Effect of changes in sodium balance on potassium/aldosterone dose–response curves in the dog

M. G. NICHOLLS,* M. TREE, J. H. LIVESEY,* R. FRASER, J. J. MORTON AND J. I. S. ROBERTSON
Medical Research Council Blood Pressure Unit, Western Infirmary, Glasgow, Scotland, U.K.

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Summary

1. Potassium was infused intravenously in an incremental fashion and the plasma aldosterone responses were measured in conscious beagle dogs at five different intakes of dietary sodium.

2. Potassium/aldosterone dose–response curves were constructed for each dietary sodium regimen.

3. The rate of increase of plasma potassium during graded potassium infusion became progressively greater with increasing sodium depletion.

4. Regression lines of plasma aldosterone on plasma potassium were progressively elevated and steepened with increasing sodium depletion.

5. The alteration of these dose–response curves could in part have been the result of chronic elevation of plasma potassium and angiotensin II, and depression of plasma sodium, with sodium deprivation.

6. By contrast, acute changes in plasma angiotensin II or sodium concentrations across incremental infusions of potassium did not explain the progressive changes in the potassium/aldosterone dose–response curves.

7. The steepest part of the plasma aldosterone response curve was in the plasma potassium range 4–6 mmol/L.

8. Maximum achieved aldosterone levels were similar to or greater than those attained during angiotensin II infusion in previous studies in beagle dogs.

9. Potassium, like angiotensin II and adrenocorticotropic hormone, becomes a more effective stimulus to aldosterone with sodium depletion, thereby facilitating the preservation of sodium homoeostasis.

Key words: aldosterone, angiotensin II, potassium, sodium.

Introduction

Aldosterone secretion is controlled by angiotensin II, potassium, adrenocorticotropic hormone (ACTH), plasma sodium concentration and perhaps dopamine. It is unlikely that these influences are independent in vivo. For example, the aldosterone response to administered ACTH and to angiotensin II is enhanced by sodium depletion [1–5] and by potassium loading [6, 7]. Potassium deprivation may impair zona glomerulosa responsiveness to various stimuli [8], perhaps by altering adrenal cell receptor number and affinity [9]. There is also evidence of a direct interaction between angiotensin II and ACTH, although the nature of this relationship remains controversial [10–12].

The inter-relations between sodium and potassium status in the control of aldosterone secretion are therefore complex. Data obtained both in vitro and in vivo show that potassium has a specific and potent stimulant action on the adrenal zona glomerulosa. However, previous reports have not clarified whether potassium/aldosterone relationships are altered by changes in cumulative sodium balance. In the present study, we have examined the effect of changes in sodium status on the response of plasma aldosterone to short-term infusions of potassium.
Beagle dogs were used so as to allow construction of more complete potassium/aldosterone dose–response curves than is possible in man.

**Materials and methods**

Eight male pedigree beagle dogs, 2–4 years old and weighing 12–18 kg, were studied. The right carotid artery had been exteriorized in a loop of skin, as previously described [3]. The animals were housed separately in metabolic cages to allow collection of faeces and urine. Diets were prepared as before [3] and were low in sodium (2–5 mmol/day) and potassium (3.7 mmol/day), being supplemented with 'Slow Potassium' (Ciba, 24 mmol/day) and 'Slow Sodium' (Ciba, 10 mmol/tablet) as required (see below). The dogs were fed and weighed once daily. They were maintained for 4 days on a sodium intake of 32–35 mmol/day, then for 4 further days on one of the following dietary regimens: (a) high sodium intake (400 mmol/day) (n = 6); (b) average sodium intake (32–35 mmol/day) (n = 20); (c) dietary sodium depletion (2–5 mmol/day) (n = 4); (d) diuretic-assisted sodium depletion [frusemide, 2 or 5 mg/kg intravenously, before (c)] (n = 6); (e) diuretic-assisted sodium depletion [frusemide, 25 mg/kg intravenously, before (c)] (n = 4).

