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Effect of Aldosterone on Ouabain-Induced Potassium Loss from Isolated Rabbit Atria.* (28652)

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Aldosterone has been shown to stimulate or enhance active sodium (Na^+)-potassium (K^+) transport in a variety of tissues both *in vivo* and *in vitro* (Gross, 1,2). On the other hand, the cardiac glycoside ouabain is recognized as a potent inhibitor of active Na-K transport across cell membranes(3), including heart muscle(4). Based on these results, therefore, a possibility exists that these 2 steroids might be mutually antagonistic insofar as their cation transport effects are concerned. Although various studies have indicated that aldosterone (as well as other corticosteroids) is ineffective in antagonizing cardiac glycoside-induced changes in cation transport in the red blood cell (RBC)(5-7), there is some evidence that aldosterone may be able to prevent the K^+ loss from isolated hearts produced with high doses of cardiac glycosides(8,9), as well as antagonize the effects of ouabain on active Na^+ transport in isolated frog skin when the steroids are given *in vivo*(10).

This conflicting evidence on the alleged ability of aldosterone to block or modify the actions of ouabain on cation transport has prompted us to investigate whether or not this naturally occurring hormone can, in fact, prevent or modify the changes in K^+ content induced with high concentrations of ouabain in stimulated isolated heart muscle. We have previously shown that insofar as the electrical and contractile parameters of isolated atrial tissue are concerned, d,l-aldosterone, in equimolar concentration, does not antagonize the actions of ouabain(11), with the apparent exception of the contracture response. In a preliminary study, we were unable to demonstrate an antagonism of ouabain-induced K^+ loss using 1×10^{-6}M aldosterone(12). The present report extends these observations and includes the effects of higher (2.2×10^{-6}M-10^{-5}M) concentrations of d-aldosterone on the K^+-depleting effect of ouabain.

Methods. Experiments were performed on electrically driven, isolated rabbit left atrial preparations. Tissue was obtained from hearts of albino rabbits weighing 1.5-2.5 kg. The general procedures for dissecting and preparing the muscle were the same as outlined previously(13,14). The left atria used were free of any pacemaker tissue, and any preparation which showed spontaneous beating during the dissection or mounting procedure was discarded. The tissue was suspended horizontally in a 30 ml muscle chamber perfused with a Ringer solution of the following composition (mM/l); NaCl, 154; KCl, 5.6; $NaHCO_3$, 5.9; CaCl, 2.2; and dextrose, 5.5. The perfusate was gassed with a 95% O_2 – 5% CO_2 gas mixture via a sintered disc placed directly in the muscle chamber. All experiments were performed at 30°C. The

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TABLE I. Effects of Ouabain, D-Aldosterone and Ouabain + D-Aldosterone on K^+ Content of Isolated, Stimulated Rabbit Left Atria.

No. of exp	Group	Treatment*	Relative K^+ content (%)†
21	I	Untreated controls	100.0 ± 2.1
7	II	d-aldosterone (1.1×10^{-6} M)	103.8 ± .6‡
16	III	ouabain (1.7×10^{-6} M)	88.0 ± 3.5§
7	IV	*Idem* + d-aldosterone (1.1×10^{-6} M)	88.0 ± 4.2‡§
7	V	" + " (2.2×10^{-6} M)	88.4 ± 2.9‡§
9	VI	" + " (1.1×10^{-5} M)	88.3 ± 1.5‡§

* See text for full description of treatment used in each group.

† Total K^+ content of tissue is expressed as % of total K^+ measured in controls (group I), designated as 100%. Values are given as mean ± S.E.

‡ $P > .05$ for group II *vs* controls (group I). $P > .05$ for all ouabain + aldosterone treatments *vs* ouabain alone (group III).

