Altered erythrocyte cation permeability in familial pseudohyperkalaemia

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SUMMARY

1. Erythrocyte cation transport pathways have been investigated in a family with pseudohyperkalaemia.

2. Ouabain- and bumetanide-resistant Na⁺ and K⁺ effluxes in three pseudohyperkalaemic patients were not different from those of control subjects when assessed at 37°C.

3. When the temperature was decreased to 20°C and 9°C, K⁺ passive permeability markedly increased and Na⁺ permeability remained unchanged in these patients. In contrast, in control subjects a reduction in temperature caused a marked reduction in Na⁺ and K⁺ passive permeability.

4. These findings could account for the marked increase in plasma K⁺ concentration observed at subphysiological temperatures.

5. The Na⁺-K⁺ co-transport pathway was reduced in all members of the family, but the Na⁺-K⁺ pump was reduced in only two of them. These alterations were independent from the pseudohyperkalaemic state.

Key words: cation permeability, erythrocyte, pseudohyperkalaemia.

INTRODUCTION

Familial pseudohyperkalaemia was first reported by Stewart et al. [1]. In this condition spuriously high plasma K⁺ concentrations are found in normokalaemic subjects. This is characterized by a several-fold increase in K⁺ concentrations when blood samples are left at room temperature for several hours. Since then a few cases of this syndrome have been diagnosed [2–7] and an impairment in erythrocyte K⁺ permeability has been reported to be responsible for the abnormal K⁺ concentrations [4–7]. In this study we report an additional family in which three out of five members showed a pseudohyperkalaemic syndrome, in which different cation transport processes have been explored. A marked increase in K⁺ and Na⁺ passive permeability was observed at subphysiological temperatures in these subjects.

METHODS

Patient

The proband, a 65-year-old woman, complained of frequent cramps, tremor of the fingers and weakness, with one episode of paralysis. Routine clinical examination was normal. Repeated blood pressure measurement revealed labile hypertension, with values exceeding 160 mmHg (21.3 kPa) (systolic) and 90 mmHg (12 kPa) (diastolic) [8]. Routine biological tests revealed a high plasma K⁺ concentration (8 mmol/l). Subsequent clinical and biological examination failed to reveal any known cause of hyperkalaemia. Plasma renin, aldosterone and creatinine concentrations were normal. Blood gases and urinary aldosterone concentrations were normal. After an oral load there was a non-significant rise in blood glucose. Routine haematological investigations, including glucose-6-phosphate dehydrogenase, pyruvate kinase and transketolase activities, did not reveal any specific abnormality. Haemoglobin, bilirubin and haptoglobin concentrations as well as erythrocyte volume were normal. The blood film, reticulocyte count, differential white cell count and platelet count were normal. There was no significant haemolysis in vitro and no abnormality of erythrocyte morphology. Erythrocyte membrane phospholipid and cholesterol analyses [9] were normal. Reassessment of kalaemia with measurement of cation concentrations immediately after blood sampling revealed a normal K⁺ plasma concentration which markedly increased with time when the blood sample was left standing at a temperature of 21°C or 9°C but not at 37°C. This strongly suggested a pseudohyperkalaemic syndrome as previ-
ously described by Stewart et al. [1]. Investigation of plasma K⁺ concentrations in the members of the family revealed a similar syndrome in two of the three children (subjects C and D) but not in the husband. This prompted us to assess the different cation transport pathways in the erythrocytes of these subjects. Routine clinical examination of the proband’s family was normal except that the father had essential hypertension and received β-adrenergic blockers as antihypertensive therapy.

