Potassium Deficiency and Cardiac Catecholamine Metabolism in the Rat

By Lawrence R. Krakoff

ABSTRACT

Norepinephrine metabolism, arterial blood pressure, and electrolytes were studied in rats maintained on either potassium-deficient or control diets. After 3 weeks on the test diets, the arterial blood pressure was significantly lower and the ratio of [Na] to [K] in cardiac tissue was significantly higher in potassium-deficient rats as compared to controls. Ten minutes after intravenous injection of \(^3\)H-norepinephrine the specific activity of cardiac norepinephrine was similar in the control and potassium-deficient groups despite a significant increase in endogenous norepinephrine concentration in the potassium-deficient hearts. But 24 hours after injection, there was a significant increase in \(^3\)H-norepinephrine specific activity, concentration, and content in the potassium-deficient group. Cardiac tissue monoamine oxidase activity was similar in both groups. The increased \(^3\)H-norepinephrine retention and concentration and the decreased arterial blood pressure of the potassium-deficient rate suggest an impairment in norepinephrine release by the sympathetic neurons.

KEY WORDS

norepinephrine release  
norepinephrine specific activity  
monoamine oxidase  
\(^3\)H-norepinephrine  
electrolytes  
arterial blood pressure  
potassium balance

Several inorganic ions have been implicated in the metabolism of the catecholamines. Sodium is required for the uptake and retention of norepinephrine by sympathetic nerve terminals in heart slices (1), brain synaptosomes (2), and isolated perfused rat hearts (3, 4). Release of epinephrine from the adrenal medulla and of norepinephrine from adrenergic nerves which have been electrically stimulated is enhanced by ionized calcium (5–7). Very high concentrations of potassium release norepinephrine from sympathetic neurons (8) and inhibit neuronal uptake of the amine (1, 2). However, lower concentrations of K\(^+\) appear to be required for optimal neuronal uptake of norepinephrine (1). All of these studies concerning the ionic requirements of catecholamine metabolism were carried out in vitro, and ionic concentrations were altered in the perfusing medium, i.e., the extracellular fluid.

In intact animals changes in the metabolism and storage of the catecholamines have been correlated with changes in Na\(^+\) balance (9). Rats made hypertensive by administration of desoxycorticosterone (DOC) and NaCl have reduced accumulations of tritiated norepinephrine, decreased content of catecholamines (10–12), and increased turnover of labeled norepinephrine in several tissues (11, 13). However, rats made Na\(^+\) deficient by low-sodium diets with or without natriuretic agents have increased tissue accumulation of tritiated norepinephrine and increased tissue content of the endogenous amine (14).

In the DOC-NaCl hypertensive rat, increased tissue Na\(^+\) has been observed in skeletal muscle, but muscle K\(^+\) is markedly reduced (15). However, in the aorta, both Na\(^+\) and K\(^+\) content are increased, indicating nonuniform tissue electrolyte changes (16).
Selective K⁺ deficiency induced by placing rats on a low-potassium diet results in reduction of responsiveness to vasopressor agents (17), lowering of arterial blood pressure in both normal and hypertensive rats (17, 18), and prevention of the development of renal hypertension (19). Reduction of the K⁺ content of the diet has also been reported to lower the arterial blood pressure in hypertensive man (20).

The studies to be described were designed to assess the role of K⁺ deficiency in catecholamine metabolism. The results indicate that K⁺ depletion increased the concentration of norepinephrine in tissue and the retention of tritiated norepinephrine but decreased the arterial blood pressure. These alterations in blood pressure and norepinephrine storage are opposite to those observed in DOC-NaCl hypertension, and they suggest a distinct role for K⁺ in catecholamine metabolism.

Methods

Animal Preparation.—Thirty-two Sprague-Dawley male rats weighing 180–200 g were placed on regular laboratory chow and tap water for 1 week. Systolic blood pressure was measured in the tail artery after warming and without anesthesia (21), and body weight was determined. The rats were then divided into two groups of 16 each which were placed on either a potassium-deficient or a control diet. Composition of the test diets was as follows: (1) Potassium-deficient diet (in g/kg): casein 200, sucrose 655.9, fiber 20.0, corn oil 70.0, potassium-free salts 40.0, NaCl 5.0, and vitamin supplement 9.08. (2) Control: identical to the potassium-deficient diet except for the addition of 1.2 g KC1/100 g diet (16 mEq K⁺/100 g). Rats were maintained on their assigned diets for 3 weeks. The rats were then redetermined prior to norepinephrine metabolism studies.