At the completion of each 4 day period the dogs were prepared for potassium infusion [3]. A fine polythene catheter was inserted into a forelimb vein under local anaesthesia and directed into the jugular vein so that the infusate mixed rapidly with a large volume of blood. The infusate was potassium chloride in 5% glucose solution and was delivered at consecutive rates of 0, 3.4, 6.5, 12.9 and 24.8 mmol/h, each rate being continued for exactly 1 h. All experiments began at 09.00 hours. The total infusion volume in each experiment was 107 ml. Carotid arterial blood samples for estimation of plasma electrolytes, aldosterone and angiotensin II were taken at the end of each hour, centrifuged at 4°C and the plasma was stored at −20°C. Up to 144 ml of blood was withdrawn on a single experimental day. Control studies have shown that this experimental procedure per se does not affect plasma electrolytes, angiotensin II or aldosterone acutely [3].

Plasma aldosterone and angiotensin II were measured by radioimmunoassay [13, 14] and electrolytes by flame photometry.

Analysis of regression was performed by a standard computer programme (Health Sciences Computing Facility, University of California) and slopes of regression lines were compared by the t-test.

**Results**

**Cumulative electrolyte balance**

The effects of altering sodium intake and of diuretic treatment on sodium and potassium balance and on body weight are shown in Table 1. Changes in cumulative sodium balance generally paralleled those in body weight. With increasingly negative sodium balance, cumulative potassium balance became progressively positive.

**Effect of sodium status on basal plasma electrolytes, aldosterone and angiotensin II**

Compared with the dogs having 32–35 mmol of sodium/day intake (group b), dogs in group (e) (25 mg of frusemide, diuretic-assisted sodium depletion) had significantly lower baseline pre-infusion levels of plasma sodium (P < 0.05); baseline plasma sodium was unchanged in the other groups (Table 2). Plasma potassium before infusion was higher as cumulative negative sodium balance increased (Table 2). Likewise, baseline aldosterone and angiotensin II concentrations rose progressively from the high sodium regimen (group a) to the most severely sodium-depleted group (e, Table 2).

**Table 1. Effect of sodium status on electrolyte balance and body weight in dogs (groups a–e) subjected to various sodium regimens**

Changes in electrolyte balance (mmol) and body weight (kg) (mean ± SEM) were measured during 4 days on each dietary regimen. To ensure a stable and uniform baseline, the dogs received the average sodium diet for 4 days before initiation of the dietary regimens (see text).

<table>
<thead>
<tr>
<th>Dietary sodium regimen</th>
<th>Dietary depletion (Group c)</th>
<th>Diuretic-assisted sodium depletion (Group d; 2–5 mg of frusemide/kg)</th>
<th>Diuretic-assisted sodium depletion (Group e; 25 mg of frusemide/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative sodium balance</td>
<td>+160 ± 63</td>
<td>+5 ± 0.5</td>
<td>−41 ± 9</td>
</tr>
<tr>
<td>Cumulative potassium balance</td>
<td>+2 ± 8</td>
<td>+20 ± 9</td>
<td>+32 ± 9</td>
</tr>
<tr>
<td>Change in body weight</td>
<td>+0.2 ± 0.15</td>
<td>−0.4 ± 0.06</td>
<td>−0.45 ± 0.16</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>
TABLE 2. Effect of sodium status on basal plasma electrolytes, angiotensin and aldosterone in dogs (groups a–e) subjected to various sodium regimens

Results are presented as means ± SEM: *P < 0.05, **P < 0.01, †P < 0.001 (comparison with data from average sodium group, unpaired t-test). n is the number of experiments performed in each group.

<table>
<thead>
<tr>
<th>Dietary sodium regimen</th>
<th>High (Group a)</th>
<th>Average (Group b)</th>
<th>Dietary depletion (Group c)</th>
<th>Diuretic-assisted sodium depletion (Group d)</th>
<th>Diuretic-assisted sodium depletion (Group e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma sodium (mmol/l)</td>
<td>145 ± 0.4</td>
<td>147.6 ± 0.8</td>
<td>147.3 ± 2.9</td>
<td>145.5 ± 1.5†</td>
<td>139 ± 1.5*†</td>
</tr>
<tr>
<td>Plasma potassium (mmol/l)</td>
<td>3.38 ± 0.10</td>
<td>3.56 ± 0.04</td>
<td>3.80 ± 0.06</td>
<td>4.23 ± 0.11†</td>
<td>4.43 ± 0.08†</td>
</tr>
<tr>
<td>Plasma aldosterone (pmol/l)</td>
<td>277 ± 55.4</td>
<td>247 ± 24.9</td>
<td>526 ± 41.6**</td>
<td>2374 ± 612†</td>
<td>4956 ± 512†</td>
</tr>
<tr>
<td>Plasma angiotensin II (pmol/l)</td>
<td>25.3 ± 4.5</td>
<td>26.2 ± 2.7</td>
<td>45.8 ± 10.1</td>
<td>88.2 ± 13††</td>
<td>151.5 ± 23.2††</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>20</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

