Potassium depletion induced by vasopressin and overhydration in the rabbit

M. A. BARRACL OUGH
Department of Medicine, St Thomas's Hospital, London

(Received 10 June 1975)

Summary

1. In order to study the effect of overhydration on body potassium, experiments were performed on pair-fed rabbits, one of which was maintained continuously on vasopressin and given extra water (60–90 ml day$^{-1}$ kg$^{-1}$) for 6–8 days, while the other served as control.

2. Overhydrated rabbits excreted significantly more potassium ($53\%$) in their urine than control rabbits and accumulated a mean potassium deficit of 65.0 mmol, significantly higher than the mean value of 37.1 mmol in the control rabbits.

3. In the overhydrated rabbits, potassium fell significantly in both erythrocytes, from 266 to 173 mmol/kg of dry cells, and also in muscle, from 435 to 341 mmol/kg of fat-free dry solids. Neither changed significantly in the control animals.

4. Overhydration in the presence of vasopressin leads to potassium depletion in the rabbit and a similar phenomenon might be expected in man. Potassium depletion due to overhydration might account for the hypokalaemia and reduction in exchangeable potassium observed in some patients with the syndrome of inappropriate secretion of antidiuretic hormone.

Key words: overhydration, potassium depletion, vasopressin.

Introduction

Although potassium depletion is not a recognized feature of inappropriate secretion of antidiuretic hormone in man (SIADH) (Bartter & Schwartz, 1967), a reduced total exchangeable potassium has been observed in some patients with this syndrome (Sousa & Jenny, 1964; Jones, Barraclough, Forsling & Petch, 1968; Barraclough, 1971). Whether this reflects a reduction in cellular potassium content or merely a reduction in cellular mass associated with the anorexia and weight loss, which often accompanies the syndrome, is uncertain. From experiments on man the effect on body potassium of overhydration induced by vasopressin is also far from clear. When the overhydration has been of short duration, renal potassium excretion has been unaffected or even slightly reduced (Leaf, Bartter, Santos & Wrong, 1953; Liddle, Bartter, Duncan, Barber & Delea, 1955; Wrong, 1956), whereas in a more prolonged study, when vasopression was given continuously over 6–14 days, there was a small negative potassium balance of 2–10 mmol/day in ten out of thirteen individuals (Stormont & Waterhouse, 1961).

Attempts to explore this problem further in man are restricted by the intolerable constitutional upset resulting from severe and prolonged overhydration. We have therefore studied it in an animal model. In order to separate the effect on body potassium of overhydration alone from that of the anorexia and starvation which ensues, experiments were performed on pair-fed rabbits, one of which was overhydrated whilst the other served as a control.

Methods

Experiments were performed on New Zealand White rabbits weighing between 2.5 and 3.8 kg.
Rabbits of the same sex and closely similar weight were paired. Two weeks before the start of the experimental period of overhydration or control pair-feeding, control plasma erythrocyte and muscle specimens were taken. Overhydration in one of a pair of rabbits was induced by giving distilled water, 60–90 ml day\(^{-1}\) kg\(^{-1}\), by stomach tube in divided morning and evening doses, along with 0.25 unit of vasopressin tannate in arachis oil (Parke Davis and Co. Ltd). The pair-fed control rabbits were intubated, but were not given a water load and were given injections of arachis oil alone. The experimental period of overhydration or pair-feeding lasted from 6 to 8 days with different pairs. Throughout this time the animals were kept in metabolism cages and fed from a single batch of standard rabbit chow containing 42 mmol/kg of sodium and 251 mmol/kg of potassium. The food intake of the overhydrated rabbits was estimated daily and their paired control was given the same intake 1 day later. In three pairs of rabbits sodium and potassium balance were estimated daily, but in the others these balances were studied over the whole of the experimental period. All animals had free access to water.

