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Potassium regulates plasma testosterone and renal ornithine decarboxylase in mice

Amelia Sánchez-Capelo[a], Asunción Cremades[b], Francisco Tejada[b], Teodomiro Fuentes[b], Rafael Peñafiel[a,*]

Departments of [a]Biochemistry and Molecular Biology, and [b]Pharmacology, Faculty of Medicine, University of Murcia, 30100 Murcia, Spain

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Potassium deficiency produced different effects in the kidney of male or female mice. While in female, potassium deficiency caused a marked renal hypertrophy with no significant changes in testosterone-regulated enzymes, such as ornithine decarboxylase and β-glucuronidase, in the male the same treatment provoked a marked fall of these enzymes owing to a dramatic decrease in plasma testosterone. Potassium replenishment restored plasma testosterone and renal enzymatic activities. These results show for the first time, that potassium modulates circulating testosterone and suggest that this cation could exert an important regulatory role in controlling androgen actions.

Potassium; Testosterone; Sexual dimorphism; Ornithine decarboxylase; Mouse kidney

1. INTRODUCTION

It is well established that potassium deficiency produces renal hypertrophy in different animal species [1,2]. The mechanisms by which renal hypertrophy is produced are unknown, although in some cases ornithine decarboxylase (ODC) and polyamines have been proposed as mediators in the process of renal growth [3–5]. In mice, testosterone produces a marked renal hypertrophy accompanied by a large increase in ODC, a key enzyme in the synthesis of polyamines and in some lysosomal enzymes such as β-glucuronidase [6,7]. β-Glucuronidase activity is elevated in the kidney of potassium-deficient female mice [8], but the implication of ODC and polyamines in this kind of hypertrophy has not yet been studied.

In the present work we have studied the effect of potassium deficiency on ODC activity in the mouse kidney. Since the existence of a clear renal sexual dimorphism in mice is known [9], we have compared the effect of hypokalemia in male and female mice, and we have found that circulating testosterone levels are regulated by plasma potassium concentration.

2. MATERIALS AND METHODS

2.1. *Animals*

Adult Swiss CD1 mice were used in these experiments. Control animals were fed with standard chow (UAR A03) containing 7.5 g/kg potassium and drinking water ad libitum. Potassium deficiency was produced by feeding the mice with a diet similar to control diet but containing 120 mg/kg potassium (UAR 212K). Potassium replenishment was achieved by supplementation of the low potassium diet with 1% KCl solution instead of drinking water. All animals were maintained at 22°C ambient temperature and 50% relative humidity, under controlled 12 h light–dark cycles.

*Corresponding author. Fax: (34) (68) 834 307.

2.2. *General procedures*

Blood was obtained under light ether anesthesia by cardiac puncture. Plasma was obtained by centrifugation at 4°C and kept frozen until analysis. Animals were killed and the kidneys collected, rinsed and weighed. They were homogenized by Polytron in buffer containing 25 mM Tris pH 7.2, 2 mM dithiothreitol, 0.1 mM pyridoxal phosphate, 0.1 mM EDTA and 0.25 M sucrose. A part of the extract was centrifuged at 20,000 × *g* for 20 min and β-glucuronidase and ornithine decarboxylase activities were determined in the crude extract and supernatant, respectively.

2.3. *Analytical methods*

ODC activity was measured as previously described [10]. The incubation mixture contained 0.4 mM L-[1-^{14}C]ornithine (New England Nuclear, specific activity 4.75 Ci/mol) and 10 μl of the kidney extract in a total volume of 62.5 μl. β-Glucuronidase activity was measured by the method of Fishman [11], using *p*-nitrophenyl-β-D-glucuronide (Sigma) as a substrate. Testosterone was determined by radioimmunoassay with a kit supplied by ICN Biomedicals (Carson). Plasma potassium was analyzed by means of a potassium-selective electrode (Beckman).

2.4. *Statistical analysis*

The results are given as means ± S.D. Group mean comparisons were made by Student's *t*-test.