Balance Study.—After 12 days of the test diets, 12 rats from each group were weighed and placed individually in metabolic cages for 24 hours during which they had free access to water and their respective diets. Urine was collected under mineral oil for volume determinations. Urine Na⁺ and K⁺ concentrations were determined by flame photometry against a lithium internal standard with an Instrumentation Laboratories flame photometer.

Norepinephrine Metabolism.—After 3 weeks of the test diets, the rats were injected in their tail veins with ³H-norepinephrine, 7–8 c/mmole, which had been purified by passage over alumina on the preceding day. Either 10 minutes or 24 hours after injection, the rats were killed by cervical fracture, and tissues were removed for assay. After weighting, tissues were placed in 8 ml of 0.4N perchloric acid and homogenized with a Duall all-glass homogenizer. Tissue homogenates were centrifuged, and 5 ml of the supernatant fluid was removed for isolation of ³H- and endogenous norepinephrine. A modified batch method was used to absorb the catecholamines (22, 23). The perchloric acid extract was placed in a 50-ml beaker containing 400 mg of acid-washed alumina, 200 mg of disodium EDTA, and 10 mg of sodium metabisulfate. Then 15 ml of 0.5N Tris acetate buffer, pH 9.0, was slowly added, and the slurry was stirred for 3 minutes with a glass rod. After stirring, the alumina was allowed to settle, the supernatant fluid was decanted, the alumina was resuspended in 5 ml of glass-distilled water, and the solution was then poured into a thistle-shaped glass column. Residual alumina in the beaker was suspended in three successive 5-ml portions of glass-distilled water and added to the column. After the water had drained through, catecholamines were eluted with 6.0 ml of 0.05N HClO₄.

Portions of the eluate were taken for tritium determination using a dioxane phosphor in a Packard Tri-Carb 3320 liquid scintillation system. For assay of endogenous norepinephrine the trihydroxyindole method with ferricyanide as the oxidant was employed (24). Recoveries of both ³H- and unlabeled norepinephrine were 74%.

Monoamine Oxidase.—Monoamine oxidase activity was determined in tissue homogenized in isotonic saline by the method of Wurtman and Axelrod (25) with ¹⁴C-tryptamine as substrate.

Serum and Tissue Electrolytes.—After 18 days on the test diets, rats were lightly anesthetized with ether, and 1.0 ml of blood was drawn from the jugular vein for plasma Na⁺ and K⁺ determinations by flame photometry. Na⁺ and K⁺ were also determined in the perchloric acid tissue extracts used for catecholamine assay.

Statistics.—For statistical comparison of the means of the two groups, Student's t-test for unpaired variables was employed (26).

Results

Blood Pressure and Body Weight.—The arterial blood pressure and the body weight of the two groups of rats were nearly identical prior to the institution of the test diets. As shown in Figure 1, rats placed on the control diet exhibited a steady gain in weight and a small but statistically significant increase in
systolic arterial blood pressure (± 6.3 ± 1.9 mm Hg, P < 0.01). The animals placed on the potassium-deficient diet gained weight much less rapidly than did those on the control diets, so that 12 days after institution of the diets the body weight of the potassium-deficient group was significantly below that of the controls. Systolic arterial blood pressure fell slightly in the potassium-deficient rats. After 3 weeks on the test diets, the systolic arterial blood pressure of the potassium-deficient rats was 121 ± 1.3 mm Hg, which was significantly lower than the control value of 128 ± 1.6 mm Hg.

**Balance Study.**—Table 1 shows the 24-hour urine volume and Na⁺ and K⁺ excretion of 12 rats from the control and low-potassium groups. Despite the smaller size of the members of the low-potassium group at this time (Fig. 1), their 24-hour urine volume was significantly greater than that of the control group. When calculated per 100 g of body weight, the urine volume of the low-potassium group was 146% greater than that of the control group, indicating significant polyuria in the potassium-deficient rats. Total Na⁺ excretion was significantly greater in the control group. When calculated on a weight basis, however, the Na⁺ excretion of the control group was only 13% greater than that of the potassium-deficient group, and the difference was not statistically significant. The K⁺ excretion of the potassium-deficient group was extremely low, less than 30 μEq/24 hours in all rats. In the control group urine K⁺ averaged 2104 μEq/24 hours and ranged from 1180 to 4620 μEq/24 hours.

