Lack of Effect of Actinomycin D on Aldosterone Induced Antinatriuresis When Administered After the Hormone.* (31783)

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Recently, Garren *et al*(1) reported that a "repressor" is involved in terminating the induction of tryptophan pyrrolase activity by the hormone hydrocortisone. Using actinomycin D, an inhibitor of DNA-directed synthesis of RNA(2), these investigators found that if the actinomycin D were administered after hydrocortisone an enhanced induction of tryptophan pyrrolase activity occurred. Previously it had been shown that the administration of this inhibitor prior to hydrocortisone would block the induction(3). Garren *et al*(1) postulated that hydrocortisone intially stimulates the enzyme formation by an actinomycin D sensitive process. The increase in enzyme activity then acts as a stimulus to initiate the synthesis of a "repressor" which inhibits further induction of enzyme activity by the hormone. The formation of the "repressor" is also actinomycin D sensitive. Thus when actinomycin D is given after hydrocortisone it inhibits the formation of the "repressor" and an enhancement of tryptophan pyrrolase activity occurs.

The purpose of this study was to determine if an actinomycin D sensitive "repressor" was involved in terminating the action induced by aldosterone, another adrenocortical steroid hormone. Actinomycin D was administered at varying times after the administration of aldosterone to determine its effect on the sodium retaining action of this mineralocorticoid.

Methods. Male rats (Simonsen strain) weighing 125-155 g, which were adrenalectomized bilaterally at least 5 days prior to the experiment and maintained on .9% sodium chloride solution for drinking water and "purina" laboratory chow, were utilized.

The duration of the antinatriuretic action of aldosterone in rats was determined as follows. At the start of the experiment aldosterone (0.5 μg/kg) was administered subcutaneously to 5 groups of animals. The corresponding control groups were given the aldosterone vehicle, subcutaneously. Urine samples were then collected from both groups for a 2 hour period by the method of Kagawa *et al*(4). The beginning of the collection period ranged from the time of aldosterone or aldosterone vehicle administration to 4 hours after such administration. At the start of the collection period a sodium chloride solution (0.9%), 17 ml/kg, was injected subcutaneously. Urinary sodium was measured with a Coleman flame photometer. Sodium excretion values of the treated group were converted to per cent of sodium excreted by the corresponding control group.

The effect of actinomycin D on the aldosterone induced retention of sodium was determined in a series of 5 experiments. In each experiment 0.6 μg/kg of aldosterone or the aldosterone vehicle was given subcutaneously with either 400 μg/kg of actinomycin D or its vehicle being administered intraperitoneally at various times so that the following combinations of treatments were obtained: 1) aldosterone and actinomycin D vehicle, 2) aldosterone vehicle and actinomycin D vehicle (control group), 3) aldosterone and actinomycin D, and 4) actinomycin D and aldosterone vehicle. All animals were loaded with a 17 ml/kg subcutaneous injection of .9% sodium chloride at the beginning of the collection period, and urine samples were collected for a 2 hours period at various times after the administration of aldosterone. The protocols of the individual experiments were as follows: experiment #1) actinomycin D was given 1 hour before aldosterone, urine was collected from 2-4 hours after aldosterone administration; experiment #2) actinomycin D was administered

* Supported in part by USPHS research grants AM-05298 from Nat. Inst. of Arthritis and Metab. Dis. and 2-T01-GM-00141 from Nat. Inst. of Gen. Med. Sciences.

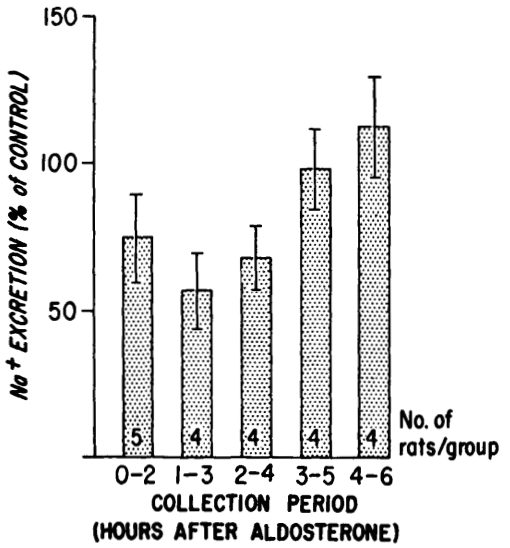

FIG. 1. Time sequence of the antinatriuretic action of aldosterone. Standard errors of means are indicated by vertical lines at top of bars.

¼ hour after aldosterone, urine was collected from 1-3 hours after aldosterone injection; experiment #3) actinomycin D was given ¼ hour after aldosterone, urine was collected from 3-5 hours after the administration of aldosterone; experiment #4) actinomycin D was administered 1 hour after aldosterone, urine was collected from 3-5 hours after aldosterone; experiment #5) actinomycin D was given 4 hours after aldosterone, urine was collected from 4-6 hours after aldosterone administration.

Again, urinary sodium was measured with a Coleman flame photometer. For each experiment within this series the sodium excretion values of the treated groups were converted to per cent of sodium excreted by the control group which received both the actinomycin D vehicle and the aldosterone vehicle.

Standard errors were determined and the data analyzed statistically using the Student "t" test(5). The 0.05 level of probability was used as the criterion of significance.

Results. Fig. 1 shows a time sequence for the antinatriuretic action of aldosterone. The antinatriuretic effect is maximal between 1 and 3 hours after administration of aldosterone.

