be embryocidal or abortion-inducing administered to pregnant rats following implantation. The mechanism of the anti-implantation effect remains to be established.

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Received August 16, 1962. P.S.E.B.M., 1962, v111.

Effects of Aldosterone on Contractile and Electrical Properties of Driven Isolated Rabbit Atria.* (27867)

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Aldosterone has been reported to produce a marked increase in left ventricular work index in the rat heart-lung preparation(1). This cardiotonic action could be demonstrated with aldosterone concentrations as low as 10^{-9} to 10^{-12}M. It was not clear from these experiments, however, whether or not this effect on ventricular work was due in part to a direct positive inotropic action on the myocardium. In preliminary experiments designed to test the possibility of a direct action of this steroid on cardiac contractility, we were unable to demonstrate any effects on driven isolated rabbit atria(2), although simultaneously and independently, Tanz reported that d,l-aldosterone could produce a direct positive inotropic effect on isolated cat heart preparations(3). These latter findings recently have been more fully described, in-

* This investigation was supported in part from grants from Nat. Heart Inst. U.S.P.H.S. and San Mateo County, Calif., Heart Assn.

cluding effects on rat and guinea pig papillary preparations(4).

In view of the striking potency of aldosterone on the isolated cat and rat heart preparations(1,3,4), we undertook additional experiments to characterize more completely the actions of this steroid on isolated rabbit atria in order to compare this possible species difference in cardiac reactivity to aldosterone. Also, since earlier reports did not include any studies on electrical properties, the effects of aldosterone on atrial excitability and effective refractory period were also examined. Studies with the biologically active d-form of aldosterone were also performed, to compare responses with the d,l form used in previous experiments(2,3,4).

Methods. Isolated, intact rabbit atrial preparations were obtained from white New Zealand rabbits weighing 1.5-1.8 kg. The animals were stunned by a blow on the head, and the heart was quickly removed and placed in a dissecting dish containing oxygenated Ringer solution(5). The atria were then transferred and suspended horizontally in a 30-ml muscle chamber containing Ringer solution. The right atrium was attached to a pair of platinum electrodes which were anchored to one wall of the muscle chamber. The left atrial appendage was then attached to a pair of hooks which were connected *via* a lever arm to the movable anode of an RCA 5734 mechanoelectronic transducer. Isometric contraction was recorded on an oscilloscope utilizing the simplified bridge circuit used with this transducer(6). After the atria were mounted, they were stimulated at a constant rate of 105 beats/min, using a square wave stimulus of 5 msec duration. Stimulus intensity was adjusted to approximately 5 times the threshold voltage needed to elicit contraction. Diastolic tension was adjusted until maximum contractile force was developed. The medium was gassed with a 95% O_2-5% CO_2 gas mixture *via* a fritted disc placed in the muscle chamber. The Ringer solution was continuously perfused through the muscle chamber at a rate of 100 ml/hr. Bath volume was maintained constant by continuous aspiration. The bath temperature was kept at 25°C throughout the experiment.

Preparations were permitted to equilibrate for 200 min after mounting before the experimental period was begun ("zero time"). At this time, all preparations were in a hypodynamic phase (see *Results*). Aldosterone or vehicle (ethanol) effects were studied by changing to a perfusing Ringer solution containing known concentrations of drug or vehicle.

The following parameters were measured: isometric contraction force; changes in diastolic tension; effective refractory period, as measured by the "maximum follow rate" method of Dawes(7); and electrical excitability, as measured by the minimum stimulus strength needed to elicit a contraction response using a constant stimulus pulse duration. Changes in contraction, threshold voltage, and maximum follow rate were expressed as per cent change from values recorded at the end of the equilibration period. Diastolic tension was expressed as centimeters of change in base line (taken as zero at the start of experimental period). Since the d,l-aldosterone used in these studies was suspended in 95% ethanol, vehicle control studies were performed using an appropriate amount of ethanol in Ringer solution. D-aldosterone was supplied as an aqueous solution, and its actions compared to untreated controls. With the concentrations used in these experiments, ethanol and untreated controls showed similar changes in all measured parameters, both as to time course and magnitude.

Results. Inotropic effects of d,l-aldosterone. Fig. 1 illustrates the changes in contraction force produced by an 8×10^{-10}M concentration of d,l-aldosterone (free alcohol) compared to control preparations. The almost identical changes seen in control and experimental groups indicated that this very low concentration of d,l-aldosterone had no significant action on the contraction force developed by driven, isolated rabbit atrial preparations. Similarly, concentrations of 2.5×10^{-10}M, and 2.5×10^{-7}M had no significant effects on contraction force compared to appropriate control groups. Even when the concentration was raised to 1.7×10^{-6}M, no statistically significant inotropic effect was seen, although the mean decreases in con-

