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THE EFFECT OF THE CHRONIC ADMINISTRATION OF BARBITONE SODIUM ON PITUITARY-ADRENAL FUNCTION IN THE RAT

B. E. LEONARD

Department of Pharmacy, The University, Nottingham, England

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Abstract—Changes in adrenal ascorbic acid, adrenal weight and plasma corticosterone levels were measured in 2 substrains of albino rats during habituation to barbitone sodium and for a short period after the animals were deprived of this drug. Little difference was found between these substrains in their responses to the period of habituation and withdrawal There was a significant decrease in the plasma corticosterone level at the end of the period of habituation, followed by a rapid rise 2 days after withdrawal and a return to the control level 1 week after withdrawal. The adrenal ascorbic acid level rose during habituation and returned to the control level within 1 week of withdrawal. These results suggest that the effect of environmental stress on the adrenal cortex is diminished during habituation but that adreno-cortical hyperactivity occurs immediately after withdrawal as a result of the increased excitability of the central nervous system.

RERUP and HEDNER[1] have shown that when rats are anaesthetized with barbiturates there is an inhibition of the release of corticotrophin (ACTH). The inhibition however is not a characteristic of general anaesthesia as both urethane[2] and ethanol[3] anaesthesia cause a prolonged hypersecretion of ACTH. Furthermore central depressant drugs such as morphine[4], reserpine and its congeners[4] and cholorpromazine[5] evoke hypersecretion of ACTH and eventually reduce its release when the rate of synthesis is insufficient to balance the rate of secretion. The present investigation was therefore undertaken to determine the effect of the chronic administration of non-anaesthetic doses of a barbiturate on adrenocortical activity.

METHODS

Female albino rats (45–55 g) originally of the Wistar strain were used. These rats were obtained from two sources*. The rats were housed in single cages and the method used to habituate the animals to barbitone sodium was the same as that described previously.[6, 6a] Groups of at least 5 rats were killed by decapitation 2 and 4 weeks after habituation and 2 days and 1 week after withdrawal. All animals were transferred from the animal house to the laboratory and handled before killing. Control and experimental rats were of the same age (6–10 weeks). After decapitation the blood was collected in ice cooled centrifuge tubes containing 0·1 ml heparin (5,000 IU/ml). Two 0·1-ml aliquots of the whole blood were taken for the determination of blood

* Boots Pure Drug Co, Ltd., Nottingham, England. A. Tuck Ltd., Rayleigh, Essex, England.

sugar.[7] The remaining blood was centrifuged and the plasma removed for the determination of corticosterone by a spectrophotofluorometric method,[8] Any haemolysed samples were discarded. The adrenal glands were removed immediately after killing, trimmed free from adhering tissue if necessary, weighed and ground with sand and cold 10 per cent trichloracetic acid. The adrenal ascorbic acid was determined by the method of Roe.[9]

RESULTS

The effect of the chronic administration of barbitone sodium on the plasma corticosterone level of the two substrains was similar. There was no change in the plasma corticosterone levels for the first two weeks of habituation but there was a significant decrease (50 per cent control value) in the plasma corticosterone by the fourth week of habituation (Fig. 1). Two days after withdrawal of the barbituate there was a rapid

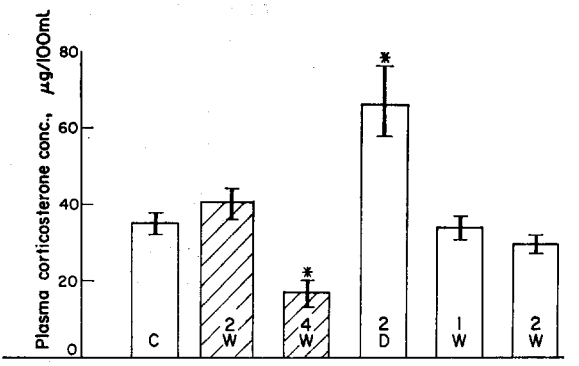

FIG. 1. Effect of the chronic administration of barbitone sodium on plasma corticosterone levels of rats (Tuck's substrain).

rats killed 2 and 4 weeks after habituation.

rats killed 2 days, 1 week and 2 weeks after withdrawal of the barbiturate.