3. RESULTS

Table I shows the effect of feeding mice with a low potassium diet on body and kidney weights. A decrease in body weight was observed in both male and female mice but while in males a decrease in kidney weight was evident, in females a clear increase in the weight of this organ was estimated. Hypokalemia appeared in mice fed with low potassium chow and the decrease in plasma

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Table I

Effect of K^+-deficiency in body and kidney weights of male and female mice

	Males		Females	
	Body weight (g)	Kidney weight (mg)	Body weight (g)	Kidney weight (mg)
Control	41.2 ± 1.1 (7)	338 ± 15	32.7 ± 2.5 (6)	208 ± 21
K^+-deficient (15 days)	32.3 ± 0.9 (10)	307 ± 31*	N.D.	N.D.
K^+-deficient (30 days)	28.9 ± 1.4 (10)	297 ± 29*	25.5 ± 1.7 (9)	246 ± 34*
K^+-deficient (15 days) + 5 days KCl replenishment	40.8 ± 0.8 (7)	347 ± 26	N.D.	N.D.

Mice were made K^+-deficient by feeding with a low K^+ diet during 15 or 30 days. K^+-replenishment was achieved by substituting the drinking water by 1% KCl solution during 5 days after day 15 of K^+ deficiency.

*$P < 0.01$. N.D.: not determined. Numbers of animals are given in parentheses.

potassium was dependent on the extension of the treatment. The replenishment with potassium by administering KCl in the drinking water reestablished plasma potassium values to control levels (Table II).

Fig. 1 shows that the high basal value of renal ODC activity of male mice was drastically decreased in kidney of potassium deficient mice and it was clearly recovered after potassium replenishment. ODC activity in female kidneys was low and was not affected by potassium deficiency. A similar effect of potassium was also observed in other renal enzyme such as β-glucuronidase. Since both enzymes are regulated by testosterone, a plausible explanation of these findings could be related to some alteration in the mechanism of control of gene expression by androgens. The possibility that potassium deficiency could hardly alter the responsiveness of mouse kidney to testosterone can be ruled out, since potassium-deficient male and female mice responded with a normal induction of renal ODC and β-glucuronidase after testosterone propionate administration (data not shown). The analysis of plasma testosterone in control and potassium-deficient mice showed that circulating testosterone dramatically decreased in male mice fed with low potassium diet. Normal testosterone values were recovered after potassium replenishment (Table III).

4. DISCUSSION

Our results show that potassium deficiency produced renal hypertrophy in female CD1 mice as reported in other mice strains [8]. However, we did not find any significant elevation of β-glucuronidase nor ODC activities in the hypertrophic kidney, which suggests that ODC is not involved in the proces of hypertrophy, as it has been claimed in other models of renal hypertrophy [3–5]. Interestingly, in male mice potassium deficiency did not produce any increase in renal weight but it affected the renal activity of ODC and β-glucuronidase, which was markedly decreased in potassium-

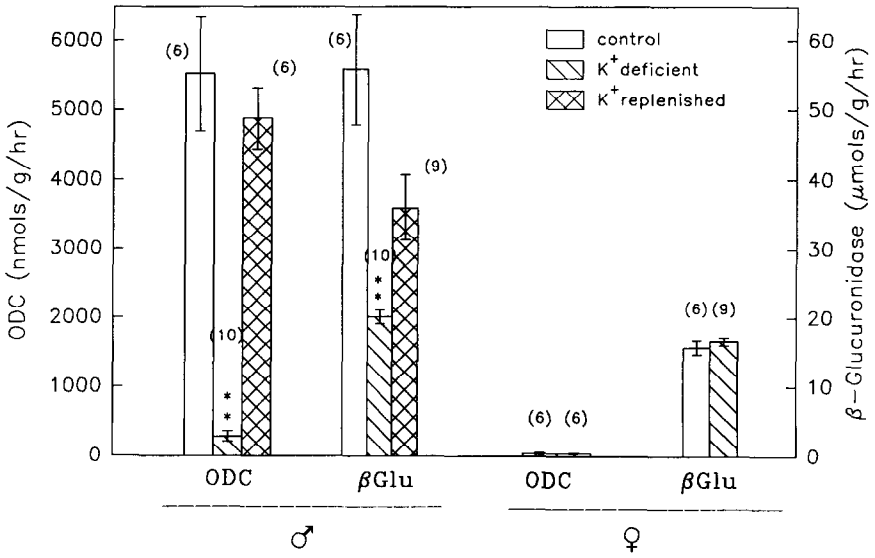

Fig. 1. Effect of K^+-deficiency on ornithine decarboxylase and β- glucuronidase in mouse kidney. Numbers of animals are given in parentheses.

Table II

Plasma potassium concentration in control and K^+-deficient mice

	$[K^+]$ (mM)	
	Male	Female
Control	4.08 ± 0.21 (11)	3.65 ± 0.13 (6)
K^+-deficient (15 days)	3.41 ± 0.20* (5)	N.D.
K^+-deficient (30 days)	2.64 ± 0.24* (5)	2.60 ± 0.50* (10)
K^+-deficient (15 days) + K^+-replenished	4.01 ± 0.46 (6)	N.D.