**Tissue ³H-Norepinephrine Retention and Endogenous Norepinephrine Content.**—Groups of rats maintained on either the potassium-deficient or control diets were given 25 μc of ³H-norepinephrine intravenously and killed 10 minutes or 24 hours later. In Figure 2 the retention of ³H-norepinephrine 10 minutes after injection is shown. It is apparent that the

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Control (12)</th>
<th>Potassium deficient (12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml)</td>
<td>7.4 ± 0.8</td>
<td>13.7 ± 2.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(ml/100 g)</td>
<td>2.6 ± 0.3</td>
<td>6.4 ± 0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sodium excretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μEq/24 hr)</td>
<td>2460 ± 314</td>
<td>1450 ± 169</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(μEq 24 hr⁻¹ 100 g⁻¹)</td>
<td>891 ± 114</td>
<td>678 ± 78</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium excretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μEq/24 hr)</td>
<td>2104 ± 260</td>
<td>14 ± 2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(μEq 24 hr⁻¹ 100 g⁻¹)</td>
<td>762 ± 95</td>
<td>6 ± 1</td>
<td>&lt;0.01</td>
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</tbody>
</table>

Twenty-four-hour urine volume and Na⁺ and K⁺ excretion are given as means ± se. P values relate control and potassium-deficient groups; NS = not significant at the 5% level. Number of animals tested is given in parentheses.
Cardiac tissue retention of $^3$H-norepinephrine in nanocuries 10 minutes after intravenous injection of $^3$H-norepinephrine in potassium-deficient (Low K*) and control rats. The mean of each group of eight rats ± se is presented as total $^3$H-norepinephrine per heart, $^3$H-norepinephrine per gram of heart, and specific activity (nanocuries per microgram norepinephrine). $P$ values for the difference between the means are given; N.S. = not significant at the 5% level.

The total amount of $^3$H-norepinephrine retained by the hearts of the potassium-deficient rats was slightly greater than that in the controls, but the difference was not significant at the 5% level. However, the concentration of $^3$H-norepinephrine in nanocuries per gram of heart was much higher in the potassium-deficient group partly because of the lower heart weight of these rats (Table 2). Ten minutes after injection of the $^3$H-norepinephrine there was no significant difference in the specific activity of cardiac $^3$H-norepinephrine between the control and potassium-deficient rats (Fig. 2), indicating that at this time $^3$H-norepinephrine retention was proportional to tissue norepinephrine content (Table 2).

Cardiac $^3$H-norepinephrine retention in control and potassium-deficient rats 24 hours after administration of the labeled amine is shown in Figure 3. At this time there was a significant increase in total cardiac $^3$H-norepinephrine in the potassium-deficient group. The $^3$H-norepinephrine concentration of the potassium-deficient group was more than twice that of the control group. Twenty-four hours after injection, the specific activity of cardiac $^3$H-norepinephrine in the potassium-deficient group was 67% greater than it was in the controls.

Comparing the $^3$H-norepinephrine retained in the potassium-deficient heart at the two times at which measurements were made, the specific activity of $^3$H-norepinephrine 24 hours after injection was 29.7% of that at 10 minutes. But in the controls, the specific activity of cardiac $^3$H-norepinephrine at the later time was only 18.7% of that observed 10 minutes after injection. Thus over 24 hours the potassium-deficient rat hearts retain 59% more $^3$H-norepinephrine than do control hearts in relation to the amount of radioactive amine present in the hearts within minutes after injection. As shown in Table 2, the endogenous cardiac norepinephrine concentration ($\mu g$/$g$) in the potassium-deficient group was significantly higher than that in the control group. When expressed as total norepinephrine ($\mu g$/heart), the slightly increased norepinephrine content of the potassium-deficient group was not statistically significant.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Control (16)</th>
<th>Potassium deficient (16)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart wt (mg)</td>
<td>974 ± 26</td>
<td>726 ± 17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart wt (% body wt)</td>
<td>0.32 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>N.S.</td>
</tr>
<tr>
<td>Norepinephrine content ($\mu g$/heart)</td>
<td>0.66 ± 0.04</td>
<td>0.72 ± 0.05</td>
<td>N.S.</td>
</tr>
<tr>
<td>Norepinephrine concentration ($\mu g$/$g$ heart wt)</td>
<td>0.68 ± 0.03</td>
<td>0.98 ± 0.06</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE; N.S. = not significant at the 5% level. Number of animals tested is given in parentheses.

