Effect of deoxycorticosterone on sodium appetite of intact and adrenalectomized rats

GEORGE WOLF

Department of Anatomy, Yale University School of Medicine, New Haven, Connecticut

WOLF, GEORGE. Effect of deoxycorticosterone on sodium appetite of intact and adrenalectomized rats. Am. J. Physiol. 208(6): 1281–1285. 1965.—The effect of DOC on sodium chloride (saline) intake was studied in intact and adrenalectomized rats under “two-bottle” self-selection conditions. It was found that in adrenalectomized rats low doses of DOC produced a decrease in saline intake (restoration of sodium-retaining ability), whereas high doses produced an increase in saline intake (stimulation of sodium appetite). At high doses, however, intact rats consumed more saline and manifested a greater preference for it than did similarly treated adrenalectomized rats. Treatment with corticosterone increased both absolute saline intake and saline preference of DOC-treated adrenalectomized rats.

It has been found in self-selection experiments that increased intake of sodium salts may be induced in rats by either a deficiency (10) or an excess (9, 16) of mineralocorticoids. Mineralocorticoid deficiency (via adrenalectomy) results in an inability to retain sodium so that the daily requirement for sodium increases sharply. It is not known why mineralocorticoid excess (via exogenous mineralocorticoids in intact rats) induces increased sodium intake. However, it appears that, unlike the polydipsia which follows mineralocorticoid overdosage, the increased sodium intake is not a result of renal tubular effects or pathological changes (17).

Although the natrorexigenic (sodium-appetite stimulating) effect of high doses of mineralocorticoids has been convincingly demonstrated in intact rats, it has not been observed in adrenalectomized rats under self-selection conditions. A number of investigators have studied the effect of “replacement therapy” with deoxycorticosterone (DOC) on the saline intake of adrenalectomized rats (4, 7, 11). They found that with restoration of sodium-retaining ability, the voluntary saline intake of the adrenalectomized rats decreased to normal levels. The doses of DOC used in these experiments were relatively high (up to 1.0 mg/day) and would be sufficient to produce a large increase in saline intake of intact rats under identical self-selection conditions (2, 6, 9).

The above experiments suggest that although adrenalectomized rats respond to the renal sodium-retaining effect of DOC, they are unresponsive or resistant to its natrorexigenic effect. It seemed likely that under the usual self-selection conditions some other adrenal secretion (perhaps a glucocorticoid) plays a role in the rat’s appetitive response to administered DOC. Thus, the present experiments compare the voluntary saline intake of adrenalectomized and intact rats given varying doses of DOC and determine if corticosterone will facilitate the natrorexigenic effect of DOC in adrenalectomized rats.

Experiment 1

The purpose of this experiment was to determine if adrenalectomized rats are completely unresponsive to the natrorexigenic effect of DOC or if they simply require larger doses than do intact rats for the elicitation of this effect. The experiment is a dose-response study and employs the standard “two-bottle” (water and saline solution) self-selection procedure. Because the palatability of saline to the rat appears to decrease with increasing degrees of hypertonicity (3), two saline concentrations were used to determine if differences between the responses of adrenalectomized and intact rats are dependent on the palatability of the solution. Thus some of the rats were given hypertonic (unpalatable) saline, while others were given isotonic (palatable) saline.

METHODS

Subjects and procedure. Eighty-two male Holtzman Sprague-Dawley rats about 100 days old and 300–350 g

Received for publication 30 July 1964.

1 This study was supported, in part, by Public Health Service Grant MH02940 from the National Institute of Mental Health, Bethesda, Md.

2 This paper is based on portions of a dissertation presented to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Drs. N. E. Miller, P. J. Mulrow, and J. P. Flynn rendered invaluable advice and guidance as members of the advisory committee.
FIG. 1. Hypertonic (.333 M) saline intake of adrenalectomized, unoperated, and sham-operated (two solid triangles) rats as a function of DOCT dosage. Sodium intake is shown in terms of milliliters of solution consumed on left ordinate and in terms of milliequivalents of sodium consumed on right ordinate. Scores are based on mean daily intake per 100 g body wt during the 6th through 10th days after a single intramuscular injection. Each point represents mean of four rats.

