Hypokalaemia stimulates prostacyclin synthesis in the rat

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Summary

1. To examine the hypothesis that the normalcy of blood pressure, despite an increase in circulating angiotensin II, and the blood pressor hyporesponsiveness to infusion of pressor agents which are associated with hypokalaemia, are due to overproduction of prostacyclin, the principal prostaglandin (PG) synthesized by the vascular endothelium, we studied the effect of experimental hypokalaemia on the urinary excretion of immunoreactive 6-keto-prostaglandin F$_{1\alpha}$, a stable metabolite of prostacyclin, in the rat.

2. The animals were fed on a potassium-deficient diet for 9 days. Twenty-four hour urine samples were collected daily for measurement of urinary excretion of immunoreactive 6-keto-PGF$_{1\alpha}$, PGE$_2$ and 13,14-dihydro-15-keto-PGF$_{2\omega}$ (PGFM).

3. Hypokalaemia caused significant increases of the three prostaglandins measured.

4. We conclude that hypokalaemia is a potent stimulus of both renal and vascular prostaglandins. The results suggest that an increase in prostacyclin synthesis in peripheral blood vessel walls may be responsible for the resistance of blood pressure to infusion of pressor substances as well as for the normalcy of blood pressure, despite the presence of high circulating angiotensin II concentrations, in conditions associated with hypokalaemia.

Key words: 13,14-dihydro-15-keto-PGF$_{2\omega}$, hypokalaemia, 6-keto-PGF$_{1\alpha}$, PGE$_2$, prostacyclin, prostaglandins.

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Abbreviations: PG, prostaglandin; PGFM, 13,14-dihydro-15-keto PGF$_{2\omega}$.

Introduction

It is well recognized that hypokalaemia impairs maximal urinary osmolality and produces polyuria in animals and man. This urinary concentrating defect is probably mediated by an increase in renal prostaglandin (PG) E$_2$ synthesis since it has been demonstrated in several experimental systems that potassium plays a role in the control of prostaglandin synthesis. Potassium depletion in dogs and patients with Bartter’s syndrome has been shown to be associated with a high level of urinary PGE$_2$ [1,2]. Furthermore, prostaglandin synthesis by rabbit renomedullary interstitial cells in tissue culture is inversely related to the potassium concentration in the incubation medium [3]. The mechanism of action of the effect of renal prostaglandins on water metabolism is probably by an antagonism of the hydro-osmotic effect of antidiuretic hormone. The hyper-renin-aemia and the resistance of the blood pressure to infusion of pressor agents in hypokalaemia is also dependent on an overproduction of prostaglandins, since both can be corrected by treatment with inhibitors of prostaglandin synthesis [2,4].

An overproduction of PGE$_2$ alone cannot satisfactorily explain the combination of high plasma angiotensin II concentration and normal blood pressure, or the blood pressure hyporesponsiveness to intravenous infusion of vasopressor agents such as angiotensin II and noradrenaline in conditions involving hypokalaemia, mainly for two reasons. First, prostacyclin, which is synthesized in the endothelial cells of arteries and veins, is the most
potent known vasodilator of the prostaglandin family and one of the most potent vasodilators known [5-7]. In rats prostacyclin is at least 20 times more potent in producing vasodilatation than is PGE₂. Second, the 6(9)-oxycyclase pathway of prostaglandin endoperoxide metabolism which leads to prostacyclin generation is the major pathway in arterial blood vessels [8]. In contrast, significant amounts of PGE₂ are probably not generated by arterial or venous tissues. Furthermore, prostacyclin, unlike PGE₂, is not metabolized on passage through the lungs; on the contrary, it is continuously released by the pulmonary endothelial cells [9].

Whereas prostacyclin cannot be measured directly in urine because of its very short half-life at physiological pH, a radioimmunoassay has been developed to measure the urinary excretion of immunoreactive 6-keto-prostaglandin F₁₀₆, one of the stable metabolites of prostacyclin [10]. Measurement of the urinary excretion of 6-keto-PGF₁₀₆ represents an index of prostacyclin synthesis. The purpose of the present experiments was to test whether experimental potassium depletion causes an increase in prostacyclin synthesis in the rat.

Methods

The experiments were performed in adult Fischer 344 rats (body weight 240-280 g, supplied by the Small Animal Section of the Veterinary Resources Branch, National Institutes of Health), which were housed at 22°C. All animals were on water ad libitum. The control diet and the synthetic potassium-deficient diet (catalogue no. TD76264) were obtained from Teklad Test Diets, Madison, Wisconsin, U.S.A., and contained reagent grade minerals. The magnesium sulphate content was 7.88 g/kg of diet. The control diet (catalogue no. TD76265) was identical in composition with the potassium-deficient diet except that potassium chloride was added at the expense of sucrose to give a concentration of 12.0 g/kg of diet. For 24-h urine collections the animals (n = 6) were placed into individual metabolic cages. They were fed on the control diet for 2 days and the potassium-deficient diet from day 3 through to day 11. The effect of potassium depletion on serum sodium and potassium and plasma renin activity was examined in separate groups of rats. One group of rats (n = 10) was fed on the control diet for 9 days; another group of rats (n = 10) was fed on the potassium-deficient diet for 9 days. For blood collection the animals were lightly anaesthetized by placing every animal into a bell jar and placing a gauze pad saturated with methoxyfluorane (Metafane, Pitman-Moore Inc., Washington Crossing, NJ, U.S.A.) into the jar. Blood was collected from the vena cava into chilled tubes for measurement of sodium and potassium in serum and plasma renin activity. Sodium and potassium in serum were measured by flame photometry (Klina Flame, Beckman Instruments), urinary creatinine on an Auto-Analyzer and plasma renin activity by Hazelton Laboratories Inc., Vienna, VA, U.S.A., under a special contract [11]. Immunoreactive PGE₂, 6-keto-PGF₁₀₆, and 13,14-dihydro-15-keto-PGF₂α (PGFM) were extracted from urine and measured by specific radioimmunoassays. Briefly, 1 ml of urine was extracted after acidification to pH 3-4 with citric acid by 6 ml of cyclohexane/ethyl acetate (1:1, v/v). The extract was evaporated under a stream of nitrogen and redissolved in buffer. The radioimmunoassays for prostaglandin E₂, 6-keto-PGF₁₀₆ and PGFM have been described previously [12-14]. The antiserum for the 6-keto-PGF₁₀₆ radioimmunoassay was obtained from Seragen Inc., Boston, MA, U.S.A.

