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Effect of D-Aldosterone on Cation Content of Isolated Rabbit Atria* (28000)

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Few substances are capable of stimulating active Na-K transport across cell membranes. Perhaps the most physiologically important of these agents is the hormone aldosterone (1). This naturally occurring steroid has been shown to stimulate active Na-K transport in a variety of tissues including isolated toad bladder(2), frog skin(3), renal tubule (4) and skeletal muscle and brain(5,6). Equivocal effects have been noted with respect to RBC ion transport, however(7,8).

Since aldosterone has been implicated in production of myocardial edema and derangements in ion metabolism in certain cardiac disease states(9), we undertook experiments to determine whether or not the biologically active d-form of aldosterone(10) was capable of modifying the Na or K content of cardiac tissue. Also, since aldosterone given *in vivo* has been shown to enhance K accumulation in skeletal muscle(5,6), we were particularly interested in determining whether the d-form of the steroid could prevent the K loss from isolated left atrial tissue which occurs with prolonged electrical stimulation *in vitro*. Thus, as a working hypothesis, we are questioning whether this steroid is capable of stimulating active Na-K transport in the isolated heart as it has been claimed to do in other tissues(2-6).

Materials and methods. Left atria from hearts of White New Zealand rabbits were studied. The animals were sacrificed by a blow on the head and the heart quickly removed and placed in a dissecting dish containing oxygenated bicarbonate-Ringer solution(11). The left atrium was dissected free from the ventricles and all visible fat, connective and vascular tissue removed. Experiments were divided into 5 groups: *Group I.* Freshly dissected tissue was blotted on filter paper to remove adhering solution and then immediately placed in a drying oven at 110°C until a constant dry weight was obtained. Total tissue K and Na were determined from nitric acid digests of the atria using a flame photometer with an internal lithium standard. *Group II.* After atria were dissected from the ventricles, they were placed in a muscle bath at a constant resting tension for 260 minutes. The muscle chamber was constantly perfused with Ringer's solution at 30°C. At the end of this period, the atria were removed from the bath and prepared for K and Na analysis as in Group I. *Group III.* Tissue prepared and handled in same manner as Group II except that the atria were stimulated at a constant rate of 200 beats/min for the entire time the tissue was *in vitro*. *Group IV.* Same as Group III except that 200 minutes after placing in the muscle chamber, a d-aldosterone (1.1×10^{-6}M)-Ringer solution was substituted for the normal Ringer perfusing the atria. Drug action continued for one hour, then the tis-

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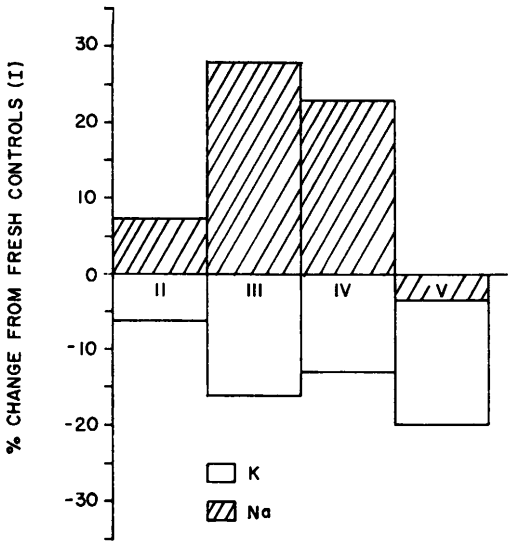

FIG. 1. Effect of d-aldosterone on potassium and sodium content of isolated rabbit left atria. See text for full description of treatment used in each group. Ion content is expressed as percentage change from normal values determined in fresh dissected atria (Group I). Results for each group based on 7-12 experiments. *Group II:* isolation, no stimulation; *Group III:* isolation + stimulation; *Group IV:* isolation + stimulation + d-aldosterone, 1.1×10^{-6}M; *Group V:* isolation + stimulation + d-aldosterone, 1.1×10^{-5}M.

sue was removed for analysis as previously described. *Group V.* Same as Group IV except that a d-aldosterone concentration of 1.1×10^{-5}M was used.