Water and saline were given in inverted graduated test tubes with drinking nozzles which protruded through the fronts of the cages about 4.0 cm apart. Each day the containers were refilled and the positions of the two solutions were reversed. Fluid ingestion was measured daily. A 10-day period of adaptation to the self-selection conditions (referred to as the “pretreatment” period) preceded the experimental treatments (adrenalectomy and/or DOC injection). Similarly, the self-selection conditions were continued for an additional 10 days after the experimental treatments (“posttreatment” period).

Experimental treatments. Adrenalectomies were performed at the end of the pretreatment period via the dorsal-approach method using pentobarbital (40 mg/kg) anesthesia. At the end of the experiment adrenalectomized rats were sacrificed and perirenal tissue was inspected for accessory adrenal nodules. In two cases in which such nodules were found the data were discarded and new rats were run.

DOC treatment involved a single intramuscular injection of DOC trimethylacetate (DOCT) given at the end of the pretreatment period. DOCT is a very slowly absorbed form of DOC. Estimations of absorption rate in humans indicate that about 1.5–3.5% of the injected dose is absorbed daily (14).

Rats given hypertonic saline. Fifty-two rats were given the hypertonic (.333 M) saline solution. The solution was presumably unpalatable to the rats; most of them ingested less than 0.5 ml/100 body wt per day during the last 5 days of the pretreatment period. Five of the 52 rats ingested more than 1.0 ml/100 g body wt per day during this time. They were discarded and replaced by new rats to avoid biasing any of the groups.

At the end of the pretreatment period 24 of the 52 rats were adrenalectomized and divided into 6 groups of 4 rats; 20 were left unoperated and divided into 5 groups of 4 rats; and the remaining 8 were sham operated and divided into 2 groups of 4 rats. Assignment to groups was made on a random basis. Each of the six groups of adrenalectomized rats received, at the time of operation, one of the following doses of DOCT: 0, 3.7, 11.0, 33.0, 100.0, or 300.0 mg/kg body wt. At the same time each of the five groups of unoperated rats received one of the above doses of DOCT from 0 to 100.0 mg. (The 300.0-mg dose was not used.) One sham-operated group received the 0 dose and the other received the 100.0-mg dose.

Rats given isotonic saline. Thirty rats were given isotonic saline. At the end of the pretreatment period 15 were adrenalectomized and divided into 5 groups of 3 rats. The remaining 15 unoperated rats were similarly divided into 5 groups of 3 rats. Each of the five groups of adrenalectomized and of unoperated rats was injected with one of the five doses of DOCT from 0 to 100.0 mg/kg noted above. Note that no rats were discarded thus controlling for the selection procedure of the preceding part of the experiment.

Analysis of data. Saline intake (ml/100 g body wt per day) and saline preference (ratio of saline intake to total fluid intake) were used as measures of sodium appetite. Since at least 5 days are necessary before the sodium intake of rats treated with DOCT reaches asymptote, only the fluid intake data of the last five days of the 10-day posttreatment period were analyzed. Individual differences in sodium appetite were partialled out by subtracting pretreatment scores (based on corresponding days of the pretreatment period) from posttreatment scores for each rat.
The results of the experiment clearly demonstrate that adrenalectomized rats will respond to the natrorexigenic effect of DOC but are less sensitive to it than are intact rats. The saline intake of adrenalectomized rats first decreased at lower doses of DOCT, but then increased again at higher doses. Both the decrease from the 0- to the 11.0-mg dose and the increase from the 0- to the 300.0-mg dose were significant \( (P < .001) \) by the method of trend analysis described by Winer (15). Finally, the saline intake of unoperated rats increased steadily as a function of DOCT dosage \( (P < .001) \) and was greater than that of the adrenalectomized rats at the 11.0- through 100.0-mg doses. This difference did not reach significance at the 11.0-, 33.0-, or 100.0-mg doses independently, but did reach statistical significance either when these three doses were combined \( (P < .02, F \text{ test}) \) or when the sham-operated and unoperated 100.0-mg groups were combined and compared with the adrenalectomized 100.0-mg group \( (P < .005, t \text{ test}) \).