§ $P < .001$ for groups III, IV, V and VI *vs* controls (group I).

atria were stimulated throughout the entire equilibration and experimental procedure at a constant rate of 200 beats/minute using a square wave stimulus of 5 msec. pulse duration at 5 times threshold voltage needed to elicit contraction. Measurements of isometric contractile tension, effective refractory period, contracture, and electrical excitability were carried out according to procedures and techniques described previously(13). All preparations were allowed to equilibrate and stabilize for 3.33 hours before experimental procedures were begun. Drug effects were studied by changing over to perfusion reservoirs containing known concentrations of ouabain†, d-aldosterone‡, or ouabain + d-aldosterone in Ringer's at the end of the 3.33 hours equilibration period. The atria were exposed to the drugs for a 1-hour period. At the end of this time (4.33 hours post mounting), tissues were removed and prepared for flame photometric analysis of total K^+ and Na^+ using nitric acid digests of the muscle.

Six groups of experiments were performed: *Group I.* Untreated controls. Atria stimulated for 4.33 hours at 200/minute (no drug). *Group II.* D-aldosterone (1.1×10^{-6}M) started at 3.33 hours and effects noted for 1 hour. *Group III.* Ouabain (1.7×10^{-6}M) started at 3.33 hours and effects noted for 1 hour. *Group IV.* Ouabain (1.7×10^{-6}M) + d-aldosterone (1.1×10^{-6}M) started at 3.33 hours and effects noted for 1 hour. *Group V.* Same as group IV except that a 2.2×10^{-6}M concentration of d-aldosterone was given simultaneously with the ouabain. *Group VI.* Same as groups IV and V except that a 1.1×10^{-5}M concentration of crystalline d-aldosterone was given simultaneously with the ouabain.

Thus, in all groups, the atria were isolated and stimulated for the same period (4.33 hours), the only difference in the groups being the presence or absence of the drugs being tested.

Results. Ion changes. Table I indicates the changes in total K^+ content of rabbit left atrial tissue with the various treatments described. Ouabain alone (Group III) produced a significant ($P < 0.001$) decrease (12%) in the K^+ content relative to the untreated controls (Group I). D-aldosterone alone (1.1×10^{-6}M) (Group II) had no significant ($P > 0.05$) effect on total K^+ when compared to the untreated controls. We have previously shown that a higher concentration (10^{-5}M) of d-aldosterone was also ineffective in changing the total K^+ content of stimulated left atrial tissue(14). When ouabain and various concentrations of d-aldosterone were administered together (Groups IV, V, and VI), the total K^+ content of the atria was not significantly different ($P > 0.05$) from the value seen with the ouabain treatment alone (Group III).

Under the conditions of these experiments, the loss of tissue K^+ associated with ouabain

† Crystalline ouabain was kindly supplied by Dr. K. K. Chen, Lilly Research Laboratories, Indianapolis, Ind.

‡ The d-aldosterone (aqueous solution and crystalline form) was kindly supplied by CIBA Pharmaceuticals, Inc., Summit, N. J. Manufacturing Lot number was #E-6403.

toxicity was not accompanied by significant change in total tissue Na^+ relative to the stimulated untreated controls. Aldosterone alone (1.1×10^{-6}M) was also ineffective in altering the Na^+ content of the atria, although we have recently shown that with higher doses (10^{-5}M), d-aldosterone may prevent the increase in total Na^+ content of isolated left atria which normally occurs with prolonged stimulation *in vitro*(14). It should be emphasized, however, that isolation + stimulation alone produces such a marked increase in tissue Na^+ content that additional drug effects on Na^+ are not easily distinguished by measurement of total Na^+.

Contractile and electrical changes. The loss of tissue K^+ which occurs with administration of ouabain is associated with a significant decrease in excitability, increase in effective refractory period (measured by the "maximum follow rate" method of Dawes (15)), and development of contracture preceded by a transient increase in contractile tension. Simultaneous administration of d-aldosterone (1.1×10^{-6}, 2.2×10^{-6}, or 1.1×10^{-5}M) and ouabain (1.7×10^{-6}M) does not significantly prevent or modify the time course or magnitude of these changes. The lack of a clear effect of d-aldosterone on ouabain contracture is in contrast to the apparent ability of d,l-aldosterone to delay ouabain contracture in isolated, intact rabbit atria(11). This discrepancy may be due in part to the fact that the experiments described here were done at a higher rate of stimulation (200/min *vs* 105/min) and temperature (30°C *vs* 25°C) than used in the studies with the intact atria. We have also noted that high concentrations of deoxycorticosterone (glucoside) (10^{-4}M) fail to prevent the onset of ouabain-induced contracture in spontaneously beating guinea pig atria in Krebs-Ringer solution at 37°C.