Except for the freshly dissected controls (Group I) total isolation time of the atria in Groups II-V was 260 minutes. Ion content was expressed as percentage change from normal values determined in the freshly dissected atria (Group I). D-aldosterone was supplied in an aqueous solution.†

Results. Fig. 1 shows the changes in total K and Na content of the isolated left atria which occurred with the various treatments described. Note that isolation for 260 minutes without stimulation (Group II) produced a slight (6.2%) but significant ($P < 0.05$) decrease in total K compared to fresh dissected controls (Group I). Stimulation at 200/min (Group III) for the same period resulted in a still greater (16.4%) loss of K. Addition of d-aldosterone in a concentration of 1.1×10^{-6}M (Group IV) or 1.1×10^{-5}M (Group V) for one hour prior to removal for analysis did not significantly ($P > 0.05$) prevent the loss of K normally seen without the steroid (Group III). The higher concentration, in fact, appeared to increase K loss in relation to the untreated stimulated controls (Group III).

With respect to the changes in total Na content of the left atria, isolation for 260 minutes without stimulation (Group II) produced a slight but significant ($P < 0.05$) rise in total Na compared to fresh dissected controls (Group I). Isolation plus stimulation for 260 minutes resulted in a marked (28.1%) and significant ($P < 0.01$) increase in total Na content of the atria compared to both Groups I and II.

Addition of d-aldosterone in a concentration of 1.1×10^{-6}M (Group IV) did not significantly ($P > 0.05$) prevent the rise in Na content of the stimulated atria when compared to stimulated atria without drug (Group III). However, in a concentration of 1.1×10^{-5}M, the steroid not only prevented an increase in Na, but actually promoted a Na loss in relation to the fresh controls.

Discussion. The finding that a high concentration (10^{-6}M) of d-aldosterone is unable to prevent the K loss and Na gain which occurs when isolated atrial tissue is stimulated for long periods fails to support the hypothesis that this hormone may promote active Na-K transport in heart muscle. In this regard, then, the data are in contrast with the results of others purporting to show a stimulation of active Na-K transport by aldosterone in other tissues(2-6). However, our results are in general agreement with the findings(1) which showed that chronic administration of very high doses of d,l-aldosterone to rabbits failed to increase the K content of the left ventricle. Indeed, it appeared that a slight loss of heart K and gain of Na occurred with aldosterone treatment(1). Our finding that a very high concentration (10^{-5}M) prevents the gain of Na normally seen in isolated, stimulated atria is, at pres-

† The d-aldosterone (Lot #E-6403) used in these studies was kindly supplied by CIBA Pharmaceutical, Inc.

ent, unexplained. Admittedly, this is not a physiological concentration (10^{-9}-10^{-10}M) of aldosterone. Therefore, some question may be raised whether or not this effect on Na actually has physiological significance *in vivo*.

It is conceivable that active Na-K transport mechanisms in the rabbit heart show less sensitivity to the "stimulant" action of aldosterone compared to other tissues from a variety of species. Another possible explanation for the failure of d-aldosterone to show a significant effect on heart K under the conditions of the present experiments is that *in vitro,* optimum conditions for demonstrating a stimulant effect on ion transport may be absent. Arguing against this, however, are the findings that show that aldosterone can stimulate active transport in certain tissues *in vitro* (*e.g.,* 2).

The possibility that this hormone may produce more subtle minute-to-minute changes in heart K and Na fluxes cannot, of course, be excluded, since terminal analysis of total tissue K or Na does not necessarily reflect active ion flux conditions. However, Briggs and Holland(12), using isotopic K to measure transmembrane fluxes in isolated rabbit atria, were unable to show any significant effect on K efflux with aldosterone in a concentration of 10^{-6}M.

The results reported elsewhere(1,12), coupled with the findings reported here, therefore indicate that aldosterone appears to have no significant action on Na-K balance of the heart, except possibly with very large doses.

Summary. The effects of the potent mineralosteroid d-aldosterone on K and Na content of stimulated and unstimulated isolated rabbit left atrial preparations have been studied. Isolation plus stimulation for 260 minutes produced a significant loss of total tissue K and significant gain of Na compared to freshly dissected controls. Addition of d-aldosterone (10^{-6}M) to the perfusing fluid for one hour prior to analysis of cations failed to prevent this K loss or Na gain. A high concentration (10^{-5}M) did significantly prevent the gain of Na, however. The data have been interpreted to mean that d-aldosterone does not promote active K accumulation in isolated heart muscle.

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