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IMPAIRMENT OF PITUITARY ADRENOCORTICOTROPHIC FUNCTION BY CORTICOSTERONE IN THE BLOOD

BY

J. R. HODGES AND JANET SADOW

From the Department of Pharmacology, Royal Free Hospital School of Medicine, London

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The release of corticotrophin (ACTH) in response to stress can be effectively prevented by large doses of corticosteroids (Hodges, 1953, 1954). It has been assumed that the degree of impairment of pituitary activity is directly proportional to the blood level of corticoids. With the exception of those of Smelik (1963a, b), there have been few reports in the literature of experiments where blood corticosteroids and the degree of pituitary inhibition have been measured simultaneously. Hodges & Jones (1964) found in the adrenalectomized rat a lack of correlation between the blood corticosterone concentration and suppression of corticotrophin release.

The experiments described in this paper were performed in order to investigate the relationship between plasma corticosteroid concentrations and the inhibition of corticotrophin release in the normal rat. Rats were subjected to stress at times when their plasma corticosterone levels were known. The consequent adrenocorticotrophic activity was assessed using adrenal ascorbic acid and plasma corticosterone changes as the parameters of corticotrophin release.

METHODS

Animals. Male Wistar rats weighing between 120 and 200 g, supplied by the Oxford Laboratory Animal Centre, were used. The animals were kept at a controlled temperature of 22° C in stock cages, 25 to a cage, for at least 5 days before use. Their diet consisted of cubes (Diet 41B, Lane-Petter & Dyer, 1952) and they were allowed free access to water. The rats were transferred to individual cages with solid sides at least 18 hr before the experiment and were left completely undisturbed during this period. Every experimental group consisted of at least 6 animals.

Blood samples were removed from the abdominal aorta under ether anaesthesia. The maximum time for completing this procedure was 2 min. Blood was rapidly transferred from the syringe into heparinized glass tubes. The samples were centrifuged and the plasma quickly separated and stored in the refrigerator. Plasma corticosterone was estimated by the method of Zenker & Bernstein (1958).

Adrenal glands were removed immediately after decapitation. The glands were rapidly dissected free from fat and connective tissue on a dry tile and weighed on a torsion balance. Each adrenal gland was placed in a separate tube and immediately ground, with the aid of a glass rod, and acid washed sand, in 8 ml. 4% trichloracetic acid solution. Adrenal ascorbic acid content was measured by the method of Roe & Kuether (1943).

Drugs. Corticosterone (Organon) was given subcutaneously to conscious rats as a suspension in 0.9% sodium chloride solution. It was administered intravenously to rats anaesthetized with pentobarbitone sodium (4.5 mg/100 g body weight) dissolved in 0.9% sodium chloride solution containing 2% ethanol.

Stress. Conscious rats pretreated with subcutaneous corticosterone were stressed by exposure to ether vapour for 2 min. Anaesthetized rats given intravenous corticosterone were subjected to sham adrenalectomy, care being taken not to pull on the mesentery.

RESULTS

Figure 1 shows the changes in plasma corticosterone concentration which occurred after the subcutaneous administration of 0.9% sodium chloride solution and suspensions of corticosterone in doses of 1 mg and 10 mg/100 g body weight. The plasma steroid concentrations reached maximal values half an hr after the injections. Control injections of 0.9% sodium chloride solution produced a small initial increase in plasma corticosterone but the concentration of the steroid returned to the resting level within 1 hr. Subcutaneous injections of suspensions of corticosterone produced large increases in the plasma concentration of the steroid, with maximal values of 51 and 77 μg/100 ml. returning to resting levels within 4 and 16 hr after the 1 mg and 10 mg doses respectively.