The first column, C, gives the control value. All results expressed as the mean ± standard error. Results significant at $*P < 0{\cdot}05$.

rise (100 per cent above the control value) in the plasma corticosterone. This corresponded with the period when the animals of both substrains were hyperexcitable and very susceptible to audiogenic seizures. The plasma corticosterone level returned to the control value by one week after the withdrawal of the barbiturate.

There were slight quantitative differences in the levels of adrenal ascorbic acid between the two substrains (Fig. 2). However, in both there was a significant rise in the adrenal ascorbic acid during habituation and this was followed by a return to the control level one week after withdrawal. There was no significant difference between the adrenal weights of the experimental and control rats of the Boots' substrain during the experiment. With the Tuck's substrain there was a significant increase in the adrenal weight at the fourth week of habituation and a further increase two days after withdrawal (Fig. 3). These differences were no longer significant two weeks after withdrawal.

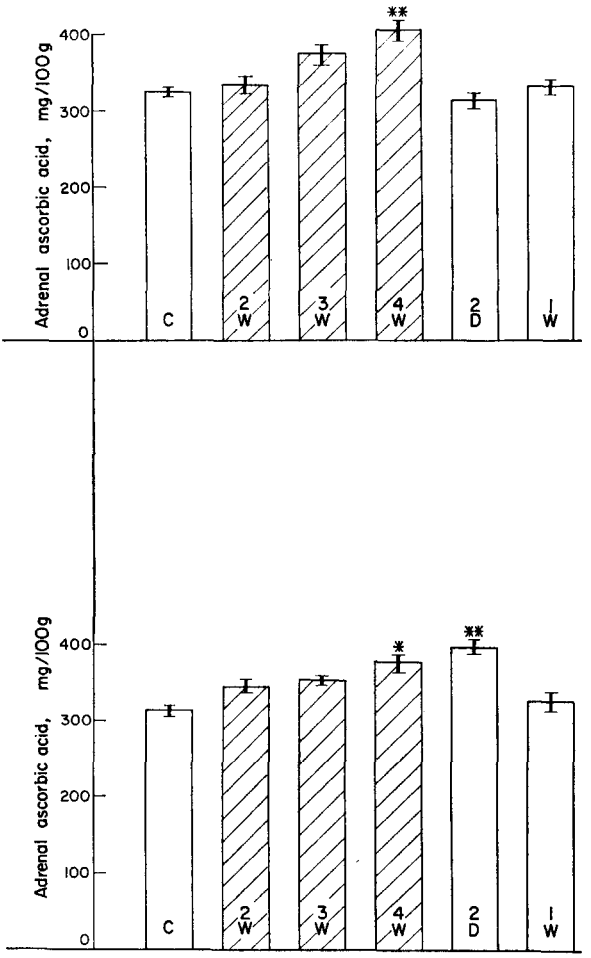

FIG. 2. Effect of the chronic administration of barbitone sodium on the adrenal ascorbic acid levels on 2 substrains of rats.

Upper histograms give results for the Tuck's substrain and the lower histograms give results for the Boots' substrain.

rats killed at 2, 3 and 4 weeks after habituation.

rats killed at 2 days and 1 week after withdrawal of the barbiturate.

Results significant at $*P < 0{\cdot}050$ $**P < 0{\cdot}01$.

There was no significant difference between the blood sugar level of the control and of the experimental animals of either substrain during the experiment.

DISCUSSION

In these experiments three parameters of pituitary-adrenal function were used to assess the possible effects of chronic barbiturate administration; these were the plasma corticosterone level, adrenal ascorbic acid level and the adrenal weight.

