FAILURE TO INHIBIT CORTICOTROPHIN SECRETION
BY EXPERIMENTALLY INDUCED INCREASES
IN CORTICOID LEVELS

By

P. G. Smelik*

ABSTRACT

The present experiments were designed in order to investigate whether physiological elevations in corticoid blood levels would inhibit the pituitary-adrenal response to stress. Plasma corticosterone (11β,21-dihydroxy-pregn-4-ene-3,20-dione) levels and the in vitro corticoid production by excised adrenals were determined in anaesthetized rats, pretreated with corticosterone solutions injected intravenously or intramuscularly. Intravenous administration of small amounts of corticosterone induced a very high but transient peak in plasma corticosterone concentrations. Corticosterone infusion caused a constant increase in plasma corticosterone levels. Increases exceeding maximal physiological values did not prevent the adrenocortical activation produced by histamine or corticotrophin.

The summation of exogenous (infused) and endogenous (produced) corticosterone in the plasma became incomplete with increasing levels. This summation was not due to an increasing inhibition of the endogenous production, but to a higher rate of disappearance from the blood.

It is concluded that these data are not in agreement with the »variable set point control theory«, and demonstrate that physiological variations in plasma corticoid concentration do not affect the acute stress-induced corticotrophin release.

* Present address: Department of Pharmacology, University of Utrecht, The Netherlands.
The secretion of corticotrophin from the adenohypophysis is said to be under a negative feed-back control of the adrenocortical hormones. This would be evident from the observations that a supra-normal production of corticotrophin results from the removal (Sydnor & Sayers 1954; Brodish & Long 1956) or pharmacological blockade (Liddle et al. 1959) of the adrenal glands, and conversely, that administration of large amounts of adrenocortical hormones block corticotrophin secretion (Ingle et al. 1938; Sayers & Sayers 1947).

However, these extreme conditions do not occur in the intact organism. It was difficult to determine whether variations in peripheral blood corticoid levels within the physiological range would influence the rate of corticotrophin secretion. Sayers & Sayers (1947) postulated that a decrease in blood corticoid levels, caused by an increased utilization in the tissues, would represent the adequate stimulus for corticotrophin release during stress. Subsequent experimental work, however, showed that this concept is untenable, since the rapid release of corticotrophin cannot be initiated by a preceding fall in peripheral corticoid levels (Bush et al. 1953; Munson & Briggs 1955). Hence it is now widely accepted that the direct stimulus for the reflex corticotrophin secretion is of hypothalamic origin (Harris 1952; Hume 1952).

Theoretically, the possibility is still open that this hypothalamic control is in its turn regulated or at least influenced by changes in blood corticoid concentration. Such a concept would meet the same objections as the original feed-back theory, unless the controller had a variable set point which was followed closely by the peripheral corticoid level. This has been proposed recently by Yates et al. (1961), who assumed that the production of corticoids is not initiated by an actual fall in corticoid levels with regard to the fixed set point of the controller, but by a virtual fall with regard to an elevation of the controller set point. A noxious stimulus would induce a higher set point and thereby cause a discrepancy between the new set point and the existing corticoid level, until this discrepancy had been compensated for by the adrenal production of corticoids.

This theory would imply that an artificial increase in the blood corticoid levels before and of the same extent as the increase produced by a certain stress stimulus, must prevent activation of the controller and consequently of adrenocortical stimulation. In fact, these authors demonstrated in rats that the rise in plasma corticosterone levels, induced by an intravenous injection of corticosterone as well as an experimental stimulus given shortly afterwards, equalled the rise induced by corticosterone injection alone.

However, in the present paper experiments will be described which seem incompatible with the variable set point theory. They indicate that preceding increments in plasma corticosterone levels are not capable of preventing the pituitary-adrenal stress response, unless they exceed considerably the maximal physiological concentrations.
MATERIAL AND METHODS

General. Female albino rats of an inbred Wistar strain, weighing between 100 and 150 g, were used. They were placed in individual cages one day before the start of the experiment. In order to avoid pituitary-adrenal activation by handling they were quickly anaesthetized before the start of the experiment with 3.5 mg/100 g body weight of sodium pentobarbital (Nembutal), injected into a lateral tail vein.