Statistical analysis was performed by repeated measures of analysis of variance (prostaglandins) and by unpaired Student’s t-test. All data are expressed as means ± SE.

Results

The effects of potassium depletion on serum potassium and sodium, urinary creatinine excretion and plasma renin activity are summarized in Table 1. Serum potassium was significantly lower

<table>
<thead>
<tr>
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<th>Serum sodium (mmol/l)</th>
<th>Serum potassium (mmol/l)</th>
<th>PRA (ng h⁻¹ ml⁻¹)</th>
<th>Urinary creatinine (mg/day)</th>
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</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>154.2 ± 0.5</td>
<td>3.46 ± 0.09</td>
<td>22.3 ± 2.3</td>
<td>7.4 ± 0.3</td>
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<tr>
<td>Hypokalaemia (n = 10)</td>
<td>157.7 ± 0.7*</td>
<td>2.42 ± 0.07*</td>
<td>44.3 ± 3.6*</td>
<td>7.4 ± 0.4</td>
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*P < 0.001.
Effect of hypokalaemia on prostaglandins

(2.42 ± 0.07 mmol/l, potassium depletion; 3.46 ± 0.09 mmol/l, control; P < 0.001) in the animals fed on the potassium-deficient diet. Plasma renin activity in the hypokalaemic rats was significantly elevated (P < 0.001). Urinary excretion of creatinine on day 9 of the potassium-deficient diet, measured as an index of glomerular filtration rate, was the same as in the control group.

Fig. 1 depicts the effect of hypokalaemia on the urinary excretion of immunoreactive 6-keto-PGF\(_{1\alpha}\), PGE\(_2\) and PGFM. Each column represents mean excretion for six rats. The animals received the control diet for 2 days, followed by the potassium-deficient diet, which was given for 9 days.

Discussion

It has been shown that PGE\(_2\) in urine predominantly reflects PGE\(_2\) made in the kidneys [15], whereas 6-keto-PGF\(_{1\alpha}\) is an index of synthesis of prostacyclin, which is mainly produced by the endothelial cells of the blood vessel walls and of the lungs [5, 6]. The results of our experiments indicate that hypokalaemia is a potent stimulus of both renal (PGE\(_2\)) and vascular (prostacyclin) prostaglandins in the rat. The effect of experimentally induced potassium deficiency on prostacyclin production has previously not been studied. It has previously been shown that patients with hypokalaemia due to Bartter's syndrome excrete supranormal amounts of 6-keto-PGF\(_{1\alpha}\) in their urine [4]. Prostacyclin, which is generated by the endothelial cells of all arterial and venous walls, is the most potent known vasodilator of the prostaglandin family. It has been shown in studies in rats and rabbits that prostacyclin is at least 20 times more potent than PGE\(_2\) with regard to vasodilatation [7]. It is also the principal and possibly only metabolite of arachidonic acid metabolism in the endothelium of vascular walls and lungs [5, 16]. Our findings suggest that an increase in prostacyclin synthesis in peripheral blood vessels is responsible for the haemodynamic changes present in conditions involving hypokalaemia, particularly for the maintenance of normal blood pressure despite an increase in circulating angiotensin II, and the hyporesponsiveness of blood pressure to infusion of pressor agents. Prostacyclin probably acts as a depressor substance by a local action in the blood vessels rather than as a circulating hormone. This does not rule out a contribution of PGE\(_2\) to the vasodilatation of hypokalaemia.

Although 6-keto-PGF\(_{1\alpha}\) probably represents a major metabolite of prostacyclin, the former can be further metabolized in blood vessels by \(\beta\)-oxidation to dinor-6-keto-PGF\(_{1\alpha}\) and possibly other metabolites [17, 18]. PGI\(_2\) may also be converted via the 9-hydroxyprostaglandin dehydrogenase pathway to 6-keto-PGE\(_1\), a biologically active, chemically stable metabolite [19].

It has been shown that only very little 6-keto-PGF\(_{1\alpha}\) can be detected in urine of prostacyclin-treated rats [20]. However, prostacyclin and 6-keto-PGF\(_{1\alpha}\), the hydrolytic product of prostacyclin, when infused into the rat, show essentially the same metabolic patterns [21]. Furthermore, a substantial amount of unchanged 6-keto-PGF\(_{1\alpha}\) was recovered in the urine after its continuous intravenous infusion. Therefore, 6-keto-PGF\(_{1\alpha}\) can
serve at least as a qualitative index of overall prostacyclin synthesis. Measurements of other metabolites are necessary in order to assess prostacyclin synthesis in a quantitative fashion.

In conclusion, our findings suggest that hypokalaemia stimulates vascular and renal prostaglandin production.

References

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