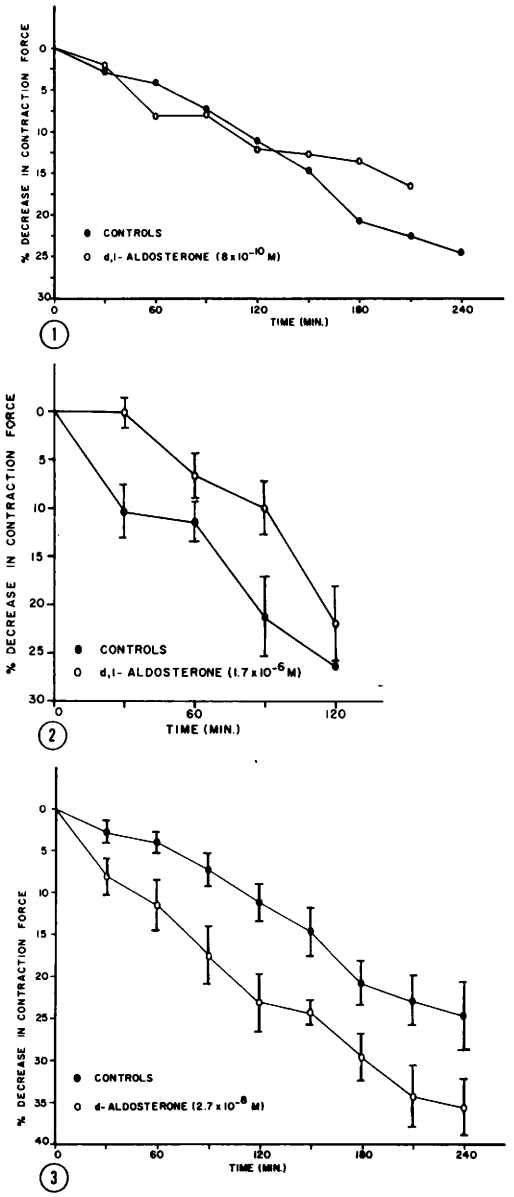

FIG. 1. Effect of d,l-aldosterone (8×10^{-10}M) on contraction force of driven, isolated rabbit atria preparations. Preparations were equilibrated for 200 min before aldosterone perfusion was started (zero time). Each point represents the mean of 5-21 experiments.

FIG. 2. Effect of d,l-aldosterone (1.7×10^{-6}M) on contraction force of driven, isolated rabbit atria preparations. Each point represents the mean of 5-7 experiments. Vertical bars indicate standard errors of means.

FIG. 3. Effect of d-aldosterone (2.7×10^{-8}M) on contraction force of driven, isolated rabbit atria. Each point represents the mean of 5-23 experiments. Vertical bars indicate standard errors of means.

traction force over a 120-minute period were slightly less than those seen in the appropriate ethanol control preparations (Fig. 2).

Inotropic effects of d-aldosterone. The natural d-isomer of aldosterone has been shown to be approximately 30% more active than the racemic form with respect to its "mineralosteroid" effects on kidney function(8). The possibility existed, therefore, that d-aldosterone might produce effects on contraction different than those seen with the d,l form.

Fig. 3 illustrates the effects of a 2.7×10^{-8}M concentration of d-aldosterone on driven, isolated rabbit atria. Results indicated that not only was there a lack of a positive inotropic effect, but that there was actually a trend toward a slightly negative inotropic action.

Effects on excitability and refractory period. In all concentrations tested, neither d,l nor d-aldosterone produced any significant changes in threshold voltage (*i.e.*, excitability) or maximum follow rate (effective refractory period) compared to appropriate controls. Moreover, there was no evidence indicating that either form of the steroid produced arrhythmias or ectopic beats, even with the highest concentrations.

As an example of the lack of effect of d,l aldosterone on electrical properties, it was found that with a concentration of 1.7×10^{-6}M, threshold voltage was only 4.7% higher than the value recorded for the control group after 120 minutes of drug perfusion. This difference was not statistically significant ($P > 0.05$). Similarly, the change in maximum follow rate was only 1.5% higher than that seen in the control group at 120 minutes.

Effects on diastolic tension. As with contraction and electrical properties, both d,l and d-aldosterone failed to produce any significant changes in diastolic tension compared to untreated controls. Only with the 2.7×10^{-8}M concentration of d-aldosterone was any evidence for contracture obtained. However, the difference of only 0.33 cm between steroid and control groups seen at 120 minutes was not statistically significant ($P > 0.05$).

Discussion. It is apparent from this study

that the potent mineralosteroid aldosterone has no significant effects on the contractile or electrical properties of driven, isolated rabbit atria. The lack of effect of very low concentrations (10^{-6}-10^{-10}M) of this steroid is in particular contrast to the results obtained in isolated rat heart-lung preparations(1), isolated cat papillary, and cat Langendorf preparations(3,4). On the other hand, these results are in general agreement with earlier findings that aldosterone had no definite effect on the isolated frog heart, even with concentrations up to 10^{-4} g/ml(9). Aldosterone also failed to affect the "staircase" response in isolated frog heart(10).

