JOURNAL OF APPLIED TOXICOLOGY, VOL. 16(3), 255–257 (1996)

Influence of Hormone Status on Enzymes Released from Renal Cortical Slices of Wistar Rats

Stefano Maso, Giampaolo Nicoletto, Livia Secondin, Aleksandrs Odinecs* and Andrea Trevisan†
Istituto di Medicina del Lavoro, Laboratorio di Tossicologia Industriale, Università de Padova, Padova, Italy

Key words: sexual hormones; aspartate aminotransferase; alanine aminotransferase; lactate dehydrogenase; γ-glutamyltransferase; alkaline phosphatase.

Release of some cytosolic (aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase) and brush border (γ-glutamyltransferase and alkaline phosphatase) enzymes from renal cortical slices was studied *in vitro*. Renal cortical slices were prepared freehand from 3-month-old male and female Wistar rats of different hormonal status. Some male and female rats were castrated at 1 month of age and a portion of castrated males and of naive males and females were s.c. treated with testosterone (10 mg kg^{-1} body wt.) on alternate days for 3 weeks. Females had higher alanine aminotransferase (77.5 ± 2.8 nmol 100 mg^{-1} tissue), lactate dehydrogenase (5.01 ± 0.24 μmol) and alkaline phosphatase (1.63 ± 0.15 mol) activities than male rats (20.4 ± 0.9, 3.99 ± 0.19 and 0.91 ± 0.02, respectively). On the contrary, aspartate aminotransferase and γ-glutamyltransferase were similar. Among cytosolic enzymes, alanine aminotransferase and lactate dehydrogenase appeared to be sexual hormone-dependent enzymes: castration significantly increased enzyme activities in males (49.6 ± 1.1 for the former; 5.30 ± 0.15 for the latter) and caused significant decreases in females (alanine aminotransferase only 47.1 ± 1.5), whereas testosterone pretreatment decreased activities in cortical slices from female (48.1 ± 3.6 and 3.81 ± 0.07, respectively) and castrated male (27.4 ± 1.8 and 4.05 ± 0.15, respectively). Moreover, exogenous testosterone increased aspartate aminotransferase in males (1.05 ± 0.01 μmol) and castration increased it in both sexes. The activity of brush border enzymes was increased by testosterone pretreatment and decreased by castration (mainly alkaline phosphatase).

INTRODUCTION

Cytosolic and brush border enzymes excreted from the kidney are used as nephrotoxic indices during biological monitoring of xenobiotics, drugs and industrial pollutants.[1]

It has been demonstrated recently[2] that urinary excretion of alanine aminopeptidase and γ-glutamyltransferase (brush border enzymes) is higher in male and testosterone-treated castrated male rats than in castrated male rats. Moreover, the rate of alanine aminopeptidase excretion is higher in male than in female rats.[3] These results suggest that the urinary excretion of these enzymes is related to the level of testosterone.[2]

Similar results on γ-glutamyltransferase are obtained in mouse:[4] the enzyme activity increases in males during sexual maturation, being low in females and castrated males. Testosterone treatment of the latter two groups increases the activity of this enzyme to the level of males.

In addition, a testosterone-mediated sexual dimorphism in mouse kidney proximal tubule was observed.[5]

The testosterone regulation of urinary output of *N*-acetyl-β-D-glucosaminidase, a lysosomal enzyme, was also demonstrated in hypertensive,[6] but not in normal, rats.[2]

The present report describes the behaviour of some cytosolic and brush border enzymes of the proximal tubule released from renal cortical slices of male and female rats with different hormone conditions, such as castration and testosterone pretreatment.

EXPERIMENTAL

Male and female albino Wistar rats (Morini, S. Polo d'Enza, RE, Italy) were purchased at 1 month of age. After 1 week of acclimatization, some males and females were surgically castrated under ketamine (20 mg kg^{-1} body wt. i.p.) and diazepam (5 mg kg^{-1} body wt. i.p.) anaesthesia. At 2 months of age, rats were subdivided into seven groups of eight animal each as follows: males, castrated males, females, castrated females, testosterone-treated males, castrated males, and females. Testosterone (testosterone propionate, Fluka, Buchs, CH), dissolved in corn oil, was injected s.c. at 10 mg kg^{-1} body wt. dose on alternate days for 3 weeks when rats were about 2 months old. Controls were treated s.c. with corn oil only. Rats were sacrificed at 3 months of age 24 h after the last treatment.

* Present address: Department of Pharmacology, Division of Clinical Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.
†Author to whom correspondence should be addressed.