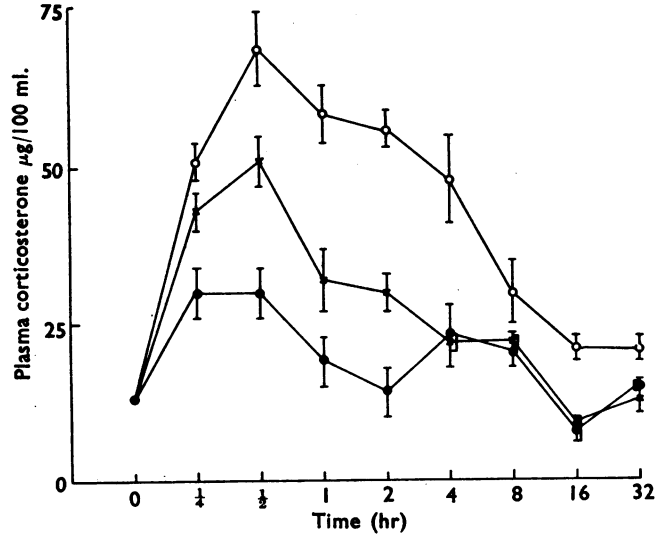

Fig. 1. Plasma corticosterone concentrations in conscious rats at various time intervals after subcutaneous injections of 0.9% sodium chloride solution (—●), 1 mg corticosterone/100 g (×——×) and 10 mg corticosterone/100 g (○——○). The vertical bars indicate S.E. of means.

The saline and corticosterone pretreated animals were subjected to stress at the times indicated on the abscissa of Figs. 1 and 2. Blood samples were removed 30 min and from different groups, adrenal glands 1 hr after the stress. Control animals were similarly dosed but not stressed. Figure 2 shows the stress-induced increments in plasma corticosterone concentration—that is, the differences between the plasma corticosterone concentrations in the animals which had been subjected to stress and those which had not. Stress increased the plasma corticosterone concentration in all the animals which had been pretreated with 0.9% sodium chloride solution. Similar increases occurred in corticosterone pretreated animals when the stress was applied immediately, ¼, ½ and 1 hr

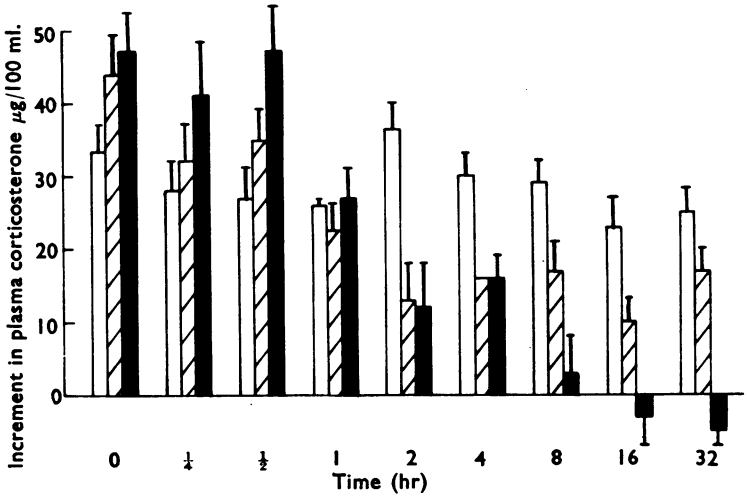

Fig. 2. Plasma corticosterone increments in conscious rats stressed at various time intervals after subcutaneous injections of 0.9% sodium chloride solution (white columns), 1 mg corticosterone/100 g (hatched columns) and 10 mg corticosterone/100 g (black columns). The vertical bars indicate S.E. of means.

after corticosterone treatment, despite the fact that the pre-existing plasma concentrations of the steroid were highest at these times. However, the increments were significantly less ($P<0.05$) 4 to 32 hr after steroid treatment. Eight, 16 and 32 hr after the larger dose of steroid, no significant ($P>0.05$) rises in plasma corticosterone occurred in response to stress. Thus, after treatment with corticosterone there was no inhibition of corticotrophin release when the plasma concentrations of the steroid were raised, but complete inhibition after the 10 mg dose and partial inhibition after the 1 mg dose when they had returned to the resting level.