Plasma samples were analysed intermittently during and together with erythrocyte and muscle specimens at the end of the experimental period. Muscle biopsy was performed under light Nembutal anaesthesia (20 mg/kg), the whole of one of the foreleg muscles being removed between its origin and insertion. The identical muscle on the opposite foreleg was used on the second occasion. The muscle was dissected free of tendon and fascia, minced with fine scissors, divided into aliquots of about 200

---

**FIG. 1.** Mean changes in the plasma concentration and balance of sodium and potassium during the experimental period in three pairs of rabbits. The intakes of sodium and potassium have been plotted downwards from the zero line and their output in faeces and urine is then plotted upwards from the intake line. Negative balance is thus shown above the zero line. Cross-hatched areas denote faecal losses.
mg and the sodium and potassium content in the fat-
free dry solids were then estimated (Litchfield &
Gaddie, 1958). All muscle analyses were made in
duplicate, which agreed to within 1·7 ± 0·44%.

Erythrocyte sodium and potassium were measured
after centrifugation of 2 ml of heparinized blood at
500 g for 5 min. Trapped plasma was estimated by
using 131I-labelled human serum albumin as a
marker. Water content was obtained by drying to
constant weight at 100°C, the sodium and potassium
content of the dried specimen then being estimated
after extraction in 0·1 mol/l nitric acid for 48 h.
Sodium and potassium were estimated by flame
photometry and osmolality was determined with an
Aminco osmometer.

The significance of differences was determined by
Student's t-test (Snedecor, 1937). Mean values are
given ± SEM, significant implying P < 0·05.

Results

Overhydration and body weight

Rabbits that were overhydrated went off their
food and usually stopped eating altogether after 3 or
4 days. They became slow and apathetic and moved
little even when disturbed. By the sixth day of
overhydration they were weak and capable of very
little movement. Brief episodes of tetanic convul-
sions were occasionally seen after the fourth day. In
contrast pair-fed control animals remained alert and
active.

Urinary osmolality was maximal in the overhydr-
ated animal at the start of vasopressin administra-
tion, ranging from 1001 to 1483 mosmol, and de-
clined as overhydration progressed. At the end of
the overhydration period it ranged from 177 to 238
mosmol.

Body weight rose during the first 2 or 3 days of
overhydration but then usually fell; at the end of the
experimental period body weight was on average
0·19 kg ± 0·12 less than during the control period. In
the control animals body weight fell continuously and
was reduced on average by 0·36 kg ± 0·5 by the end of
the experimental period.

The mean changes in sodium and potassium
balance, in the three pairs of rabbits in which this
was determined daily, are shown in Fig. 1. Observa-
tions during the control period and at the end of the
experimental period on all pairs of animals are
shown in Table 1. Erythrocyte and muscle electro-
lytes have been expressed as mmol/kg of dry tissue
and as mmol/kg of fat-free dry solids respectively,
thereby minimizing the effect of extracellular fluid
contamination on either weight or potassium con-
tent.

Potassium

There was no consistent change in plasma potas-
sium concentration during overhydration, for by the
end of the experimental period it had fallen only
from 3·9 to 3·0 mmol/l in the overhydrated animals
and from 4·1 to 3·3 mmol/l in the control animals.
There was no significant difference in the fall between
paired control and overhydrated animals.

Urinary potassium excretion was consistently
greater in the overhydrated animal of each pair and
was maintained at a high level throughout the period
of overhydration despite progressive potassium
depletion. Overhydrated rabbits excreted 53·4% more
potassium than did control rabbits (P < 0·005).

Mean faecal loss of potassium during the experi-
mental period was 4·3 ± 1·1 mmol in the control and
6·48 ± 1·1 mmol in the overhydrated animals, a
difference which was not significant. Mean negative
potassium balance during the experimental period
was greater by 27·93 ± 5·04 mmol in the overhydrated
animals (P < 0·001).

