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Effect of Ouabain on Adrenal Potassium Balance in Sheep[1] (39015)

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(Introduced by E. Knobil)

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The hypothesis has been advanced on several occasions that the effect of K on aldosterone secretion could be mediated by a change in intracellular [K] (1–10). We previously reported that in sheep there is a marked inhibition of aldosterone secretion consequent to administration of ouabain in the arterial supply of an autotransplanted adrenal (11). Currently no data are available regarding the magnitude and time course of change in adrenal K balance during administration of ouabain *in vivo*, but *in vitro* evidence indicates that ouabain produces a striking reduction of adrenal intracellular [K] (3).

The present study was undertaken to evaluate the magnitude and time course of the changes in adrenal [K] that are produced by infusion of ouabain into the adrenal arterial blood supply of conscious sheep.

A technique was developed for adrenal arterial infusion and collection of the total adrenal venous effluent in conscious animals. This method allowed for acute studies to be performed in conscious sheep with collection of the total adrenal venous effluent and minimal systemic contamination by the infused ouabain.

Methods. Surgical preparation. Prior to experimentation, crossbred Merino ewes were maintained in metabolism cages with free access to water and received a standard diet containing between 50–100 mequiv of Na/day.

Anesthesia was induced with pentothal and maintained with halothane–oxygen. Sterile technique was observed throughout the surgical procedure. The technique for collecting the total adrenal venous effluent used an extracorporeal circuit similar to that described by Blair-West *et al.* (12). To allow infusion directly into the adrenal arterial blood supply, the lumbar artery which provides part of the arterial blood to the adrenal was visualized at its origin from the aorta. This artery has a 5-mm course from the aorta and divides into two branches, one to the back muscles and a smaller one to the adrenal. The larger muscular branch was ligated distally, and a small polyethylene catheter (PE20) inserted and advanced a short distance but not so far as to occlude the blood flow down the adrenal branch. Heparinized saline was infused into this catheter at 0.1 ml/min. Thus, the infusion solution was carried along with the much greater blood flow down the adrenal arterial branch with minimal systemic contamination. A similar cannulation procedure was likewise used to provide infusion to the renal branch of the adrenal arterial supply. Figure 1 provides a schematic representation of the catheter placements. The extracorporeal circuit allowed access to the adrenal venous blood without interfering with the animal after recovery from anesthesia.

The animals recovered rapidly and usually were standing in their cages an hour after completion of the surgery.

Experimental procedure. After a 2–3-hr recovery period during which heparinized saline (250 U/ml) was delivered to each arterial cannula at 0.1 ml/min, a slow, whole blood infusion was started into a jugular vein. This blood had been obtained from a donor sheep the same day as the experiment and was administered at a rate estimated to match the loss of blood from the collection procedures. The adrenal vein–jugular vein bypass was

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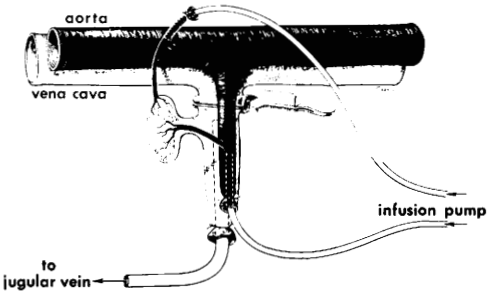

FIG. 1. Schematic drawing illustrating catheter placements for collection of total adrenal venous effluent and arterial infusion in conscious sheep.

opened by cutting through the Silastic cannula about midway along its length. The cannula could be reconnected by a Teflon joint which fitted the inner diameter of the cannula.

Adrenal venous blood and systemic arterial blood from a left carotid artery cannula were collected simultaneously; the arterial collection rate being matched with the spontaneous adrenal venous blood flow. Collection periods were 10-min-long and either the entire flow for 10 min was collected and sampled or alternatively 20 ml of blood was collected at each sampling period with the bypass reconnected between collections. Three control blood samples were obtained and the infusion was changed to 2.4×10^{-4} *M* ouabain dissolved in 250 U/ml of heparin–saline solution for a further 30 min. This solution was delivered at the rate of 0.1 ml/min to each adrenal arterial cannula. During the time of the ouabain infusion and for 30 min immediately afterward, the entire adrenal venous effluent was always diverted from the animal to avoid contamination of the systemic circulation by the ouabain.

The animal was fed and watered and, if possible, the experiment repeated the following day.