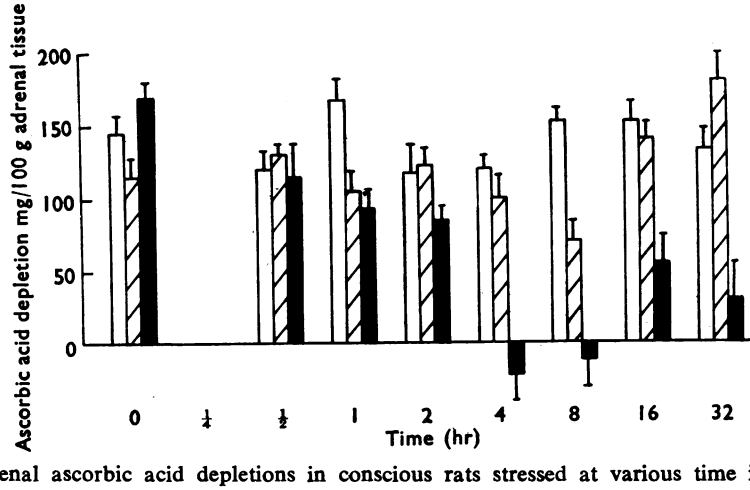

Fig. 3. Adrenal ascorbic acid depletions in conscious rats stressed at various time intervals after subcutaneous injections of 0.9% sodium chloride solution (white columns), 1 mg corticosterone/100 g (hatched columns) and 10 mg corticosterone/100 g (black columns). The vertical bars indicate S.E. of means.

Figure 3 shows the results of a similar experiment using adrenal ascorbic acid changes as the index of pituitary adrenocorticotrophic activity. Stress caused corticotrophin release and adrenal ascorbic acid depletion occurred in the saline-treated groups and in the corticosterone-treated animals when the plasma steroid levels were highest. Inhibition of corticotrophin release developed, there being less or no adrenal ascorbic acid depletion, as the plasma corticosterone concentration fell, which persisted after the plasma corticosterone had returned to resting values. The time interval taken for the development of complete inhibition of corticotrophin release was similar to that seen in the previous experiment.

In another series of experiments, 20 μg or 100 μg corticosterone, or the vehicle alone, were injected intravenously to rats under pentobarbitone anaesthesia. The plasma corticosterone concentrations were measured at various time intervals after the injections. Figure 4 shows the rapid decline in plasma steroid concentration. Rats which had been similarly treated were subjected to sham-adrenalectomy. Plasma corticosterone concentrations were measured 30 min and, from different groups, adrenal ascorbic acid concentrations 1 hr after the stress, as before. Figure 5 shows the results using plasma corticosterone changes as the index of corticotrophin release. The larger dose of corticosterone resulted in marked inhibition of corticotrophin release $2\frac{1}{2}$ min after its administration. The adrenocorticotrophic response to stress was not inhibited during the