Discussion. Our findings showing that d-aldosterone fails to inhibit or antagonize the K^+-depleting effect of ouabain on isolated heart muscle fail to support the speculative view this hormone may competitively antagonize the actions of the cardiac glycosides on heart muscle K^+ transport(8,9,16). In this regard, the data are in accordance with results obtained on the RBC where a similar lack of antagonism of glycoside effects has been noted(3,5,6). The results obtained here with the isolated atria are in contrast, however, to the findings of a purported antagonism by aldosterone of glycoside effects on isolated hearts(8,9). No explanation for this discrepancy of results is available. However, the fact that contractile and electrical changes induced by ouabain are not antagonized by equimolar or 10 × concentrations of aldosterone either in intact atria(11) or left atrial tissue (present study), casts further doubts on the view that there may be a competition between these two steroids. Corticosterone has also been found to be ineffective in antagonizing the contractile effects of strophanthin on rat ventricle strips(17).

The ability of certain adrenal steroids to antagonize the effects of cardiac glycosides on electrolyte balance in intact animals or the ability of cardiac glycosides to support life in adrenalectomized animals has been offered as support for the mutual antagonistic action of these agents(16). However, we interpret this "competition" as being mediated through renal effects and unrelated to any specific competitive antagonism between these two groups of steroid substances. Thus, the ability of aldosterone to "antagonize" ouabain effects on kidney electrolyte excretion(18) for example, does not distinguish whether or not aldosterone is increasing the number of carrier sites available for the transport of Na^+ as proposed by Vander (19). Also, in the intact animal, aldosterone might be acting to reverse ouabain effects by changing the Na^+-K^+ ratio in the kidney tubule unrelated to any competitive antagonism. However, it is of interest that ouabain may inhibit the stimulating effect of aldosterone on active Na^+ transport in isolated toad bladder under conditions in which ouabain alone does not inhibit active Na^+ transport(20).

Summary. D-aldosterone (1.1×10^{-6} – 1.1×10^{-5}M) has been found to be ineffective in preventing the K^+ loss from isolated rabbit atrial tissue caused by a high concentration of ouabain (1.7×10^{-6}M). This lack of effect in preventing the K^+-depleting effect

of ouabain was paralleled by a lack of antagonism of ouabain-induced changes in contractile and electrical properties of the atrial muscle. Therefore no evidence was found to support the view that d-aldosterone may competitively antagonize the K^+-depleting actions of ouabain on isolated atrial myocardium.

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Influence of Dietary Saturated and Unsaturated Fats on Blood Coagulation In Miniature Pigs.*† (28653)

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Following the observations of Keys and coworkers(1,2) that the fat content of a diet bears a direct relationship to the plasma cholesterol level of the population and to the incidence of coronary heart disease, it was shown by other investigators(3,4) that unsaturated fats decreased the plasma cholesterol level and that saturated fats increased it. Whether saturated and unsaturated types of dietary lipids can affect coagulability of blood, as well as possibly contributing to the development of atherosclerosis, is not known. Numerous studies have attempted to ascertain whether, and to what extent, dietary fats bring about changes in blood coagulation and fibrinolytic systems. Both animal and vegetable fats produced the same shortening of the recalcification time of plasma of patients, 3 and 4 hours after ingestion of fat(5). Keys *et al*(6) found that the silicone clotting time of blood was much shorter on a butterfat or coconut oil diet than on a corn oil diet. Using pigs as experimental animals, Rowsell *et al*(7) suggested that feeding butterfat increases both the incidence and severity of aortic lesions, perhaps due to alterations in the blood clotting mechanisms.

At The Hormel Institute, miniature pigs are being used in studies of the fundamental causes of atherosclerosis, including the influence of various dietary fats on blood coagulation. This paper presents some of the differences observed in the coagulability of blood on miniature pigs maintained for a

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