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Cardiac tissue retention of $^3$H-norepinephrine in nanocuries 24 hours after intravenous injection of $^3$H-norepinephrine in potassium-deficient (Low K$^+$) and control rats. The data is presented as in Figure 2.

Plasma and Tissue Electrolytes.—After 18 days on the test diets, blood was drawn for plasma Na$^+$ and K$^+$ determinations. Samples in which gross hemolysis was observed were discarded. In Figure 4, the plasma Na$^+$ and K$^+$ concentrations of the control and potassium-deficient groups are shown. The plasma Na$^+$ was 136.2 ± 0.6 mEq/liter in the potassium-deficient rats; this was slightly, but significantly, lower than the control value of 139.9 ± 0.5 mEq/liter. Plasma K$^+$ was 5.0 ± 0.3 mEq/liter in the potassium-deficient rats and 5.2 ± 0.1 mEq/liter in the controls. There was no significant lowering of plasma K$^+$ in the potassium-deficient rats.

Cardiac tissue Na$^+$ and K$^+$ concentrations and the ratio of [Na] to [K] in the control and potassium-deficient rats were determined on tissue extracts after 21 days of the test diets. The results are shown in Figure 4. Tissue K$^+$ was 69.4 ± 1.6 mEq/kg wet weight in the potassium-deficient hearts, significantly lower when compared to 78.5 ± 1.7 mEq/kg in the controls. The tissue Na$^+$ in the potassium-deficient hearts was 60.0 ± 2.7 mEq/kg compared to 49.8 ± 1.4 mEq/kg in the controls. Thus both decreased tissue K$^+$ concentration and increased tissue Na$^+$ concentration in the potassium-deficient hearts contributed to a significantly higher tissue ratio of [Na] to [K] as compared to that observed in controls.

Monoamine Oxidase Activity.—Cardiac monoamine oxidase activity was determined in tissue homogenates. There was no significant difference between the level of monoamine oxidase activity in the potassium-deficient (4.9 ± 0.7 nmoles mg$^{-1}$ hr$^{-1}$) and control (5.4 ± 0.6 nmoles mg$^{-1}$ hr$^{-1}$) hearts.

Discussion

Dietary K$^+$ deficiency is associated with a reduction in arterial blood pressure (17, 18, 20, 27) and prevents the development of experimental hypertension (19). In the present study, when rats were maintained on a potassium-deficient diet for 3 weeks their arterial blood pressure fell slightly but was significantly lower than that of the control group in which a rise in blood pressure was observed.

Prior studies have suggested that one of the factors participating in the effect of K$^+$ deficiency on arterial blood pressure is reduced responsiveness of the peripheral vasculature to vasoconstrictor agents such as norepinephrine and angiotensin. Friedman et al. (17) observed that rats on potassium-deficient diets had reduced arterial blood pressure responses to norepinephrine, renin, and angiotensin which could be partially
reversed by infusion of KCl. Reid and Laragh (28) found that potassium-deficient rats had diminished pressor responses to angiotensin which were increased to control levels by simultaneous Na⁺ and K⁺ deficiency. Reduced arterial blood pressure responses to renin and angiotensin were observed by Fisher and Funckes (19) in both intact potassium-depleted rats and potassium-depleted rats with unilateral renal artery clips. In this study pressor responsiveness to norepinephrine did not differ in potassium-deficient and control groups. In view of recent studies demonstrating a marked increase in plasma renin concentration in potassium-deficient rats (29), the reduced pressor responsiveness to angiotensin and renin observed in these animals could in part be due to tachyphylaxis in addition to a primary reduction in vascular smooth muscle responsiveness.