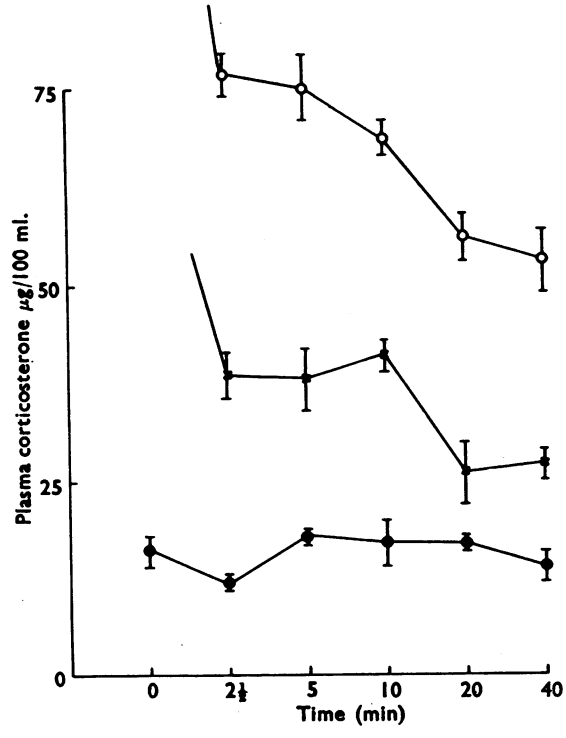

Fig. 4. Plasma corticosterone concentrations in anaesthetized rats at various time intervals after intravenous injections of 0.9% sodium chloride solution (●——●), 20 μg/100 g corticosterone (×——×) and 100 μg/100 g corticosterone (○——○). The vertical bars indicate S.E. of means.

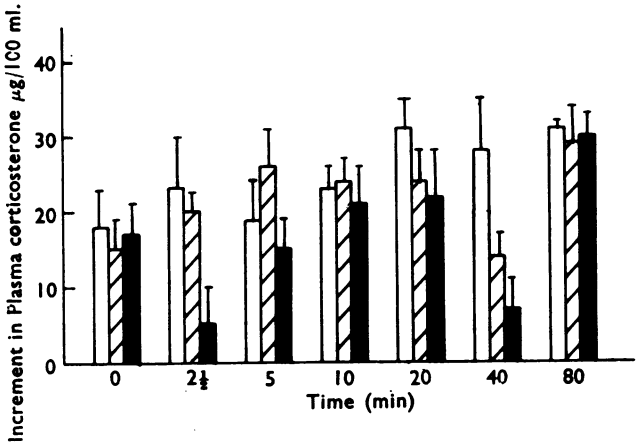

Fig. 5. Plasma corticosterone increments in anaesthetized rats stressed at various time intervals after intravenous injections of 0.9% sodium chloride solution (white columns), 20 μg/100 g corticosterone (hatched columns) and 100 μg/100 g corticosterone (black columns). The vertical bars indicate the S.E. of means.

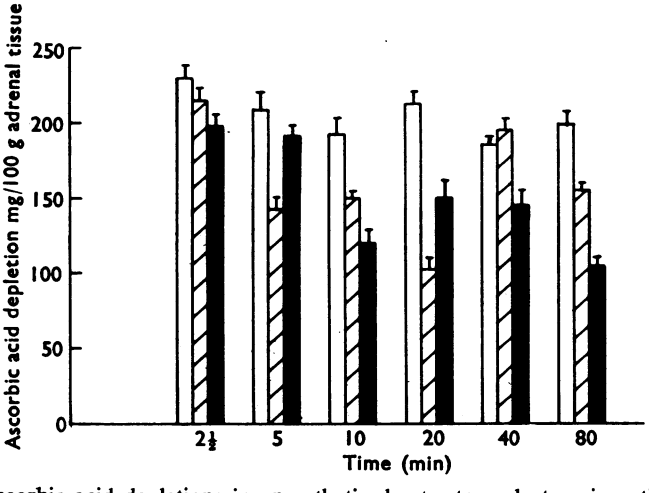

Fig. 6. Adrenal ascorbic acid depletions in anaesthetized rats stressed at various time intervals after intravenous injections of 0.9% sodium chloride solution (white columns), 20 μg/100 g corticosterone (hatched columns) and 100 μg/100 g corticosterone (black columns). The vertical bars indicate the S.E. of means.

subsequent 20 min but partially reduced again 40 min after the treatment. The 20 μg dose produced partial inhibition of corticotrophin release only 40 min after injection and at all other time intervals studied the increments in plasma corticosterone were not significantly different ($P>0.05$) from those in rats given 0.9% sodium chloride solution.

The results of similar experiments done using adrenal ascorbic acid changes as the index of corticotrophin release are shown in Fig. 6. The depletion in ascorbic acid was significantly reduced ($P<0.05$) by corticosterone treatment, 10 to 80 min after the injection, but the degree of inhibition of corticotrophin release was never very great.