Treatments as indicated in legend of Table I.
*Statistical significance: $P < 0.001$ versus control. The number of animals is indicated in parentheses.

deficient mice and was recovered after potassium replenishment. Since these enzymes are regulated by testosterone in the mouse kidney [6,7,12] the effect produced by potassium deficiency could be ascribed either to an impairment in the responsiveness of mouse kidney to androgens or to a decrease in the amount of testosterone reaching this organ.

Our results clearly show that the fall in ODC and β-glucuronidase is caused by the diminution of circulating plasma testosterone produced by potassium deficiency, and suggest that potassium concentration plays an important role in regulating plasma testosterone.

To our knowledge, this result shows for the first time that potassium modulates circulating testosterone, and hence, it might regulate androgenic actions in both accessory sex organs and extragenital tissues. The fact that a variation of about 0.6 mM in plasma potassium concentration could greatly decrease the concentration of testosterone may have physiological relevance since the range of human plasma potassium concentration is 3.4–4.6 mM [13]. Moreover, this effect of potassium on testosterone levels could have certain significance in some pathological situations in which hypokalemia is produced [13–15]. This result and other observations showing that hypokalemia could affect aldosterone secretion [13,16] or insulin release [13,17,18] suggest a major role of potassium in regulating different endocrine systems.

Our model provides a good experimental approach to learn about the regulatory mechanisms of testosterone secretion. Experiments designed in order to test whether potassium directly affects the synthesis or liberation of testosterone by testicular Leydig cells or indirectly through modulation of the hypothalamo–hypophyseal axis are presently carried out in our lab.

Table III

Plasma testosterone levels in control and K^+-deficient male mice

	Testosterone (nM)
Control	48.9 ± 24.6 (13)
K^+-deficient (15 days)	2.9 ± 2.5* (10)
K^+-replenished	67.3 ± 30.9 (5)

*$P < 0.001$.

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REFERENCES

[1] Brokaw, A. (1953) Am. J. Physiol. 172, 333–346.
[2] Fine, L.G. and Norman, J. (1989) Annu. Rev. Physiol. 51, 19–32.
[3] Brand, J.T., Pierce, D.A. and Fausto, N. (1972) Biochim. Biophys. Acta 279, 184–193.
[4] Austin, H.A., III Goldin, H., Gaydos, D. and Preuss, H.G. (1983) Kidney Int. 23, 581–587.
[5] Goldstone, A., Koening, H. and Lu, C. (1982) Biochem. Biophys. Res. Commun. 104, 165–172.
[6] Koening, H., Goldstone, A., Blume, G. and Lu, C.Y. (1980) Science 209, 1023–1026.
[7] Berger, F.G. and Watson, G. (1989) Annu. Rev. Physiol. 51, 51–65.
[8] Cleveland, C.E. and Swank, R.T. (1978) Biochem. J. 170, 249–256.
[9] Bardin, C.W. and Catterall, J.F. (1981) Science 24, 1285–1294.
[10] Cremades, A., Tovar, A. and Peñafiel, R. (1992) Biochem. Int. 27, 823–830.
[11] Fishman, W.H. (1974) in: Methods of Enzymatic Analysis (Bergmeyer, H.U., Ed.) 2nd Edn., Vol. 2, pp. 928–943, Academic Press, New York.
[12] Seely, J.E., Pöso, H. and Pegg, A.E. (1982) J. Biol. Chem. 257, 7549–7553.
[13] Tannen, R.L. (1991) in: The Kidney (Brenner, B.M. and Rector, F.C., Eds.) Vol. 1, pp. 805–839, Saunders, Philadelphia.
[14] Hood, V.L. (1986) in: Fluids and Electrolytes (Kokko, J.P. and Tannen, R.L., Eds.) 1st Edn., pp. 712–732, Saunders, Philadelphia.
[15] Adrogue, H.J., Lederer, E.D., Suki, W.N. and Ekonyan, G. (1986) Medicine 65, 163–168.
[16] McKenna, T.J., Island, D.P., Nicholson, W.E. and Liddle, G.W. (1978) Endocrinology 103, 1034–1041.
[17] Gordon, O. (1973) Diabetes 22, 544–549.
[18] Grunfeld, C. and Chappel, D.A. (1983) Am. J. Med. 75, 553–558.