Modulation of aldosterone secretion in frusemide-induced hypokalaemia

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Abstract. To study the effect of hypokalaemia in the regulation of aldosterone secretion, repeated injections of frusemide (3 mg/kg) plus saline with or without simultaneous infusion of potassium chloride (1 mEq/kg/h) were performed in 24 conscious female rabbits for 7 h. Without potassium supplementation, the plasma renin activity (PRA) remained elevated throughout the study, while an initial increase (1 h to 3 h) in plasma aldosterone (PA) gradually returned to normal with reduction of the serum potassium. In rabbits on potassium supplements to prevent the development of hypokalaemia, both PRA and PA remained elevated. The incremental aldosterone response to administration of potassium chloride, angiotensin II or ACTH, was considerably smaller in potassium-depleted rabbits than in potassium-repleted rabbits. These results suggest that serum potassium modulates the effects of angiotensin II or ACTH on aldosterone secretion, and that a certain level of potassium is necessary to maintain the aldosterone secretory capacity of the adrenal gland.

Aldosterone and potassium are regulated by each other either directly or indirectly (Williams & Dluhy 1972). Aldosterone is the key hormone determining potassium balance, while potassium, either serum or intracellular, modulates aldosterone secretion independently of the renin-angiotensin system and ACTH (Fraser et al. 1979; Saruta et al. 1972). In addition to the direct effect of potassium upon aldosterone secretion, potassium enhances the stimulatory effect of angiotensin II and ACTH (Gaillard et al. 1979; Linde et al. 1981). In vitro studies, these effects have been clearly demonstrated (Kaplan 1965; Fredlund et al. 1977). Furthermore, dietary changes in potassium and sodium balance alter aldosterone secretion and modulated the effects of angiotensin II and ACTH (Williams et al. 1970; Douglas et al. 1978). However, few investigations have considered modulation in the stimulatory effects of angiotensin II and ACTH due to the changes in potassium balance in an acute, in vivo study.

Therefore, in this study, we tried to elucidate the modulatory effects of changes in serum potassium induced by repeated injections of frusemide on aldosterone secretion in response to angiotensin II and ACTH in conscious rabbits.

Materials and Methods

Female rabbits, weighing 2.5 to 3.5 kg, were used. They were fed on a standard laboratory diet and tap water ad libitum. Under general anaesthesia with iv sodium pentobarbital, the femoral artery and vein were cannulated for blood sampling and infusion of agents, respectively. A urethral catheter was inserted for separate urine collections. Five hours after the surgical procedures, the rabbits were studied in the conscious state. All experiments were performed at between 14.00 and 22.00 h to minimize diurnal variations in steroid production. Blood samples were collected in tubes containing EDTA (1 mg/ml), and were kept iced until the plasma was separated.

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Experiment 1
Twenty-four female rabbits were used in this study and were divided into two groups. In the rabbits of group 1, bolus injections of frusemide, 5 mg/kg, were given 4 times at 0, 1, 3 and 5 h. Blood samples were collected just prior to each frusemide injection and at 2 h after the last injection. Urine volume was measured in each period during the study. In order to maintain the diuretic effect of frusemide, saline equivalent to 80% of the urine volume of the previous period was infused. In the rabbits of group 2, with administration of frusemide and saline, potassium chloride, 1 mEq/kg/h, was infused for 6 h (1 h to 7 h). The infusion rate of potassium chloride was that sufficient to preserve serum potassium levels within the normal range during repeated frusemide injections, as determined in a preliminary study.

Experiment 2
Immediately after experiment 1, the rabbits of group 1 and group 2 were each divided into 3 subgroups, and were given potassium chloride, angiotensin II (Hypertensin, Ciba) or ACTH (1-24α-Cortrosyn, Organon). The potassium chloride and angiotensin II were infused at a rate of 1.2 mEq/kg/min and 20 ng/kg/min, respectively, using a Harvard infusion pump, while an iv bolus injection of ACTH, at a dose of 0.125 mg, was given. Blood samples were collected before and at 1 h after these administrations.