TABLE 3. Effect of potassium infusion on plasma sodium and angiotensin II in dogs (groups a–e) subjected to various sodium regimens

Means (with SEM in parentheses) are shown. Basal values are means of two estimations before infusing potassium; values obtained in samples drawn at the end of each of the incremental rates of potassium infusion (i–iv) are also shown. Comparison with basal means by paired t-test: *P < 0.05.

<table>
<thead>
<tr>
<th>(a) Plasma sodium concn. (mmol/l)</th>
<th>(b) Plasma angiotensin II concn. (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal i ii iii iv</td>
<td>Basal i ii iii iv</td>
</tr>
<tr>
<td>Group (a): high sodium intake</td>
<td>145 (0.4) 145.6 (1-1) 145.2 (1-0) 145.7 (0.7)</td>
</tr>
<tr>
<td>Group (b): average sodium intake</td>
<td>147.6 (0.8) 147.2 (1-1) 147.2 (0-8) 147.3 (0.5)</td>
</tr>
<tr>
<td>Group (c): dietary sodium depletion</td>
<td>147.3 (2.9) 147.8 (3-1) 145.8 (1-9) 148 (2.8)</td>
</tr>
<tr>
<td>Group (d): dietary sodium depletion plus 2 or 5 mg of frusemide/kg</td>
<td>145.5 (1-5) 145.7 (1-5) 145.2 (1-4) 144-3 (1-6)</td>
</tr>
<tr>
<td>Group (e): dietary sodium depletion plus 25 mg of frusemide/kg</td>
<td>139.0 (1-5) 138.3 (1-5) 138.8 (1-4) 139.3 (1-6)</td>
</tr>
</tbody>
</table>

FIG. 1. Regression lines of plasma potassium on infusion rates of potassium for the five dietary regimens together with their respective correlation coefficients; see the Materials and methods section for key to the five groups (a)–(e).

Effects of potassium infusion

Plasma potassium concentration. In Fig. 1, the regression lines of plasma concentrations on infusion rates of potassium are plotted for each of the five groups. The correlation coefficients were highly significant (P < 0.001) for each regimen. The slope of the regression lines increased monotonically from the high sodium to the most sodium-depleted group. The probability that the four regression lines represented the same data population was less than 0.00001.

Plasma sodium concentration. Details of plasma sodium concentrations in each of the five groups of dogs before and at the end of each of the four incremental periods of potassium infusion are shown in Table 3(a). The only significant change occurred in group (d), where there was a slight fall (P < 0.05) in plasma sodium at the highest potassium dose.

Plasma angiotensin II concentration. Details of the changes in plasma angiotensin II concentration in each of the five groups of dogs before, and at the end of each of the four incremental periods of potassium infusion, are shown in Table 3(b). In the four groups (a)–(d) plasma angiotensin II concentrations fell as the dose of potassium was increased, and this fall achieved conventional levels of statistical significance (P < 0.05) at the highest potassium dose in groups (b) and (c). By contrast, in the most sodium-depleted group (c) there was a significant rise (P
FIG. 2. Plasma potassium/plasma aldosterone dose–response curves in individual dogs from three dietary regimens: group (a), high sodium diet ( ), group (c), dietary sodium depletion ( ) and group (e), diuretic-assisted sodium depletion, 25 mg of frusemide/kg ( ).

