intact and denervated gland since the intact gland showed a significant increase whereas the denervated gland demonstrated a continuous decrease (45% increase for intact and 42% decrease for denervated, respective to control values). After 30 and 120 minutes the denervated gland started to recover towards the control value. The intact gland showed a return to normal values after 30 min, but after 120 min adrenal noradrenaline was lower than the controls when expressed in μg/adrenal or in μg/mg of adrenal protein.

Table 1 provides variations in total catecholamine content in intact and denervated gland at various intervals after 5 min immobilization stress. The total catecholamine content has been shown to facilitate the interpretation of results and to distinguish the effects of individual catecholamines (adrenaline and noradrenaline) from the entire medullary function.

DISCUSSION

The adreno-cortical response to immobilization stress does not appear to be dependent on nerve supply, at least in the presence of ACTH. The results are similar to those obtained after cerebral hemidecortication [6]. Under normal conditions corticosterone levels are slightly higher in the denervated gland, but the stress produces a similar response in both glands [6]. Some direct effects of the nervous system on the adrenal cortex have been reported. After cerebral hemidecortication some hyperactivity is detected in the gland contralateral to the lesion, as compared to the homolateral, when karyometric and other morphological methods are used [15, 16]. Unilateral hypothalamic lesions give similar results, less pronounced [17]. Mid-brain transection gives an increase in basal levels of corticosterone [18] and spinal cord transection is followed by an increase in nuclear volume [19]. Unilateral splanchnicotomy [20] induces an increase in the stocks of birrefringent material. In some of these cases the apparent hyperactivity of the denervated gland turns into a reduced response to stress [15, 17, 19, 25]. It should be pointed out that many of the morphological studies are based on increase in adrenal size or in nuclear volume as indexes for adrenocortical activity. Even if good correlation has been found between morphological and biochemical methods [21], it has also been shown that increase in adrenal weight can be a neurally-mediated reflex without corresponding elevation in corticosterone levels [22, 23].

Biochemical data only partially confirm these findings. Corticosterone levels are higher in the gland contralateral to hemidecortication, but the response to stress, although lower, is not significantly different to that of the homolateral gland [6]. The present study gives similar results, but in this case none of the side differences are significant.

It is well established that ACTH evokes the steroidogenic response *via* c-AMP, but only some part of the nucleotide released is needed to obtain the maximal effects [25, 26]. The rise in c-AMP after immobilization stress is greatly reduced in the denervated gland [27], but it could be enough to provide a normal corticosterone elevation. It could explain the similar levels in intact and denervated gland.

Previous studies in hypophysectomized rats have shown that the adrenal cortex of the animals is able to increase its steroid content, as well as blood levels, in response to short immobilization stress [8]. This response is different to that in the intact rat, since the rise takes 15 min instead of 3 min to appear, and the elevation is obviously much smaller. The stress level in hypophysectomized animal is just below the basal level in intact animals [8]. This response could be attributed to the nervous system. In view of the present results this possibility cannot be excluded, since such a slight modification could have been overlapped by the massive response due to ACTH. How-

ever, the physiological importance of this hypothetical nervous control seems far from that of ACTH.

The catecholamine content of the adrenal medulla in response to denervation depends on the length of post-operatory interval. After 3 days no significant change occurs [28], but one to two weeks later a significant decrease has been reported [29]. Our results are in agreement for adrenaline and also for total catecholamine content, but there is no difference between control and denervated gland for noradrenaline. Stress and trauma induce an increase in blood catecholamines [30] as well as in their urinary metabolites [3] with a concomitant decrease in the adrenal medulla [3]. This decrease appears after 90 min of immobilization for adrenaline, but noradrenaline requires a longer time interval [2]. Nevertheless, the urinary excretion increases significantly for each time interval of immobilization studied. There is a rapid resynthesis of adrenaline after splanchnic nerve stimulation or chemically induced release of this hormone [31]. This acceleration of the synthesis of catecholamines has also been found after stress [32]. It can explain the urinary and blood increase without relevant changes in the adrenal gland, indicating an adjusted balance between synthesis and release of these hormones.

In response to mild stress (5 min immobilization) we have found a significant increase in adrenal noradrenaline 15 min after the beginning of the stress in the intact gland. This quick response can be attributed to the activation of tyrosine-hydroxylase by removal of end-product inhibition. The further decrease could be related to the increase in adrenaline found after the initial fall. In the denervated gland, the levels of noradrenaline after stress were similar to those found in the intact gland, except the fact that the early increase at 15 min was abolished. One possible interpretation can be given by the fact that that there is no nerve supply to stimulate early release of noradrenaline which could lead to increase in tyrosine hydroxylase activity with subsequent increase in noradrenaline. On the other hand, the adrenaline levels remained significantly below the controls and the time-course was practically similar in both glands.

The effect of denervation on adrenal medullary response to stress has been studied after long-term immobilization, but only data on enzyme levels were published [2]. After insulin injection (hypoglycemic shock) the catecholamine levels in the intact gland fall to 26% of the controls while the denervated glands show no change [30]. In our case denervation prevented the slight rise in noradrenaline levels 15 min after the onset of the stress.

In conclusion denervation does not modify the response of the adrenal cortex to short immobilization stress. On the other hand it seems to lower the levels of adrenaline without change in the pattern of response to intact as well as denervated gland. The early stress induced increase in noradrenaline is abolished by denervation.

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