Analytical procedures. Plasma [K] and [Na] were determined by flame photometry using a Technicon AutoAnalyzer. Each sample was divided before centrifugation into two equal aliquots and duplicate determinations were performed on each, yielding quadruplicate estimations of the electrolyte concentrations. These four values were averaged and the resultant mean used as an individual datum point.

Statistical analyses were performed by Student's *t* test for grouped observations.

Results. Ten animals were studied in this series of experiments, but three provided no data because of technical problems associated with the bypass. That is, clotting of the extracorporeal circuit occurred in two animals, and in one animal the adrenal vein was partially occluded resulting in extremely low flow. In addition, one animal had an adverse reaction to the blood transfusion in one experiment, and two others developed signs of acute ouabain toxicity in one experiment. Thus, four animals provided data for the complete experiments performed on separate days, and three animals provided data for one experiment on one day only.

Figure 2 illustrates the changes in adrenal venous and peripheral arterial plasma [K] during a typical experiment. In this animal the adrenal plasma flow was 4.3 ml/min which resulted in a ouabain concentration of 8.4 μg/ml. During the control period when only heparinized saline was administered, no difference was observed between the peripheral plasma and adrenal venous plasma [K]. When ouabain was infused into the adrenal arterial supply for 30 min, there was a rapid rise in adrenal venous and a smaller rise in systemic arterial plasma [K]. These changes resulted in a net loss of 72.4 μequiv of K from the gland. The left adrenal from this animal weighed 2.2 g. The loss of K from the adrenal was most marked during the ouabain infusion but persisted for 30 min after the infusion had ended. By 30 min after ending the ouabain infusion, K had begun to move back into the adrenal.

Figure 3 presents the composite adrenal K balance data from all of the animals of this study. These data were derived by multiplying the adrenal venous–arterial plasma [K] difference by the adrenal plasma flow over the 10-min collection period. Similar to the data of the individual animal displayed in Fig. 2, the composite data reveal the same changes in adrenal K balance after ouabain. The maximum loss of K occurred during the ouabain infusion then decreased to control levels by 30 min after ending the infusion. All values of negative K balance were statistically significant when compared to the final 10-min control value. Between 90 and

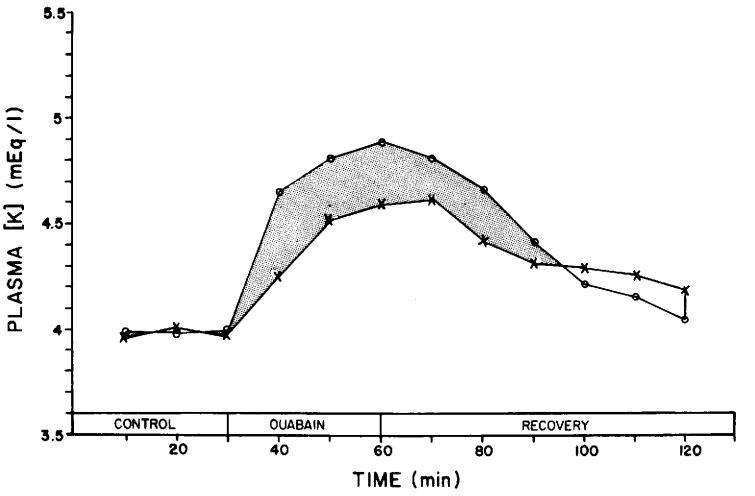

FIG. 2. Time course of the effects of adrenal arterial administration of ouabain on the adrenal venous (○) and systemic arterial (×) plasma [K] of one animal.