At the end of the experiment the animals were decapitated, the blood was collected in heparinized tubes and the adrenal glands were excised. The free plasma corticosterone content and the adrenal corticoid production in vitro were determined according to the method of van der Vies et al. (1960). These parameters were used as indices respectively of plasma corticosterone levels resulting from exogenously administered and endogenously produced corticosterone, and of the rate of adrenocortical activation by endogenous or exogenous corticotrophin. Crystalline corticosterone was dissolved in 0.4–0.8 ml ethanol and then diluted with saline to a volume of 14–30 ml. All injections are given here as amount per 100 g body weight.

Experiment 1. A solution containing 12 or 60 μg corticosterone was injected intravenously 15 minutes after Nembutal administration. The animals were decapitated after the following time intervals: immediately at the end of the injection (0 secs), and after 10, 30 or 60 seconds.

Experiment 2. An intramuscular infusion of corticosterone was performed in the following way. Ten anaesthetized rats, lying on a wooden board under a heat lamp, were connected with an infusion apparatus holding 10 syringes which were discharged at a rate of 1 ml/hour. The syringes were filled with a corticosterone solution so made up as to produce an infusion rate of either 2, 10 or 40 μg/minute · 100 g body weight. Infusions of saline containing the same amount of ethanol served as controls. The infusion started 30 minutes after Nembutal anaesthesia. An intraperitoneal injection of 1 mg histamine phosphate or of saline was given 25 minutes after the start of the infusion. Since the elevation in plasma corticosterone levels and in the rate of in vitro corticoidogenesis is almost constant between 15 and 60 minutes after the injection of histamine, but has reached the maximal level at 30 minutes, the animals were decapitated 30 minutes after the injection.

Experiment 3. The same experimental design was used, except for the following modifications. The infusion rate was either 3 or 30 μg/minute · 100 g body weight; 25 minutes after the start of the infusion 1 mU ACTH (USP Standard) was given intravenously; 15 minutes later the rats were decapitated.

Experiment 4. In this experiment the infusion rates were 2, 10 and 30 μg/minute · 100 g body weight; 100 μg corticosterone was injected subcutaneously 25 minutes after the start of the experiment, and the animals were sacrificed 15 minutes later.

RESULTS

1. Plasma corticosterone levels after a single intravenous injection of corticosterone

At first an attempt was made to induce an increased corticosterone blood level by the intravenous injection of 12 or 60 μg of corticosterone. It appeared that very high values were almost immediately produced, but then decreased rapidly, reaching physiological values within one minute (Table 1, cf. Fig. 4).
Table 1.
Plasma free corticosterone levels shortly after intravenous injection of corticosterone into anaesthetized rats. Corticosterone concentrations are given as µg/100 ml plasma ± standard deviation. The number of experimental animals is given in brackets.

<table>
<thead>
<tr>
<th>Decapitation</th>
<th>Plasma corticosterone concentrations after intravenous injection of</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>12 µg corticosterone</td>
</tr>
<tr>
<td>0 secs</td>
<td>179.8 ± 11.4 (9)</td>
</tr>
<tr>
<td>10 secs</td>
<td>122.5 ± 10.6 (10)</td>
</tr>
<tr>
<td>30 secs</td>
<td>57.0 ± 3.0 (10)</td>
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<tr>
<td>60 secs</td>
<td>41.8 ± 2.6 (10)</td>
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Since this method appeared to be unsuitable for the production of a constant elevation of the plasma levels, corticosterone was given as an infusion in subsequent experiments. Pilot experiments showed that an intramuscular infusion produced a fairly constant corticosterone level within 15 minutes.

This method was therefore adopted for testing the pituitary responsiveness to stress with different corticosterone levels.