DISCUSSION

These results show that the inhibition of corticotrophin release which is produced by the administration of corticosterone is not directly correlated with the blood corticosteroid concentration. They confirm the work of Smelik (1963a) who, using a different parameter of pituitary adrenocorticotrophic activity, clearly demonstrated the same phenomenon.

The possibilities that the secretion of corticotrophin is initiated by an actual fall in blood corticosteroid concentration (Sayers & Sayers, 1947) or by a resetting at a higher level of the blood corticosteroid concentration necessary to inhibit corticotrophin release (Yates, Leeman, Glenister & Dallman, 1961) are difficult to reconcile with recent work. The Sayers' hypothesis became untenable when it was shown that stress causes a rise and never a fall in circulating corticosteroids (Nelson, Samuels, Willardson & Tyler, 1951). The Yates " reset " hypothesis was challenged by Smelik (1963b) and by Hodges & Jones (1963), both of whom were unable to confirm that impairment of pituitary adrenocorticotrophic activity in response to stress is directly correlated with the existing level of blood corticoids. However, Hodges & Jones (1964) concluded that it is not reasonable to exclude the possibility that corticoids play some part in controlling corticotrophin release but they found a lack of direct correlation between the plasma corticosteroid concentration and inhibition of pituitary adrenocorticotrophic activity in the adrenalectomized rat given corticoid replacement therapy.

In the present series of experiments it was found that corticosterone administered subcutaneously to normal rats produces inhibition of corticotrophin release some time after the blood corticoids have returned to pre-experimental concentrations. Similarly, corticosterone administered intravenously produces only slight and transient inhibition of corticotrophin release when the plasma concentration is very high and a greater effect after it has declined.

Recently, Kawai & Yates (1966) showed that intravenous administration of corticosterone together with its binding proteins produces no inhibition of pituitary adrenocorticotrophic activity as distinct from injection of the free steroid and suggested that the ratio bound/unbound corticosterone may be the regulating factor for corticotrophin release. In the present experiments, total corticosterone only was measured. The possibility exists that our results with corticosterone given subcutaneouly could be explained on this basis. The failure of intravenous corticosterone to produce pituitary inhibition could also be explained in this way if the binding process were sufficiently rapid.

Corticoids must exert some effect in controlling corticotrophin secretion but variations in plasma corticosteroid concentration do not appear to affect the acute stress-induced corticotrophin release. Inhibition of ACTH release does occur after corticoid treatment, but only after a considerable time delay, when the plasma concentrations of the steroid have returned towards the resting values. Slusher, Hyde & Laufer (1966) studied this problem more directly by analysis of neuronal unit activity in the posterior diencephalon and midbrain of cats after intravenous injections of cortisol. They demonstrated a similar time delay before the appearance of alterations in the firing rate in the hypothalamus. Such time lags suggest the existence of a corticoid sensitive controller of adrenocorticotrophic activity in some tissue not readily accessible to the systemic circula-

tion. Data obtained from experiments involving placement of discrete lesions or corticoid implants in the central nervous system are in agreement with this view (Fortier, 1966).

It appears that the release of corticotrophin in conditions of stress is regulated by factors other than the total corticosterone concentration in the blood. Inhibition of corticotrophin secretion is a delayed process requiring large doses of corticoids. It is unlikely, therefore, that the corticoids regulate the rapid release of corticotrophin in conditions of acute stress.

SUMMARY

1. The time relationships of the changes in plasma corticosterone concentration were studied in rats after subcutaneous and intravenous injections of the steroid.

2. Corticosterone-treated animals were subjected to stress at times when the plasma steroid concentrations were known.

3. Corticotrophin release in response to stress was not inhibited when the plasma corticosterone concentrations were highest.

4. Release of the trophic hormone was completely or partially impaired when the plasma corticosterone concentrations returned to resting levels.

5. The possible significance of the findings is discussed.

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