During the experimental period erythrocyte
potassium content did not change significantly in
control animals, but fell from 266 to 173 mmol/kg of
dry cells in the overhydrated animals (P < 0·001).
Muscle potassium did not change in the control
animals but fell from 435 to 341 mmol/kg of fat-free
dry solids in the overhydrated animals, a fall which
was highly significant between paired animals (P <
0·001).

Sodium

During the experimental period plasma sodium
concentration did not change significantly in the
control animals, but fell considerably in the over-
hydrated animals from a mean control value of 138
to 88 mmol/l by the end of the overhydration period
(P < 0·001). The mean difference in the fall of plasma
sodium concentration between paired control and
overhydrated animals was 47·3 ± 4·3 mmol/l (P <
0·001).

Erythrocyte and muscle sodium content did not
change significantly between the control and the end
<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Plasma concentration (mmol/l)</th>
<th>Erythrocyte potassium (mmol/kg of dry cells)</th>
<th>Muscle (mmol/kg FFDS)</th>
<th>Muscle water (%) dry wt.</th>
<th>Urinary excretion experimental period (mmol)</th>
<th>Negative balance experimental period (mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodium</td>
<td>Potassium</td>
<td>Sodium</td>
<td>Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>134</td>
<td>139</td>
<td>3.9</td>
<td>3.1</td>
<td>288</td>
<td>225</td>
</tr>
<tr>
<td>A2</td>
<td>139</td>
<td>90</td>
<td>3.8</td>
<td>4.5</td>
<td>277</td>
<td>180</td>
</tr>
<tr>
<td>B1</td>
<td>133</td>
<td>131</td>
<td>5.0</td>
<td>2.8</td>
<td>328</td>
<td>304</td>
</tr>
<tr>
<td>B2</td>
<td>136</td>
<td>94</td>
<td>3.5</td>
<td>2.9</td>
<td>265</td>
<td>175</td>
</tr>
<tr>
<td>C1</td>
<td>133</td>
<td>116</td>
<td>4.5</td>
<td>3.3</td>
<td>316</td>
<td>299</td>
</tr>
<tr>
<td>C2</td>
<td>132</td>
<td>91</td>
<td>5.1</td>
<td>2.8</td>
<td>269</td>
<td>173</td>
</tr>
<tr>
<td>D1</td>
<td>140</td>
<td>137</td>
<td>4.5</td>
<td>3.0</td>
<td>271</td>
<td>236</td>
</tr>
<tr>
<td>D2</td>
<td>140</td>
<td>81</td>
<td>4.2</td>
<td>2.3</td>
<td>243</td>
<td>215</td>
</tr>
<tr>
<td>E1</td>
<td>132</td>
<td>136</td>
<td>3.1</td>
<td>4.6</td>
<td>304</td>
<td>296</td>
</tr>
<tr>
<td>E2</td>
<td>142</td>
<td>76</td>
<td>4.0</td>
<td>4.8</td>
<td>274</td>
<td>125</td>
</tr>
<tr>
<td>F1</td>
<td>133</td>
<td>131</td>
<td>4.2</td>
<td>3.5</td>
<td>278</td>
<td>298</td>
</tr>
<tr>
<td>F2</td>
<td>138</td>
<td>91</td>
<td>3.3</td>
<td>2.5</td>
<td>283</td>
<td>168</td>
</tr>
<tr>
<td>G1</td>
<td>137</td>
<td>138</td>
<td>4.2</td>
<td>3.0</td>
<td>358</td>
<td>246</td>
</tr>
<tr>
<td>G2</td>
<td>140</td>
<td>92</td>
<td>3.8</td>
<td>2.0</td>
<td>258</td>
<td>175</td>
</tr>
<tr>
<td>H1</td>
<td>140</td>
<td>123</td>
<td>3.9</td>
<td>2.9</td>
<td>260</td>
<td>187</td>
</tr>
<tr>
<td>H2</td>
<td>139</td>
<td>82</td>
<td>3.6</td>
<td>2.1</td>
<td>260</td>
<td>175</td>
</tr>
<tr>
<td>Mean values</td>
<td>Control rabbits</td>
<td>Overhydrated rabbits</td>
<td>Mean difference (C - E) between paired control and overhydrated rabbits</td>
<td>±SEM</td>
<td>Significance (P)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
<td>Potassium</td>
<td>Control rabbits</td>
<td>Overhydrated rabbits</td>
<td>Mean difference (C - E) between paired control and overhydrated rabbits</td>
<td>±SEM</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>131</td>
<td>4.1</td>
<td>3.3</td>
<td>292</td>
<td>264</td>
</tr>
<tr>
<td>Mean difference (C - E) between paired control and overhydrated rabbits</td>
<td>138</td>
<td>88</td>
<td>3.9</td>
<td>3.0</td>
<td>266</td>
<td>173</td>
</tr>
<tr>
<td>±SEM</td>
<td>±0.04</td>
<td>±0.56</td>
<td>±17.1</td>
<td>±14.0</td>
<td>±15.8</td>
<td>±0.58</td>
</tr>
<tr>
<td>Significance (P)</td>
<td>&lt;0.001</td>
<td>N.S.</td>
<td>&lt;0.005</td>
<td>N.S.</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
of the experimental period in either control or overhydrated animals, nor was there any significant difference in the change of erythrocyte and muscle sodium between paired animals.