2. Stimulation of the pituitary-adrenal axis by histamine

Fig. 1 illustrates the results of this experiment. Infusion with saline or with several doses of corticosterone up to 40 µg/minute did not activate the adrenal cortex of nembutalized rats, as evidenced by the basal values of the in vitro corticoidogenesis (6.8 ± 0.2 µg/100 mg·h), indicating that this procedure did not act as an unspecific stress stimulus. In rats infused with saline, a histamine injection induced an increase in plasma corticosterone levels to 32.6 ± 1.0 µg/100 ml, and in the rate of the in vitro corticoid production up to 31.5 ± 1.1 µg/100 mg·h. In the presence of plasma corticosterone levels elevated to 34.1 ± 2.2 µg/100 ml by constant infusion of 2 µg/minute corticosterone, the effect of histamine on the in vitro corticoidogenesis was not changed (32.6 ± 1.7 µg/100 mg·h), and the plasma corticosterone levels showed an almost complete summation of the endogenous and exogenous corticosterone (54.7 ± 2.2 µg/100 ml).

A higher infusion rate of corticosterone (10 µg/minute) induced plasma levels of 72.7 ± 2.7 µg/100 ml. These high levels did not depress the adrenocortical activation brought about by histamine, the in vitro corticoid production being 31.6 ± 1.0 µg/100 mg·h. Plasma corticosterone levels amounted to 88.3 ± 4.3 µg/100 ml, which is considerably lower than would be expected if complete summation had occurred.
The effect of an intraperitoneal injection of saline or 1 mg histamine phosphate on plasma corticosterone levels and in vitro corticoidogenesis in anaesthetized rats, receiving an intramuscular infusion of saline or corticosterone at different rates of infusion. The vertical line on top of each column indicates the standard error. Dotted lines indicate the calculated sum of corticosterone plasma concentrations induced by infused corticosterone and endogenously produced corticosterone.

An infusion rate of 40 μg/minute corticosterone produced plasma corticosterone levels as high as 103.8 ± 6.8 μg/100 ml. Histamine injection under these conditions caused a significantly reduced activation of the adrenal gland in vitro production (20.6 ± 2.2 μg/100 ml·h; $P = 0.005$), but the plasma levels were not further increased (107.2 ± 6.1 μg/100 ml).

3. Stimulation of the adrenal cortex by exogenous corticotrophin

The summation of administered and endogenously produced corticosterone appeared to become less complete with increasing pre-existing corticosterone levels. In order to decide between possible explanations for this phenomenon, in a subsequent experiment similar infusions were combined with ACTH injection, as a direct stimulus for corticoid production (Fig. 2).

In animals in which the plasma corticosterone level had been elevated by an infusion rate of 3 μg/minute to 46.7 ± 2.2 μg/100 ml, injection of 1 mU ACTH induced an increment to 65.4 ± 3.1 μg/100 ml, which is an almost complete summation, since ACTH in saline-infused rats induced a level of 30.7 ± 1.6 μg/100 ml.

An infusion rate of 30 μg/minute corticosterone resulted in a plasma con-
The effect of an intravenous injection of saline or 1 mU ACTH on plasma corticosterone levels and in vitro corticoidogenesis in anaesthetized rats, receiving an intramuscular infusion of saline or corticosterone at different infusion rates. The vertical line on top of each column indicates the standard error. Dotted lines indicate the calculated sum of corticosterone plasma concentrations induced by infused corticosterone and endogenously produced corticosterone.

In contrast, the adrenal corticoid production in vitro in saline-infused and corticosterone-infused rats, provoked by ACTH, appeared to be the same in all cases.

3. Summation of infused and injected corticosterone

In the last experiment, corticosterone infusions at several dose levels were combined with the subcutaneous injection of 100 µg corticosterone. Although in saline-treated animals, corticosterone injection at a dose level of 100 µg induced a plasma corticosterone level of 60.3 ± 4.7 µg/100 ml within 15 minutes, the effect of a similar injection in animals infused with 2, 10 or 30 µg corticosterone/min was drastically reduced (Fig. 3). The procedure did not cause any adrenal activation, since the rate of corticoid production in vitro remained at a basal level.
DISCUSSION

In the present study the effect of an experimentally elevated blood level of adrenocortical hormones on the reflex pituitary-adrenal response to acute noxious stimulation was investigated. In order to differentiate between the effects of exogenous administration and endogenous production of corticosterone in the rat, the plasma corticosterone levels (reflecting the sum of administered and endogenously produced corticosterone) as well as the in vitro corticoid production of excised adrenals (as an index of the rate of adreno-cortical activity) were used as parameters.