In K⁺ depletion associated with the syndrome of primary aldosteronism in man, impairment of cardiovascular reflexes mediated by the sympathetic nervous system has been observed and related to the state of K⁺ balance (30). Distler et al. (31) have demonstrated, however, that such patients do not have reduced responsiveness to norepinephrine or angiotensin but do have a reduced blood pressure response to tyramine. Since the pressor action of tyramine is mediated by norepinephrine released from sympathetic nerve terminals (32), the selective reduction of pressor responsiveness to tyramine observed in patients with primary aldosteronism suggests an impairment in catecholamine release mechanisms within the sympathetic nervous system or a reduced releasable norepinephrine pool.

The concentration of norepinephrine in cardiac tissue was significantly increased in potassium-deficient rats in the present study. Tissue retention of intravenously administered [³H]norepinephrine was proportional to tissue norepinephrine content 10 minutes after injection, indicating that sympathetic neuronal uptake was intact. Twenty-four hours after injection, however, cardiac [³H]norepinephrine in the potassium-deficient rats was increased significantly and was out of proportion to endogenous tissue norepinephrine. This resulted in a marked increase in the specific activity of cardiac norepinephrine in the potassium-deficient rats.

Tissue retention of intravenously administered labeled norepinephrine is related to several processes. Active uptake of circulating catecholamines by sympathetic neurons is followed by binding in storage vesicles (33). Subsequently, release of the unchanged amine may occur or intraneuronal metabolism by monoamine oxidase may result in inactivation prior to release from the nerve terminal of the deaminated catechol (34).

More rapid disappearance of norepinephrine from the tissue stores occurs in association with cold exposure or strenuous exercise (35, 36). Increased turnover of [³H]norepinephrine has also been observed after administration of phenoxybenzamine (37) and hydralazine (38), both of which tend to lower arterial blood pressure and thus stimulate baroreceptor sympathetic reflexes. In DOC-NaCl hypertension (10) and neurogenic hypertension (39) cardiac tissue [³H]norepinephrine disappears more rapidly than it does in normotensive controls. The diphasic pattern of [³H]norepinephrine disappearance and the results of subcellular distribution studies suggest that there is an abnormality in [³H]norepinephrine binding by adrenergic neuron storage vesicles in DOC-NaCl hypertension (11). However increased norepinephrine turnover is also observed (13). Also, ganglionic blocking agents reduce arterial blood pressure and increase [³H]norepinephrine tissue retention to control levels (14). These findings suggest that altered sympathetic tone may play a significant role.

Ganglionic blocking agents can reverse the increased norepinephrine synthesis and turnover associated with cold exposure or action of alpha-receptor blocking agents (40). Inhibition of monoamine oxidase also increases tissue norepinephrine and reduces turnover of catecholamines (23, 41). This effect is similar to that seen with ganglionic blockade, but a proposed mechanism suggests that it is due to
reduced intraneural catabolism of norepinephrine, which results in feedback inhibition of catecholamine synthesis (23, 34).

The pattern of cardiac norepinephrine metabolism observed in the potassium-deficient rats resembles that seen with either ganglionic blockade or monoamine oxidase inhibition. In K⁺ deficiency there is increased retention of ³H-norepinephrine and elevated endogenous norepinephrine concentration without apparent change in norepinephrine uptake. Direct assay of tissue monoamine oxidase activity, however, revealed no significant difference between potassium-deficient and control rats. Nonetheless, a selective reduction in intraneuronal monoamine oxidase cannot be excluded at this time. It seems more likely that K⁺ deficiency may result in impairment of some part of the normal norepinephrine release mechanism. Whether this occurs as a result of impaired ganglionic transmission or because of alteration in the release mechanisms of the adrenergic nerve terminal remains to be clarified.

In this study there was a reduction in cardiac tissue K⁺ concentration and an increase in tissue Na⁺ in the potassium-depleted group. Plasma Na⁺ however, was reduced. The changes in Na⁺ probably represent a shift from the extracellular to the intracellular compartment in compensation for a loss of intracellular K⁺. The extent to which similar intracellular changes occur within pre- or postganglionic sympathetic neurons or both remains conjectural. Although K⁺ depletion from some tissues does occur with mineralocorticoid administration, it is apparent that the changes in arterial blood pressure and in norepinephrine metabolism in dietary K⁺ deficiency are opposite to those observed in DOC-NaCl hypertension. It is therefore suggested that the lowered arterial blood pressure of potassium-deficient rats and their resistance to the development of experimental hypertension may in part be due to an impairment of release of norepinephrine, the sympathetic neurotransmitter.

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