Analytical methods
PRA was estimated by the method of Skinner (1976). PA concentration was determined by a direct radioimmunoassay (Ogihara et al. 1977). Plasma corticosterone was also determined by radioimmunoassay after chromatographic separation on a Sephadex LH-20 column (Makino et al. 1974). Serum potassium and sodium were measured with a flame photometer.

The values are presented as mean ± se. To assess the effect of treatment an analysis of variance with a repeated-measures design was performed using a BMDP2V programme (Jennrich et al. 1981). To assess differences within a group a randomized blocked analysis of variance was used. This was followed by Dunnett's test for multiple comparison. Also comparison between groups was analyzed by Student's t-test for unpaired data. Linear regression analysis and correlation coefficient were calculated by the method of least square. Changes were considered to be significant at $P < 0.05$.

Results

Experiment 1
Response to frusemide without potassium supplements.
The changes in PRA and PA in response to iv administration of frusemide in group 1 are illustrated in Figs. 1 and 2. At 1 h after the injection of frusemide, PRA increased from $3.2 ± 0.1$ ng/ml/h to $6.3 ± 0.5$ ng/ml/h, and remained at an increased level during the next 6 h. In contrast to the PRA, PA increased from $305 ± 20$ pg/ml to $633 ± 53$ pg/ml initially, and then decreased to the control

![Fig. 1](image_url)
Changes in PRA during the administration of frusemide with or without potassium supplements. *$P < 0.01$ compared to the basal value.

![Fig. 2](image_url)
Changes in plasma aldosterone during the administration of frusemide with or without potassium supplements. *$P < 0.01$ compared to the basal value.
Table 1.
Responses of corticosterone, serum electrolytes and haematocrit to repeated frusemide injections with or without potassium supplements.

<table>
<thead>
<tr>
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<th>Time (h)</th>
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<tr>
<td></td>
<td>0</td>
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<tr>
<td><strong>Corticosterone</strong></td>
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</tr>
<tr>
<td>group 1</td>
<td>11.4 ± 1.4</td>
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<tr>
<td>(ng/ml)</td>
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<tr>
<td>group 2</td>
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<tr>
<td>Serum potassium</td>
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<td>(mEq/l)</td>
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<tr>
<td>Serum sodium</td>
<td>144 ± 1</td>
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<tr>
<td>(mEq/l)</td>
<td>2</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>40 ± 2</td>
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<td>(%)</td>
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Group 1: without potassium supplements. Group 2: with potassium supplements. Each value represents the mean ± SE for 12 rabbits. *P < 0.01 compared to the basal value.

level during the next 6 h. The serum potassium gradually decreased during the study, while serum sodium and plasma corticosterone remained unchanged (Table 1). There was also no significant change in haematocrit.

Response to frusemide with potassium supplements. Figs. 1 and 2 also show the PRA and PA responses to frusemide in the presence of 1 mEq/kg/h potassium chloride replacement in rabbits of group 2. Unlike the changes of PA in group 1, it increased from a basal level of 286 ± 16 pg/ml to 558 ± 36 pg/ml at 1 h and remained elevated. A peak response of 770 ± 58 pg/ml was observed at 5 h. There was significant differences in the PA at 5 h and 7 h between groups 1 and 2. In group 2, no significant changes were seen in serum potassium, sodium, plasma corticosterone or haematocrit during the study (Table 1).

Relationships of PA and PRA or serum potassium. Figs. 3 and 4 illustrate the relationships of PA and PRA
or serum potassium in group 1. During the first 3 h (0 h to 3 h), a significant correlation was noted between PRA and PA ($r = 0.75, P < 0.01$). There was, however, no significant correlation between serum potassium and PA of the same period. At a later period (3 h to 7 h), serum potassium, but not PRA, was correlated well with PA ($r = 0.67, P < 0.01$). There was a significant correlation between PRA and PA throughout the study in group 2 ($r = 0.61, P < 0.01$).