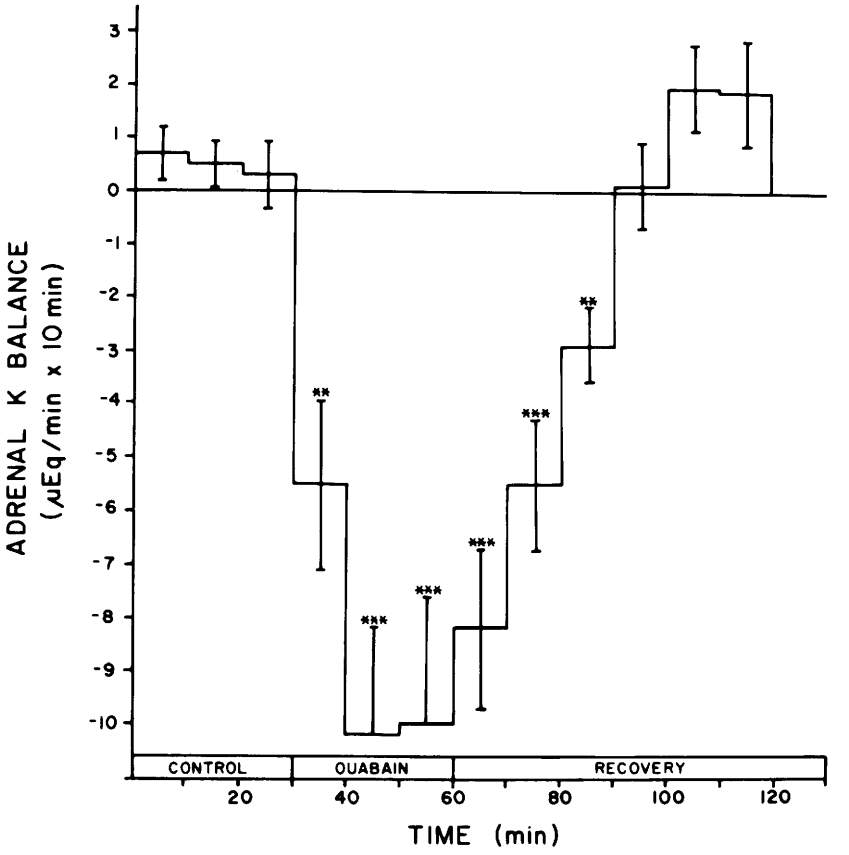

FIG. 3. Time course of changes in adrenal K balance for all animals before, during and after ouabain administration. Mean ± SE, $n = 11$; **, $P < 0.01$; ***, $P < 0.001$.

120 min the adrenal began to take up K, but these values were not statistically different from control.

Sodium concentration in either adrenal venous or peripheral arterial blood did not change during the course of the experiment.

We have previously shown (11) that *in vitro* addition of this concentration of ouabain to whole sheep blood did not change plasma [K] between the time the blood was collected and the plasma separated from the red cells.

Discussion. We reported earlier (11) that adrenal arterial infusion of ouabain producing an adrenal arterial plasma ouabain concentration of approximately 10^{-5} *M* caused a striking reduction in moderately elevated aldosterone secretion which did not occur until approximately 1 hr after cessation of the ouabain infusion. By this time the adrenal venous plasma [K] had returned to control levels from a previous elevation associated with the ouabain infusion. In this previous study, the ouabain was administered so that the systemic plasma contained significant levels of ouabain, and it was not clear what the actual K balance of the adrenal might be; although it was suggested that the aldosterone secretion fell while K was actively moving back into the adrenal. While this study is not directly comparable because the sheep had undergone recent surgical intervention and were not Na depleted, the data do suggest that the previously observed aldosterone inhibition occurred at a time when K was moving back into the adrenal, although certainly the adrenal total K content was still depressed.

In the present study it was apparent that the ouabain-sensitive Na–K ATPase was markedly inhibited as evidenced by the large negative K balance exhibited by the adrenal during and for 30 min after the ouabain infusion. The rapid loss of K from the adrenal during this period contrasts with the much slower uptake of K subsequently. In two animals in which K balance was followed for up to 3 hr post-ouabain, the adrenal did not recover its total K complement. Thus, it is likely that, during aldosterone inhibition previously observed, intracellular K is decreased. The difficult point to reconcile is the slow time course for the "turn-off" of aldosterone as compared to the very rapid "turn-on" response when K is administered into the adrenal artery (13). The explanation for this observation remains obscure, and it is entirely possible that turn-on and turn-off of aldosterone secretion may be influenced by different control factors.

Summary. A previous study revealed that ouabain caused a marked decrease in aldosterone secretion, but the adrenal K status was not clear from those data. The present study investigated the magnitude and time course of change in adrenal K balance when ouabain was administered into the adrenal arterial supply of the *in situ* adrenal of conscious sheep. Ouabain at an adrenal arterial plasma concentration of approximately 2.4×10^{-4} *M* produced a striking negative adrenal K balance within 10 min of beginning the infusion. The adrenal continued to lose K during the 30-min infusion and for 30 min thereafter. The mean total K loss was 42.3 ± 7.9 μequiv/adrenal ($n = 11$). Thirty minutes after ending the ouabain infusion, the adrenal began taking up K but had not recovered its normal K complement by 60 min.

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