**Analyses**

Na⁺ and K⁺ transport pathways were studied using a technique previously described [10]. Samples of freshly drawn venous blood, collected in heparinized tubes, were centrifuged at 1750 g for 10 min, and the plasma and buffy coat were removed. The erythrocytes were washed twice with 10 vol. of buffered 150 mmol/l NaCl and re-centrifuged for 3 min at 1750 g. All steps were carried out at 4°C. The erythrocytes were then immediately processed for flux measurements. Na⁺ and K⁺ ground membrane permeability was assessed in fresh cells. The maximal velocity of Na⁺-K⁺ co-transport and of the Na⁺-K⁺ pump was measured in Na⁺-loaded, K⁺-depleted cells. Na⁺ loading was achieved using nystatin [10]. The cells were then washed five times with ice-cold magnesium-sucrose medium containing 75 mmol/l MgCl₂, 85 mmol/l sucrose and 10 mmol/l 4-morpholinepropanesulphonic acid–Tris (pH 7.4, 37°C). The cells were added to three tubes containing magnesium-sucrose medium with (a) 2 mmol/l KCl (K⁺ medium), (b) 0.1 mmol/l ouabain (ouabain medium) and (c) 0.1 mmol/l ouabain plus 0.02 mmol/l bumetanide (bumetanide medium). The resulting suspensions were incubated at 37°C and duplicate samples were removed at 10 min and 30 min for the K⁺ medium and at 1 h and 2 h for both the ouabain and the bumetanide media. The supernatant Na⁺ and K⁺ concentrations were measured by flame photometry. In order to study the temperature dependency of the ouabain- and bumetanide-sensitive effluxes, fresh cells were added to bumetanide medium and incubated at 9°C, 21°C and 37°C. Triplicate samples were removed at 45, 90 and 120 min and centrifuged. The supernatant was carefully removed, avoiding pellet contamination, and its Na⁺ and K⁺ concentrations were measured by flame photometry. Data relating extracellular Na⁺ and K⁺ concentrations as a function of time were subjected to linear regression analysis, and the slope and its SD were calculated. The ouabain-sensitive Na⁺ efflux was obtained by subtracting the efflux value in ouabain medium from that in K⁺ medium. The bumetanide-sensitive Na⁺ and K⁺ effluxes were obtained by subtracting the efflux values in bumetanide medium from those in ouabain medium.

Ouabain blocks the exchange of internal Na⁺ for external K⁺ catalysed by the Na⁺-K⁺ pump. Bumetanide blocks the Na⁺-K⁺ efflux mediated by Na⁺-K⁺ co-transport. The ouabain-and bumetanide-resistant Na⁺ and K⁺ effluxes represent the ground membrane 'leak' for monovalent cations.

**RESULTS**

When freshly drawn blood samples were incubated for 4 h at 37°C, 21°C and 9°C, a marked increase in plasma K⁺ concentration was observed at subphysiological temperatures in the pseudohyperkalaemic subjects, while kalaemia was maintained constant in the other members of the family. The study of ouabain- and bumetanide-resistant Na⁺ and K⁺ effluxes from fresh cells incubated at 37°C did not reveal any significant differences between pseudohyperkalaemic and control subjects. However, when the incubation temperature was lowered to 21°C and 9°C, a marked increase in the ouabain- and bumetanide-resistant Na⁺ and K⁺ effluxes was observed. (See Fig. 1).

**Fig. 1.** Ouabain- and bumetanide-resistant Na⁺ (a) and K⁺ (b) effluxes at different temperatures. Erythrocytes were suspended in magnesium-sucrose medium in the presence of ouabain (0.1 mmol/l) and bumetanide (0.02 mmol/l) and incubated at different temperatures (37°, 21° and 9°C). Fluxes were calculated as described in the Methods section. Filled symbols represent effluxes in two pseudohyperkalaemic patients (●, ▲) and open symbols, two control subjects (○, △).