**Experiment 2**

*Alcohol responses to potassium, angiotensin II and ACTH.* The effects of potassium chloride, angiotensin II and ACTH on aldosterone secretion in groups 1 and 2 after experiment 1 are shown in Fig. 5. The incremental aldosterone responses to all stimuli were considerably smaller ($P < 0.01$) in group 1 (potassium-depleted) than in group 2 (potassium-repleted).

**Discussion**

This study has demonstrated that during repeated injections of frusemide, PA initially increased, then gradually returned to the basal level in spite of a sustained increase in PRA. There was a significant correlation between the reduction of PA and that of serum potassium. When potassium chloride was supplemented to preserve a normal level of serum potassium, there was no reduction in PA during repeated injections of frusemide.

In in vitro studies using isolated adrenal cells, it has been reported that the concentration of potassium in the incubation medium is an important determinant of the activity of angiotensin II or ACTH in stimulating aldosterone production (Fredlund et al. 1977; Foster et al. 1979). Dietary balance studies showed that the prior dietary intake of both potassium and sodium can alter the magnitude of the aldosterone response to acute stimula-
tion of angiotensin II and ACTH as well as that of potassium (Williams & Braley 1977; Campbell & Schmitz 1978).

With the acute in vivo preparation, we determined that the activities of angiotensin II, ACTH and potassium in stimulating aldosterone secretion are decreased when a hypokalaemic state exists. These results confirm the findings of the study mentioned previously.

The several mechanisms by which potassium modulates angiotensin II- and ACTH-induced steroidogenesis have been proposed: two have gained some acceptance. One mechanism proposed by Douglas & Catt (1976) suggests that potassium exerts modulatory effects on the concentration of adrenal cortical angiotensin II receptors without any constant change in receptor affinity. However, in the incubation medium used to suspend isolated adrenal glomerulosa cells, angiotensin II receptor concentration and affinity were not changed by the various levels of potassium (Fredlund et al. 1977). In the present study, the activities of angiotensin II, ACTH and potassium in aldosterone secretion were reduced in an acute hypokalaemic state, and it therefore seems unlikely that the changes in angiotensin II receptor concentration and affinity would play a major role in steroidogenesis after the modulation of potassium balance.

The other proposed mechanism suggests that the modulation of steroidogenesis by potassium exists in the late steps of aldosterone biosynthesis (conversion of corticosterone to aldosterone) (Baumann & Müller 1979; Müller & Baumann 1974; Komor & Müller 1979). An earlier study has indicated that dietary potassium loading produces a significant increase in the activity of the late steps of aldosterone biosynthesis induced by angiotensin II and ACTH (Williams & Braley 1977). On the other hand, in a chronic in vivo study, Haldy & Müller (1981) have shown that treatment with frusemide in rats kept on a potassium-deficient diet, resulted in a high PRA and a decreased conversion of corticosterone to aldosterone. In our laboratory, Saito et al. (unpubl. commun.) found that deprivation of potassium in the incubation medium inhibited angiotensin II- or ACTH-induced aldosterone production by reducing the conversion of corticosterone to aldosterone in vitro.

Therefore, the present study suggests that even in acute in vivo hypokalaemic conditions, aldosterone reduction induced by potassium depletion may be due to the inhibition of late steps of aldosterone biosynthesis, and this inhibition may be observed in spite of elevated angiotensin II levels. Similarly in our previous study on patients with hyporeninaemic hypoaldosteronism (Saruta et al. 1981), the late steps of aldosterone biosynthesis were inhibited by a reduction of angiotensin II levels even when serum potassium levels were elevated, suggesting that a balance between potassium and angiotensin II is necessary for the biosynthesis of aldosterone. Combining our previous report and present study, the interrelationship between potassium and angiotensin II on aldosterone biosynthesis appears to be more complicated than previously expected, and therefore, more studies are necessary.

In summary, we have confirmed using an acute in vivo preparation that potassium has a modulatory role in the biosynthesis of aldosterone induced by angiotensin II and ACTH.

Acknowledgment

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References


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