Fig. 2 shows the results obtained when actinomycin D was administered either before aldosterone or at various times after administration of aldosterone. Actinomycin D alone did not significantly alter sodium excretion when compared to the control group ($P>.05$). Therefore, data presented in this Figure were compared only to the control group. As previously reported(6), actinomycin D, when administered before aldosterone, completely blocks the antinatriuretic action of aldosterone. When actinomycin D was administered only 15 minutes after aldosterone no blockade was observed. No enhancement of the antinatriuretic action of aldosterone occurred as indicated by the results of the 1-3 hours collection period. Also no prolongation of the antinatriuretic action of aldosterone occurred as indicated by the results of the 3-5 hours and the 4-6 hours collection periods. In one case, actinomycin D did appear to inhibit ($P<.05$) the antinatriuretic action of aldosterone when administered after aldosterone. This occurred in the group receiving the inhibitor one hour after the hormone. The reason for the difference would seem to be due to a greater sensitivity of aldosterone in the group receiving only the hormone. The excretion of sodium in this group (3-5 hours after aldosterone) was greater than usually seen (Fig. 1). However, while the difference was significant, it was in the opposite direction.

Discussion. In the series of experiments shown in Fig. 2 actinomycin D was administered during various phases of the antinatriuretic curve obtained in Fig. 1. Actinomycin D was given 15 minutes after aldosterone, whereby it was present during maximal aldosterone action, it was given 1 hour after aldosterone such that it was present during the beginning of the upward phase of the curve; and actinomycin D was given 4 hours after aldosterone whereby it was present during the later phase of the curve. It was assumed that if a "repressor" were functioning in connection with the antinatriuretic action of aldosterone, actinomycin D given after aldosterone would inhibit the synthesis of the "repressor" and thereby either enhance or prolong the antinatriuretic action of aldosterone.

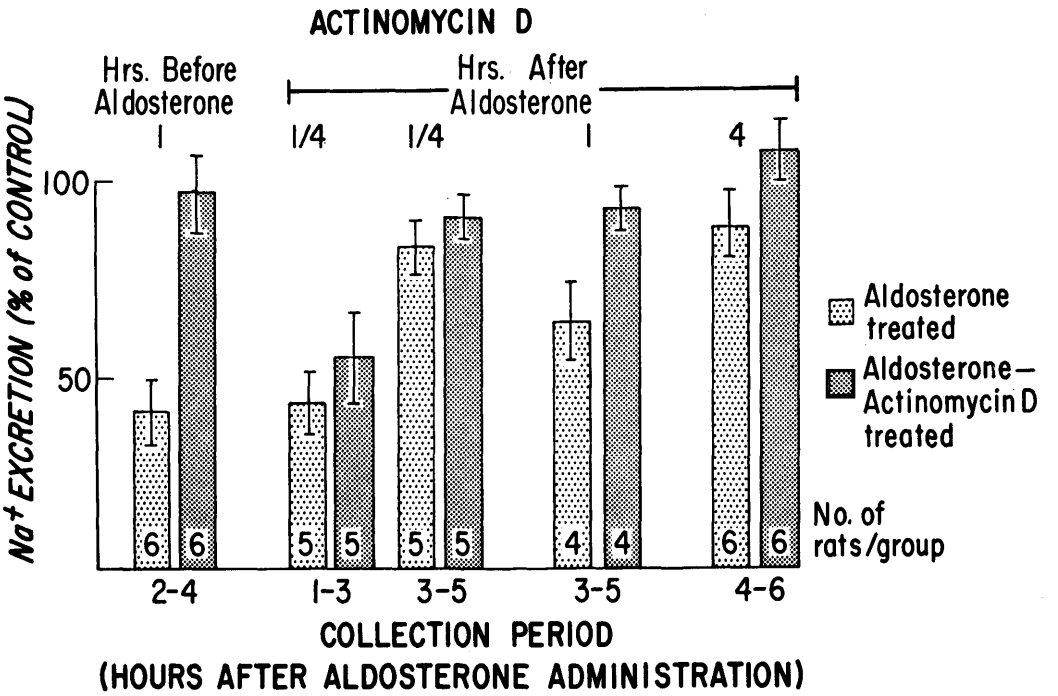

FIG. 2. Effect of actinomycin D on antinatriuretic action of aldosterone when administered either before or at varying times after the hormone. Standard errors of means are indicated by vertical lines at top of bars.

It is concluded that a "repressor" which might function in depressing the antinatriuretic action of aldosterone can not be demonstrated with the present experimental conditions. The present experiments by no means eliminate all possibilities of a controlling-repressor, but do indicate that if a "repressor" is involved it is not actinomycin D sensitive as was the "repressor" reported by Garren *et al*(1).

The lack of effect of actinomycin D on aldosterone induced antinatriuresis when administered as little as 15 minutes after aldosterone indicates that the hormone is involved only transiently in mediating its effect on sodium transport.

Summary. Actinomycin D was administered at varying times after the administration of aldosterone to rats in order to determine if this inhibitor would either enhance or prolong the antinatriuretic action of aldosterone. No such effects were found, thus it would appear that an actinomycin D sensitive "repressor" is not involved in terminating the sodium retaining action of the adrenocortical steroid hormone, aldosterone.

Technical assistance of Mrs. Penny Lang is gratefully acknowledged.

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Received August 24, 1966. P.S.E.B.M., 1967, v124.