3, which summarizes all potassium–aldosterone data, regression lines and correlation coefficients, individual potassium/aldosterone dose–response curves on semi-logarithmic plots were markedly steepened and shifted upwards in sodium-depleted dogs (groups c, d and e) compared with those on the high sodium (group a; Fig. 2) or average sodium intake (group b). Correlations between plasma potassium and concurrent aldosterone concentrations were highly significant both within each group ($P < 0.01 - P < 0.001$, Fig. 3) and also when all data were combined ($r = 0.63, P < 0.001, n = 240$). A progressive increase in the slope of individual dose–response curves (Fig. 2) and in potassium–aldosterone regression lines (Fig. 3) was observed from the high sodium (group a) through the average sodium (b) to the sodium-depleted groups (c, d and e). The slopes of the regression lines for groups (c) (dietary sodium depletion) and (e) (frusemide, 25 mg/kg, assisted sodium depletion) were significantly steeper than those of the average sodium uptake (group b) and high sodium (group a) regimens ($P < 0.01$ in each instance).

Alterations in cumulative sodium balance, therefore, resulted in changes in the relationship between plasma potassium and its infusion rate (Fig. 1) and between plasma potassium and aldosterone concentrations (Figs. 2 and 3). These two relationships were further examined by plotting, for each group, the slope of the respective potassium/aldosterone regression lines against the mean of the log plasma potassium (this includes all data, both before and during potassium infusion). The slopes of the regression lines increased as mean log plasma potassium rose. When the squared reciprocals of the standard errors of the slopes were used as weighting factors, the weighted correlation coefficient was 0.916 ($P < 0.05$).

Discussion

There is an abundant literature attesting to the aldosterone-stimulating potential of potassium. From studies in vitro utilizing adrenal tissue of the rat [15–18], dog [19], ox [20] and man [21] and from experiments in sheep [22] and dogs [23], where potassium was infused directly into the blood supply of the isolated adrenal gland, it is apparent that this ion can act directly on the zona glomerulosa to increase aldosterone production. Further confirmation that potassium can stimulate aldosterone in its own right, independently of angiotensin II, ACTH and plasma sodium concentration, is available from the studies of Davis et al. [23], who demonstrated...
that potassium infusion in the hypophysectomized–nephrectomized dog elicited a rise in aldosterone secretion rate without concurrent change in plasma sodium concentration. This latter study made the important additional observation that the stimulating ion was potassium itself, rather than the accompanying anion, since substituting sulphate for chloride did not alter the aldosterone response. Studies in vitro [16, 17] and in vivo [22–26] indicate that even minor increments in the concentration of potassium regularly raise aldosterone. Dluhy et al. [27] consider potassium to be as potent as the renin–angiotensin system in controlling aldosterone secretion in sodium-restricted man.

Comparison of plasma potassium and angiotensin II as a stimulus to aldosterone

Comparison of the present data with those from a similar study on the effects of angiotensin II shows that changes in plasma potassium within the physiological range, in various states of sodium balance, can achieve changes in plasma aldosterone as great as, or greater than, those induced by alterations in plasma angiotensin II, which are also within the physiological range [3].

An unanswered question, of major importance, is whether the aldosterone-stimulating potency of potassium is dependent upon body sodium status. Cannon et al. [28] reported that, in normal man, sodium depletion amplified the aldosterone secretory and excretory response to oral potassium administration. Contrary results were, however, reported by Scholer et al. [29] and by Dluhy et al. [25] in man and by Funder et al. [22] in sheep.

The present work shows, nevertheless, that in pure-bred beagle dogs, potassium/aldosterone dose–response curves are progressively elevated and steepened with progressive sodium depletion. It is unclear why most earlier workers failed to show this effect of sodium balance on potassium/aldosterone relationships. In much of the previous work, very few dose rates of administered potassium were studied. As a result, extensive dose–response curves could not be constructed, thus making interpretation difficult. Differences in the method of inducing sodium depletion could also be important. For example, loss of saliva in sheep [22] may be associated not only with a severe and relatively sudden sodium deficit, but also with profound changes in acid–base balance which may alter zona glomerulosa function [30]. Furthermore, the changes in cumulative potassium balance which accompany changes in sodium balance, will alter aldosterone responsiveness to a number of stimuli [16, 31]. Unfortunately, most earlier studies do not describe changes in potassium balance produced by sodium depletion. In the present experiments we observed progressive potassium retention as sodium loss increased. Finally, the possibility remains that there are species differences in the response of the zona glomerulosa to potassium, although man and the beagle dog exhibit similar changes in aldosterone responsiveness to both angiotensin II [3, 4] and to ACTH [5, 32] with alterations in cumulative sodium balance.