Firstly, the effect of intravenous injection of corticosterone was studied. It has been reported that the adrenocortical secretion due to stress can be blocked not only by a previous intravenous administration of large doses of corticoids (Sayers & Sayers 1947; Munson & Briggs 1955), but also with doses lower than 20 µg (Yates et al. 1961). The latter suggests that even small variations in corticoid blood levels within the physiological range are capable of exerting an effective negative feed-back action on the corticotrophic function of the adenohypophysis. Yates et al. (1961) observed that the elevation in corticosterone plasma levels, determined 15 minutes after application of a
stressful stimulus or after corticosterone injection, did not add up if the corticosterone was injected intravenously at a dose level of 12 µg, 15–30 seconds prior to the noxious stimulus.

In our experiments, determination of the corticosterone concentration in the plasma during the first few minutes after intravenous injection of 12 or 60 µg corticosterone revealed that this procedure induces a high but transient peak in plasma corticosterone levels, which exceeds by far the physiological range. Within a minute the corticosterone levels decrease towards values below the maximal physiological concentration (which is about 70 µg/100 ml according to Yates & Urquhart 1962). It is clear, however, that the application of a noxious stimulus during the first 30 seconds after injection would coincide with a supra-physiological corticosterone level in the plasma (Fig. 4). It would appear, therefore, that the conclusion that corticosterone in physiological concentrations may act as a potent inhibitor of corticotrophin release (Yates et al. 1961), has been based on the unjustified assumption that the intravenous injection of this small dose of corticosterone induced a plasma level equivalent to that produced by the stressed animal itself.

Our data show that not only the height of the initial peak, but also the

![Course of plasma corticosterone concentration following intravenous injection of 12 µg corticosterone, as compared with levels attained by endogenous corticosterone production.](image)

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**Fig. 4.**
rate of disappearance from the plasma is proportional to the amount injected. This is in agreement with earlier observations that supra-normal concentrations of corticoids are effectively and rapidly cleared from the blood into the extravascular space (Peterson et al. 1955; Peterson & Wyngaarden 1956; Samuels et al. 1957).

In subsequent experiments an intramuscular infusion of corticosterone was used, since in this way a standard stress stimulus could be applied during a constant elevation of the plasma corticosterone concentration equal to that produced by such a stimulus itself. Under these conditions no inhibition was observed, since an almost complete summation of exogenous and endogenous corticosterone occurred and the in vitro corticoidogenesis was not depressed. These results seem to exclude any instantaneous negative feed-back action of an elevation in corticosterone titers which is equivalent to the discrepancy between the stress-induced higher set point of a controller and the pre-existing corticoid level. Our data contradict therefore the variable set point control theory, and confirm studies by Hodges and co-workers who have maintained that only supraphysiological corticoid levels have an inhibiting effect on the acute release of corticotrophin (Hodges 1953, 1954; Hodges & Vernikos-Danellis 1962; Hodges & Jones 1963). Recent clinical data also indicate that low doses of corticoids do not interfere with the rise in corticoid production by laparotomy (Liddle et al. 1962; Estep et al. 1963).