The possibility that species differences might be an important factor in the response of the heart to exogenous aldosterone is further emphasized by the finding that left ventricle papillary preparations from guinea pig hearts showed only a weak positive inotropic effect over a wide concentration of aldosterone(4). Moreover, experiments with rat papillary and trabeculae carnae tissue failed to reveal a positive inotropic action of aldosterone(4). Thus, it appears that not only species difference may be involved in explaining discrepancies of results, but also the type of isolated tissue preparation used (in any given species) may be important.

Differences in methodology may also be cited as possibly contributing to discrepancies in results. For example, in the present study, the factors of K/Ca ratio in the perfusate, rate of stimulation, temperature, perfusion technics, time of "equilibration" before drug additions were all different than the conditions described in studies utilizing isolated cat papillary(4). It is now well established that the first 3 factors are particularly important in the study of the actions of cardiotonic steroids such as digitalis(11). Whether varying these and other parameters would yield results different from those described here cannot be answered completely. However, experiments (unpublished) with isolated rabbit left atrial preparations driven at a rate twice that used in the present report, and maintained at 30°C, failed to reveal any significant response to d-aldosterone perfusion, except with high concentrations (10^{-6}M) where a slight negative inotropic effect was seen.

Another factor to be considered is whether or not one must have a markedly hypodynamic heart preparation before a positive inotropic effect of aldosterone can be seen. In the experiments with cat papillary, a definite and rapid failure of the control preparations was evident in alkaline media(4). When pH was lowered by reducing the $NaHCO_3$, the control preparations actually improved slightly over a 2-hour period. Nevertheless, both "non-failing" and "failing" cat papillary still responded to low concentrations of aldosterone(4). Thus it appears that "failure" is not a requirement to show the positive inotropic effect of aldosterone. This also has been shown for other isolated tissues treated with cardiac glycosides(12).

The lack of effect of aldosterone on excitability and refractory period seen here is in agreement with results reported by Briggs and Holland(13) using the same preparation. These investigators found that the lack of effect of aldosterone on refractory period was also paralleled by a lack of effect on potassium efflux.

While experiments with isolated tissues are informative, it should be re-emphasized that different tissues may react differently to aldosterone under physiological conditions present in the intact organism(14). However, under the conditions of the experiments described here, aldosterone has a notable lack of effect on driven, isolated rabbit atria. This lack of action may be helpful in better understanding the factors which possibly influence the response, if any, of the heart to this hormone.

Summary. The effects of aldosterone on contractile and electrical properties of driven, isolated rabbit atrial preparations have been determined. Very low concentrations (*e.g.*, 10^{-10}M) failed to produce significant effects on contractile force, excitability, effective refractory period, or diastolic tension. Higher concentrations of both the d,l and d-form tended to produce a slight negative inotropic effect except at a d,l-aldosterone concentration of 1.7×10^{-6}M, where the normal decrease in contractile force was slightly de-

layed compared to control preparations. These higher concentrations also had no significant action on electrical parameters compared to appropriate controls.

The d,l and d-aldosterone used in these studies were kindly supplied by CIBA Pharmaceuticals, Inc., Summit, N. J.

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Received August 27, 1962. P.S.E.B.M., 1962, v111.

Relationship of Thymolytic and Glycogenic Activities of Adrenocorticoids. (27868)

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The disparity between estimates of adrenocorticosteroid efficacy, assessed by different criteria of response (*e.g.*, liver glycogen deposition, thymus involution, anti-inflammatory action) is prevalent in the bioassay literature. Stephenson(1) has stated, "ideally the various assays for glucocorticoid-like activity should be carried out simultaneously in test animals from the same group, and using the same route of administration and injection medium for all of the tests." Ringler *et al.*(2) indicated that liver glycogen deposition and thymus involution relative potencies compared favorably, suggesting that potency estimates of glucocorticoid activity would be similar if assessed simultaneously.

To study more critically the relationship between glycogenic and thymolytic efficacy ratios, 64 adrenocorticoids of varied structure were assayed utilizing a 5-day adrenalectomized rat bioassay.

Materials and methods. In a 5-day bioassay, liver glycogen deposition and thymus involution were simultaneously determined in adrenalectomized, immature (40-60 g Sherman strain) male rats. Animals were maintained with Purina Lab Chow and 1% NaCl drinking fluid *ad lib*. Six concentrations of test steroids* were assayed in parallel with 6 concentrations of hydrocortisone, using 4 rats per group. Twenty-four hours after adrenalectomy the rats were injected subcutaneously with steroid suspended in 0.2 ml of carboxymethylcellulose vehicle(3), excluding benzyl alcohol. Control animals received vehicle only. Steroid was administered daily for an additional 4 days. The rats were fasted 15 hours before and 7 hours after receiving the last injection to insure maximum depletion of liver glycogen. Animals were anesthetized with sodium pentobarbital and the livers quickly excised, weighed and hydrolyzed in 30% potassium hydroxide(4). Thymi were removed and weighed on a Roller-Smith torsion balance. Thymus response was expressed as mg thymus weight/100 g final body weight. Liver glycogen was measured

* Steroids kindly supplied by the Organic and Biochemical Research Sections, Lederle Laboratories.