Saline preference (ratio of saline intake to total fluid intake) as a measure of sodium appetite yielded results which were identical to the above. Of greatest importance was the fact that the unoperated rats given the 11.0-, 33.0-, 100.0-mg doses preferred the saline solution more than did the corresponding adrenalectomized groups \( (P < .005, F \text{ test}) \). Since the preference measure does not depend on absolute levels of saline intake per se but only on the relation between saline and water intake, it cannot be argued that the diminished appetitive response to DOCT of the adrenalectomized rats is simply a reflection of a nonspecific depression of fluid intake after adrenalectomy.

Figure 2 shows .154 M saline intake of adrenalectomized and intact rats as a function of DOCT dosage. Although over-all intake of the palatable isotonic solution was much higher and more variable than that of the hypertonic solution, the relations between the groups are similar to those observed in the rats given hypertonic saline. The isotonic saline intake of adrenalectomized rats first decreased and then increased again as a function of DOCT dosage \( (P < .001) \), and the saline intake of the intact rats was greater than that of the adrenalectomized rats at the higher doses \( (P < .005) \).

Finally, the saline-preference measure for the groups given isotonic saline again yielded results similar to those obtained by analysis of absolute saline intake. There was an initial decrease and then an increase in the saline preference of the adrenalectomized rats as a function of DOCT dosage \( (P < .01) \), and at the higher doses the intact rats preferred the saline more than the adrenalectomized rats did \( (P < .05) \).

The results of the experiment clearly demonstrate that adrenalectomized rats will respond to the natrorexigenic effect of DOC but that under ad libitum self-selection conditions they are less responsive to this effect than are intact rats. Furthermore, the difference between the responses of intact and adrenalectomized rats treated with high doses of DOC seems to be independent of the concentration of the saline. It is of interest to note, however, that the amount of sodium ingested by the untreated adrenalectomized rats appears to be less dependent on the concentration of the solution than does that of any of the other groups.

**Experiment 2**

The foregoing experiment showed that under ad libitum self-selection conditions adrenalectomized rats are less responsive to the natrorexigenic effect of DOC than are intact rats. The present experiment investigates the possibility that corticosterone, the major glucocorticoid of the rat, plays a role in the appetitive response to DOC. The effect of corticosterone on saline intake and preference of DOC-treated adrenalectomized rats was studied.

The secretion of corticosterone, unlike that of aldosterone, is not depressed by DOC administration (13), and thus it may exert an effect in the intact DOC-treated rat. Although corticosterone has insignificant direct effects on sodium metabolism, under the present conditions it may facilitate the natrorexigenic effect of DOC in some indirect, permissive manner, for example, by facilitating the excretion of ingested sodium; that is, the accumulation of body sodium in the DOC-treated rat might inhibit further sodium ingestion. Facilitation of sodium excretion would diminish this inhibitory effect. There already exists suggestive evidence that glucocorticoids may potentiate the appetitive effect of DOC in intact rats in some indirect way (1).
Asymptotic scores of the final analyses. As in the previous experiment, individual differences were partialed out by subtracting corresponding pretreatment from posttreatment scores.

Results

Figure 3 shows the mean daily saline intake of each of the nine groups of rats during the last 2 days of the injection period. As in the two preceding experiments, increasing doses of DOCA produced first a decrease and then an increase in saline intake. This highly significant effect ($F < .001$, $F$ test) was not disrupted by the addition of corticosterone. In general, saline intake increased monotonically with increasing doses of corticosterone ($P < .001$) and the interaction between the effects of the two steroids was insignificant ($F = 1.1$). The effects of 0.5 and 2.5 mg of corticosterone given alone (i.e., with 0 mg DOCA) were not significant, although there may be a tendency for the 2.5-mg dose to produce increased saline intake. Saline-preference scores were again similar in direction and statistical reliability to the saline-intake scores.

Discussion

The present experiments have shown that under ad libitum self-selection conditions: a) adrenalectomized rats are resistant to the natrorexigenic effect of DOC, but b) they will respond when given sufficiently high doses, and c) their resistance can be reduced by treatment with corticosterone.