Next, it became of interest to investigate how high the artificial increase in corticosterone levels should be, in order to block the pituitary-adrenal activation. Accordingly, plasma corticosterone levels were raised to about 70 or 105 μg/100 ml by an intramuscular infusion of large amounts of corticosterone. It appeared that the summation of exogenous (infused) corticosterone and endogenous corticosterone (produced in response to stress) became less complete with increasing dose levels of corticosterone, suggesting an increasing inhibition of pituitary activation. On the other hand, since the in vitro corticoid production was partially depressed only by the highest corticosterone level attainable, it was possible that the increasing incompleteness of the summation did not result from pituitary-adrenal inhibition, but from other factors. Following direct stimulation of the adrenal cortex by exogenous ACTH under different corticosterone levels, the in vitro corticoid production was not reduced, but again a similar reduction in summation of plasma corticosterone occurred. From this it can be concluded that the phenomenon of incomplete summation cannot be accounted for by a blockade of pituitary of adrenocortical function. Since the removal rate of corticosterone from the plasma rapidly increases with increasing supra-normal plasma levels, the findings may be interpreted as indicative of a peripheral mechanism responsible for the loss of summation. Additional experiments in which a combination of infused and injected corticosterone was employed without any concomitant
pituitary-adrenal activation, showed that indeed an elevation in plasma corticosterone levels is counteracted by an increased rate of clearance from the blood.

Hence, in the present experiments the only indication for an acute blocking action of high plasma corticosterone levels is the reduction of the in vitro corticoidogenesis by the adrenals of stressed animals with a plasma corticosterone concentration of about 105 µg/100 ml. This suggests that a complete blockade would only result from plasma corticosterone levels markedly exceeding this value. However, it appeared that it was not possible to raise this level further by increasing the amount of infused corticosterone. A maximal level was obtained with an infusion of 30 µg corticosterone/minute, but no further increase was induced by an infusion of 40 µg/minute. It may be that this plasma concentration could not be exceeded because the clearance rate from the blood became higher than the maximal uptake rate.

With intravenous administration, the limiting factor of uptake is ruled out and much higher levels can thus be obtained. Intravenous injection of 12 and 60 µg corticosterone appeared to induce a short peak in corticosterone plasma levels amounting to about 180 and 724 µg/100 ml, respectively (Table 1), and according to Yates et al. (1961) the 12 µg dose level blocked the adrenocortical response to histamine when given within 15–30 seconds. Munson & Briggs (1955) were able to prevent the adrenal ascorbic acid response to an intravenous injection of histamine by the intravenous injection of 1 ml of an aqueous adrenocortical extract only two seconds earlier. Since the amount of corticoids in this extract is roughly equivalent to 0.1 mg cortisol/ml, it can be estimated from our data (Table 1) that in the plasma of these rats a concentration must have been present of more than 1000 µg cortisol/100 ml, the blocking potency of which is about 4 times higher than that of corticosterone (Sayers & Sayers 1947).

On the other hand, much lower blood levels are capable of inhibiting pituitary-adrenal responses to stress. Sayers & Sayers (1947) prevented the adrenal ascorbic acid depletion induced by cold stress for one hour by previous subcutaneous administration of 0.2 mg corticosterone, which in our hands induces a maximal plasma level of 97.1 µg/100 ml.

Thus, it seems that a corticoid blockade is dependent not only on the amount of circulating cortical hormones, but also on the time during which they are present in the body. Thus, the shorter the time available for the hormone to act, the higher the induced blood level should be. This may explain why in our acute experiments plasma corticosterone levels of about 105 µg/100 ml did not exert a full blockade, whereas subcutaneous administration, which does not result in higher level inhibits the pituitary response to stress.

In summarizing our conclusions, it can be stated that in the intact animal noxious stimulation results in a reflex corticotrophin secretion by way of a
direct hypothalamic drive, which is independent of any changes in the homeostatic controlling mechanism induced by the stimulus itself. Only excessive changes in corticoid levels induced by experimentation or pathological conditions, are capable of influencing the excitability of the adenohypophyseal response to stress. The blocking action of large amounts of corticoids seems to be dependent on the mode and time of administration, and can be considered (within certain limits) as a function of dosage and time. It should be emphasized that these conclusions apply only for conditions of acute noxious stimulation. Apart from this, a feed-back control of the basal corticotrophic function of the pituitary gland, sensitive to small but persistent changes in corticoid levels, may well exist.

REFERENCES

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