Urinary sodium excretion was significantly greater in the overhydrated animals, exceeding that of the paired controls by 22.3 \pm 4.32 mmol over the experimental period (P < 0.001). Sodium excretion in the overhydrated animals tended to be maximal at the beginning of the experimental period and then tended to decline (Fig. 1).

Mean faecal loss of sodium during the experimental period was 1.84 \pm 0.36 mmol in the control and 3.4 \pm 0.79 mmol in the overhydrated animals, a difference which was not significant.

Mean negative sodium balance during the experimental period was greater by 23.76 \pm 3.9 mmol in the overhydrated animals (P < 0.001).

Discussion

Overhydration induced by water loading in the presence of vasopressin has been shown to deplete the rabbit of potassium as well as sodium. Depletion of sodium under these circumstances is well documented in man (Leaf et al., 1953; Stormont & Waterhouse, 1961), and results from increased renal sodium excretion due to greater glomerular filtration (Wrong, 1956), as well as inhibition of sodium reabsorption in both the proximal (Jones, Barraclough & Mills, 1963) and distal tubules (Bartter, Liddle, Duncan, Barber & Delea, 1956), the latter associated with a reduction in aldosterone secretion. The salient finding of the present study is the simultaneous marked depletion of potassium.

A number of factors may be involved in the negative potassium balance which results from overhydration. Overhydration causes anorexia and a reduction in food and potassium intake. Excess of potassium loss under these circumstances may in part reflect a transient imbalance before renal conservation adapts to the reduced potassium intake. The deficit from this might be large in an animal such as the rabbit, which normally eats an herbivorous diet with a high potassium content, but would be equal in the pair-fed and overhydrated rabbits as they had the same food intake. In addition there may be some loss of potassium due to a reduction in cellular mass if food intake declined to starvation levels. Any such loss, however, would tend to be small during the brief period of the experiment and since it would be associated with a loss of cell solids, it would not influence dry-weight muscle potassium content (Muntwyler & Griffin, 1955). The latter did not change in the control animals over the experimental period. Similarly the fall in dry-weight muscle potassium content in the overhydrated rabbits cannot be due to greater negative nitrogen balance. The much more marked potassium loss seen in the overhydrated animals and the attendant reduction in erythrocyte and muscle potassium content is thus clearly an effect of overhydration in the presence of vasopressin. Here it is the overhydration rather than the vasopressin which is the cause, since there is no significant effect on potassium balance in the rabbit if vasopressin is given and water balance is maintained constant at the same time (M. A. Barraclough, unpublished work).