It is possible that under the present ad libitum self-selection conditions, the sodium-retaining effect of DOC antagonizes its natrorexigenic effect by retarding the excretion of ingested sodium. Corticosterone may indirectly facilitate the natrorexigenic effect of DOC via its actions on glomerular filtration rate (GFR). It is well known that glucocorticoids, while having relatively little effect on sodium reabsorption, greatly potentiate GFR (5, 8), and thus can actually block the sodium-retaining effects of DOC (12). Adrenalectomized, DOC-treated rats may retain sodium as well as intact rats when deprived, but because of a deficient GFR not excrete it as rapidly under load. By facilitating the excretion of sodium in the DOC-treated rat, corticosterone could allow it to be ingested at a higher rate.

Ingestion of sodium produces pathological effects in mineralocorticoid-overdosed animals. Thus, under the usual experimental conditions, the appetitive response to mineralocorticoid excess is clearly maladaptive. This is not necessarily the case under natural conditions (e.g., in a sodium deficient animal). It has recently been proposed that the potentiation of sodium appetite may be a natural adrenocortical function (17). According to this theory, in sodium-deficient animals, increased mineralocorticoid secretion functions not only to reduce sodium excretion and to mobilize sodium from body reservoirs but also to potentiate the appetitive for sodium salts. Although neither elevated mineralocorticoid levels nor decreased body sodium levels are necessary conditions

**Methods**

Subjects and maintenance. Thirty-six rats were used. All of them were adrenalectomized and immediately after the operation given 250 M saline and water ad libitum. The self-selection conditions were like those of the previous experiment. The 250 M concentration was used in the hope of avoiding both the aversiveness of .333 M saline and the variability of intake of .154 M saline.

Steroid injections. After 6 days of adaptation, daily subcutaneous injections of steroids were given for an additional 6 days. Each rat received only one type of steroid (or steroid combination) at one dosage. The steroids used were corticosterone acetate (henceforth called corticosterone) and DOCA acetate (DOCA). The steroids were suspended in sesame oil containing 0.5 % chlorobutanol and were given in 0.5 ml of oil per injection. Unlike the previous experiment, dosage was not calculated on a body weight basis. Three doses of DOCA (0, 0.5, and 2.5 mg/rat per day) and three doses of corticosterone (0, 0.5, and 2.5 mg/rat per day) were used. It is necessary to use relatively larger doses of DOCA than of DOCT, probably because blood levels are less stable with the more rapidly absorbed DOCA.

Design. A 3 x 3 factorial design was used with four rats in each cell. All the combinations of the three doses of each of the two steroids comprised the nine cells of the factorial design. In the cell representing 0 mg of both steroids was a group given vehicle injections.

Analysis of data. Saline intake and saline preference were used as measures of sodium appetite. Fluid intake did not begin to stabilize until about the 4th day after commencement of steroid treatment. Thus, only the asymptotic scores of the final 2 days were included in the analyses. As in the previous experiment, individual differences were partialed out by subtracting corresponding pretreatment from posttreatment scores.
for the elicitation of sodium appetite, they are both sufficient conditions and may have summative effects.

The doses of mineralocorticoid which are necessary to induce increased sodium intake in nondeficient rats under self-selection conditions such as those of the present experiment are very high. The inability to elicit the effect with doses within the physiological range would, at first, seem to contradict a theory that endogenous mineralocorticoids play a role in the regulation of sodium appetite. On the assumption that sodium appetite is a homeostatic mechanism, the necessity of using high doses can be accounted for as a result of the antagonistic effects of sodium retention which were suggested by the present experiments. The tendency of DOC to retard the excretion of sodium would, to some extent, inhibit the sodium intake of intact rats as well as that of adrenalectomized rats under the present conditions. High doses of mineralocorticoids would thus be necessary to overcome the inhibitory effects of elevated body sodium levels. (Increasing the dosage would presumably cause no further increment in the rate of tubular sodium reabsorption since this effect appears to reach a maximum with relatively low doses (12).)

REFERENCES