Control studies have previously shown that in beagle dogs infused with 5% glucose solution alone, and subjected to periodic blood sampling over 5 h, as in the present experiments, there are no significant changes in plasma sodium, potas-
sium, angiotensin II or aldosterone [3], neither in usual sodium status nor after sodium depletion. This applies both to animals with average sodium uptake and to those subjected to sodium depletion.

Moreover, systematic changes in plasma angiotensin II or sodium concentration during the incremental infusion of potassium cannot readily be invoked to explain the progressive steepening of the plasma potassium/aldosterone dose-response curves with advancing sodium depletion. In groups (a), (b), (c) and (d), mean plasma angiotensin II concentration fell slightly with increasing doses of administered potassium (Table 3). This might indicate suppression of renin secretion by potassium loading [39]. By contrast, however, in the most severely sodium-depleted group (e), plasma angiotensin II concentration had risen significantly at the highest potassium dose and this rise in plasma angiotensin II could have reinforced the effect of potassium in elevating plasma aldosterone.

Similarly, the only significant change in plasma sodium concentration was a slight fall in group (d) (Table 3). The magnitude of this was, however, such as to have only a marginal effect on aldosterone secretion [23].

The mechanism of the enhanced response of aldosterone to potassium is not established. Sodium depletion is associated with a fall in plasma sodium and a rise in potassium and sodium concentration was a slight fall in group e, with plasma sodium and potassium dosage having risen significantly at the highest potassium dose and this rise in plasma angiotensin II could have reinforced the effect of potassium in elevating plasma aldosterone.

Characteristics of potassium/aldosterone dose-response curves have been defined in several studies in vitro [15, 17, 18, 20, 35]. In general, the steepest portion of the dose-response curve is in the range 4-5-7 mmol/l for changes in ambient potassium concentration. The maximum aldosterone response is usually reached around 8-9 mmol/l, with a fall in aldosterone production at higher potassium levels. Fewer studies in vivo have been reported, and construction of complete dose-response curves cannot safely be attempted in man. Partial dose-response curves from chronic potassium infusions in dogs were reported by McCaa et al. [36] and showed increasing steepness of the aldosterone response up to plasma potassium levels of approximately 5.5 mmol/l. The present study in vivo shows the steepest portion of the response curves of plasma aldosterone to be in the plasma potassium range 4-6 mmol/l, with the suggestion of a stable plateau above 6.5 mmol/l. We found no evidence of a fall in plasma aldosterone at the higher plasma potassium concentrations, although from experiments in vitro potassium values greater than 8 mmol/l may be required before this occurs. However, in some animals in the most severely sodium-depleted group, there was evidence that a maximum aldosterone response had been achieved, indicating that the top of a sigmoid dose-response relationship might have been reached.

The present experiments have considered plasma aldosterone concentration, which may be influenced by changes in both aldosterone secretion and metabolic clearance. Most [2, 3, 37] but not all [38] studies suggest that the former is the dominant factor in sodium depletion. Our present studies were not designed to, and do not, illuminate this aspect further.

The mechanism by which potassium stimulates aldosterone secretion remains to be clarified. The current state of research has recently been reviewed [39]. In summary, neither the renin-angiotensin system nor stimulation of adenyl cyclase is involved. Despite earlier suggestions to the contrary, intracellular potassium in the zona glomerulosa cells is not markedly altered during changes of secretion. However, potassium may have its effect by altering cellular calcium metabolism.

The main aim of the present study was to examine the interaction of sodium and potassium status in the control of plasma aldosterone over as wide a dose range of potassium as possible. Our results leave no doubt that sodium depletion enhances the acute response of aldosterone to potassium in the conscious dog. In this respect, therefore, potassium resembles both angiotensin II [2-4] and ACTH [1, 5, 32]. Thus the three major stimuli of aldosterone become progressively more effective with advancing sodium depletion, facilitating the maintenance of sodium balance and preserving homeostasis.

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References