Many factors influencing renal potassium excretion are altered with overhydration. On the one hand, reduction in the aldosterone secretion rate resulting from expansion of extracellular fluid volume will tend to reduce potassium loss, so explaining the failure to demonstrate any increase in excretion in acute overhydration (Leaf et al., 1953; Liddle et al., 1955; Wrong, 1956). On the other hand, a number of factors will tend to increase potassium excretion. An increase in glomerular filtration rate and reduction in sodium reabsorption by the proximal tubule will increase sodium and fluid delivery and thus the transtubular potential difference and flow rate in the distal tubule, both of which will favour potassium secretion at this site (Malnic, Klose & Giebisch, 1966; Giebisch, 1969). These factors alone, however, may not account fully for the renal potassium wasting, nor may the latter account fully for the disturbance in body potassium seen with overhydration. It may be significant that, in the present study, there was no consistent fall in plasma potassium concentration in the overhydrated animals nor was the mean fall in their plasma level during the experimental period greater than in the control animals. Such a discrepancy might be explained by a failure of cellular binding of potassium in the overhydrated state and be part of a general disturbance in the distribution of sodium and potassium across the cell membrane as a result of the fall in tonicity. Renal tubular cells (Little & Robinson, 1967) and leucocytes (Patrick & Hilton, 1973) gain sodium and lose potassium when they are incubated in hypotonic media. The lack of change in muscle sodium content in the present study would fit in with this. Since
extracellular sodium accounts for over 70% of the sodium in whole muscle (Litchfield & Gaddie, 1958; Flear, Crampton & Mathews, 1960), and even if one assumes that all of the small increase in muscle water during overhydration was in the extracellular phase, a fall in mean plasma sodium concentration of the magnitude seen in the overhydrated animals should have been reflected in a considerable reduction in whole muscle sodium content. That this was not the case suggests that there was a simultaneous increase in intracellular sodium at the time that its extracellular concentration was declining. Evidence for such an intracellular shift of sodium during overhydration can be deduced from the failure of changes in sodium and water balance to account fully for the severity of the hyponatraemia in normal subjects given vasopressin (Stormont & Waterhouse, 1961) and in subjects with the inappropriate secretion of antidiuretic hormone syndrome (SIADH) (Kaye, 1966; Nolph & Schrier, 1970). An increase in muscle sodium which paralleled the severity of overhydration was also observed by Kaye (1966) in a patient with SIADH.

The effect of overhydration on body potassium therefore is complex and probably consists both of increased renal excretion of the ion and of an inability of the cell to retain potassium normally, so that the plasma concentration is less depressed than would otherwise be the case. It is also possible that whatever the precise mechanism by which hypotonicity impairs the ability of the cell to retain potassium, it will affect the renal tubular cell in a similar way and this might be an additional mechanism acting to increase potassium excretion.

Is the potassium depletion caused by overhydration in the rabbit of relevance to the potassium status of patients with SIADH? In this study, the increase in renal potassium excretion with overhydration was consistently greater than that observed by Stormont & Waterhouse (1961) in man and probably reflected the greater fall in body tonicity. In their study the potassium loss was most marked when overhydration was most severe. Lowance, Garfinkel, Mattern & Schwartz (1972), who studied hypotonic volume expansion in the dog, also observed increased potassium excretion when plasma osmolality had fallen to 240 mosmol or below. Osmolalities well below this are seen in some patients with SIADH. In addition to the fall in tonicity, the degree of potassium depletion will also depend on the duration of the overhydration period. Whereas the loss of sodium is maximal at the beginning, the loss of potassium is much more sustained, and in the present study was considerable even on the sixth day of overhydration, despite the already considerable potassium deficit. Taken in conjunction, these various studies strongly suggest that potassium depletion is likely to be present in patients with SIADH with marked or prolonged overhydration. This might contribute to the apathy, muscular weakness and hypotonia often seen in such patients.

Acknowledgment

I thank Mr Firoz Nilam for his invaluable assistance with this study.

References

LOWANCE, D.C., GARFINKEL, H.B., MATTERN, W.D. &