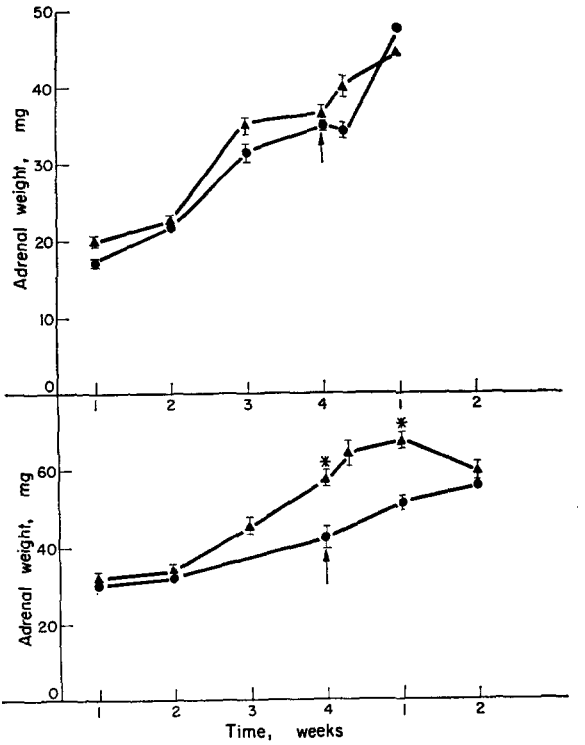

FIG. 3. Effect of the chronic administration of barbitone sodium on the adrenal weight of 2 substrains of rats. Results given for the Boots (upper curves) and Tuck's (lower curves) substrains. ▲ gives the mean ± standard error for the experimental and ● control rats respectively. Results significant at $*P < 0{\cdot}05$. The experimental animals for both substrains were withdrawn from the barbiturate after 4 weeks (↑) and killed 2 days, 1 week and 2 weeks later.

Change in the plasma corticosterone level is a measure of the intensity and duration of ACTH release,[4] and therefore it is evident that the secretion of this hormone is partially inhibited at the end of the period of habituation, but increases very rapidly soon after withdrawal of the barbiturate presumably because of the hyperexcitability of the central nervous system.

The adrenal ascorbic acid level rose during the period of habituation, and as this test can be used to measure the approximate intensity of ACTH discharge but not the duration of the discharge,[4] these results also suggest that ACTH release is reduced during habituation. It is possible that the adrenal ascorbic acid rises during habituation because the barbiturate stimulates its synthesis.[10]

Adrenal hypertrophy occurred during the period of habituation with one of the substrains of rats. Results from this laboratory[6a] suggest that this is due to a significantly greater increase in the body weight of these animals, compared with their controls, during this period.

Although the level of adrenal ascorbic acid can be used as an index of the intensity of ACTH discharge it may be fallacious to draw conclusions about the effect of a drug on the release of this hormone when the level of adrenal ascorbic acid is the

only criterion used.[1] Thus in the present investigation the change in plasma corticosterone is probably the most accurate parameter of the intensity and duration of ACTH discharge, and the rise in adrenal ascorbic acid is a verification that ACTH release is blocked during habituation.

There is a good agreement between the figures obtained in the present experiments and those published by other investigators[1, 8, 11] for rats which were moved from the animal house and handled before killing.

Several investigations have lead to the conclusion that barbiturates depress ACTH secretion. Sayers[12] reported that pentobarbitone reduces the level of circulating ACTH in the rat and Egdahl[13] found that this barbiturate also depressed the elevation of corticosterone in the decorticate dog. Rerup and Hedner[1] have suggested that rats anaesthetized with barbiturates could provide a possible assay system for exogenous ACTH. However other investigators[14] have reported that the release of ACTH following histamine and vasopressin induced stress in rats can only be blocked by morphine and pentobarbitone, the latter drug being ineffective when injected alone. Furthermore Barrett and Stockham[11] found evidence to suggest that barbiturate anaesthesia does not produce an inhibition of ACTH release. Undoubtedly the physiological state of the rats following a single, anaesthetic dose of barbiturate differs considerably from that occurring in rats which have been chronically treated with barbiturate and which do not show any obvious signs of behavioural depression. It is nevertheless possible that during the initial period of pretreatment with the barbiturate there was an initial hyper-secretion of ACTH followed by a marked fall in ACTH release due to depletion of the hormone from the anterior pituitary. This appears to be the mode of action of several central depressant drugs.[2–5] However there is no evidence from the present investigation to suggest that barbitone sodium is affecting ACTH release in this way since 2 weeks after habituation there is no significant difference between the corticosterone levels of the control and experimental groups and moreover there is a decline in the corticosterone levels until the end of the period of habituation in the latter group. Similar changes have been observed in these laboratories for the excretion of 17-ketosteroids by rats of the Boots' substrain (unpublished).

In conclusion, these results suggest that the chronic administration of barbitone sodium in increasing doses for 4 weeks diminishes the effect of the stress on the adrenal cortex that results from a change in the environment and in handling the animals. The increased excitability occurring in these animals 2 days after they have been withdrawn from the barbiturate is accompanied by adrenocortical hyperactivity.

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