Statistical significance was determined by Student's *t*-test (for further details see [10–12]).
All fluxes were assessed in Na⁺-loaded, K⁺-depleted cells by using the nystatin technique \[lo\]. The co-transport efflux represents the ouabain-resistant, bumetamide-sensitive Na⁺ and K⁺ fluxes. The Na⁺-K⁺ pump represents the ouabain-sensitive Na⁺ efflux. The passive permeability constant represents the ouabain- and bumetanide-resistant fluxes. Data represent the flux value ± SD obtained by regression analysis in one flux determination (see the Methods section). Data obtained in the family members were compared with those of control subjects by using Student’s t-test. \*P < 0.005.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Cellular content (mmol/l)</th>
<th>Co-transport efflux (mmol h⁻¹ litre⁻¹ of cells)</th>
<th>Na⁺-K⁺ pump (Na⁺ efflux) (mmol h⁻¹ litre⁻¹ of cells)</th>
<th>Passive permeability constant (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Mother)</td>
<td>65</td>
<td>39 74</td>
<td>0.103 ± 0.020*</td>
<td>0.123 ± 0.035*</td>
<td>4.560 ± 0.088*</td>
</tr>
<tr>
<td>B (Father)</td>
<td>62</td>
<td>35 75</td>
<td>0.258 ± 0.035*</td>
<td>0.207 ± 0.028*</td>
<td>6.920 ± 0.080</td>
</tr>
<tr>
<td>C (Son)</td>
<td>29</td>
<td>43 70</td>
<td>0.192 ± 0.057*</td>
<td>0.295 ± 0.020*</td>
<td>4.440 ± 0.094*</td>
</tr>
<tr>
<td>D (Daughter)</td>
<td>38</td>
<td>42 70</td>
<td>0.142 ± 0.024*</td>
<td>0.146 ± 0.055*</td>
<td>4.560 ± 0.201</td>
</tr>
<tr>
<td>E (Daughter)</td>
<td>33</td>
<td>44 66</td>
<td>0.196 ± 0.009*</td>
<td>0.107 ± 0.044*</td>
<td>5.910 ± 0.120</td>
</tr>
<tr>
<td>Control subjects (from [10])</td>
<td>25 ± 4</td>
<td>38 ± 11</td>
<td>0.438 ± 0.117</td>
<td>0.436 ± 0.088</td>
<td>6.880 ± 2.000</td>
</tr>
</tbody>
</table>

DISCUSSION

The rates of various transport pathways fall as the temperature is reduced, and cooling is often used to terminate biological reactions. The temperature sensitivity of erythrocyte cation passive permeability has been previously described by Stewart et al. [13], who reported a paradoxical temperature response of Na⁺ and K⁺ permeability below 12°C.

In the pseudohyperkalaemic subjects investigated in this study, reducing the temperature did not affect the passive permeability rate constant for Na⁺, while the permeability for K⁺ markedly increased. This could not be related to haemolysis in vitro and is consistent with the hyperkalaemia observed when blood samples were left standing at subphysiological temperatures.

Previous workers have attempted to define the mechanism responsible for the cellular K⁺ loss observed in pseudohyperkalaemia. In all cases the increase in plasma K⁺ concentrations was attributed to an abnormal erythrocytic permeability for K⁺. Thus, an abnormal temperature sensitivity of passive permeabilities was reported in three studies [5–7], one of which was associated with hereditary xerocytosis [5]. In another case impaired K⁺ leak was reported at physiological temperature [4]. Although all these studies suggest an abnormality in the same cation transport pathway, this impairment does not share the same characteristics in all pseudohyperkalaemic subjects, suggesting heterogeneity in the mechanism underlying this defect.

Other transport systems investigated were not found to be implicated in the massive K⁺ loss. A reduced maximal activity of the Na⁺-K⁺ pump was observed in two of three pseudohyperkalaemic patients in this study, while in previous studies no alteration of this pathway was reported [4–7]. On the other hand, the bumetanide-sensitive Na⁺-K⁺ co-transport system was found to be reduced in all members of the family. This could be in relation to the hypertension diagnosed in both parents. In fact, a defect in this pathway was reported to be present in patients with essential hypertension and in some members of their families [14]. This pathway was not found to be altered in three studies of pseudohyperkalaemia [4–6], while in one other study a defect in the temperature sensitivity of this pathway was reported [7].

In conclusion, the present study in a family with pseudohyperkalaemia identifies an impairment of erythrocytic Na⁺ and K⁺ passive permeability at sub-physiological temperatures. It stresses the importance of the investigation of cation fluxes mediated by different pathways in the diagnosis of this rare syndrome.

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REFERENCES