CCC 0260–437X/96/030255–03
© 1996 by John Wiley & Sons, Ltd.

Received 20 October 1995
Accepted 8 January 1996

Kidneys were quickly removed, placed in cold saline and thin freehand renal cortical slices (100 ± 10 mg wet tissue, approximately 25 mg/slice, thickness approximately 0.5 mm) were prepared with a scalpel according to Berndt[7] and placed into incubation medium (sodium phosphate buffer 7.4 mM, pH 7.4, enriched with 97 mM NaCl, 40 mM KCl and 0.74 mM $CaCl_2$) until all the slices could be prepared. Slices were transferred to 25-ml Erlenmeyer flasks containing 4 ml of the incubation medium. The flasks were stoppered, gassed with 100% O_2 for 5 min and incubated at 37°C for 90 min in a Dubnoff metabolic shaker (100 cycles min^{-1}). Afterwards, the medium was centrifuged and aspartate aminotransferase (AST, E.C. 2.6.1.1), alanine aminotransferase (ALT, E.C. 2.6.1.2), lactate dehydrogenase (LDH, E.C. 1.1.1.27), γ-glutamyltransferase (GGT, E.C. 2.3.2.2) and alkaline phosphatase (ALP, E.C. 3.1.3.1) were determined using commercial kits (Boehringer, Mannheim, Germany).

Spectrophotometric determinations were carried out using a UV–Vis supectrophotometer Perkin-Elmer lambda 5 model.

Variance analysis (ANOVA) was used for statistical evaluation of the results. Significance was set at $P \leq 0.05$.

RESULTS

Table 1 shows the values of enzyme activities released into the incubation medium from renal cortical slices of Wistar rats with different hormonal status. The enzymatic patterns were quite different.

Female rats (Table 2) showed a significantly higher enzyme release from renal cortical slices than males, with the exception of AST and GGT, although AST values were lower in males than in females.

Testosterone pretreatment significantly increased (Table 2) AST activity in males only, decreased female ALT activity and almost completely prevented the increase caused by male castration reduced LDH activity in females, and castrated males at the male levels, and increased GGT (but not in females) and ALP activities in all groups.

Finally, castration significantly increased (Table 2) AST activity in both sexes and ALT and LDH activities in males, whereas it decreased ALT activity in females and GGT and ALP activities in both sexes.

Table 2. Statistical evaluation of the results by means of variance analysis[a]

	AST	ALT	LDH	GGT	ALP
Males vs females	=	<	<	=	<
Castrated males vs naive males	+	+	+	−	−
Castrated females vs naive females	+	−	=	=	−
Males + testosterone vs naive males	+	=	=	+	+
Females + testosterone vs naive females	=	−	−	=	+
Castrated males + testosterone vs naive males	+	+	=	+	+
Castrated males + testosterone vs castrated males	=	−	−	+	+

[a] Legend (=) Not significant; (<) significantly lower than females ($P < 0.05$); (+ or −) significantly increased or decreased ($P < 0.05$) after castration, testosterone pretreatment or both.

DISCUSSION

A renal cortical slice model is used to evaluate kidney metabolism and nephrotoxic effects of xenobiotic substances.[8] Several enzymes released into the incubation medium allow the nephrotoxicity of metals, drugs and solvents to be studied.[8–11] Furthermore, cytosolic enzymes appear to be better markers than brush border enzymes.[8]

The results of the present work show that:

(i) enzyme activities are generally higher in females than in males;

(ii) artificial changes of hormone pattern induced by means of surgical castration and/or of testosterone pretreatment caused different effects on cytosolic or brush border enzymes;

(iii) the behaviour of the studied enzymes under these conditions may explain the role of sexual hormones in enzyme expression.

The behaviour of ALT and LDH, both cytosolic enzymes, suggests an influence by sexual hormones, in particular oestrogens. Therefore, sexual hormones could play a pivotal role in enzyme regulation.

Table 1. Cytosolic and brush border enzymes released into incubation medium from renal cortical slices of naive, castrated and testosterone-pretreated (or not) Wistar rats[a]

	AST (μmol)	ALT (nmol)	LDH (μmol)	GGT (μmol)	ALP (μmol)
Males	0.77 ± 0.04	20.4 ± 0.9	3.99 ± 0.19	2.83 ± 0.15	0.91 ± 0.02
Castrated males	0.95 ± 0.03	49.6 ± 1.1	5.30 ± 0.15	2.21 ± 0.04	0.68 ± 0.02
Males + testosterone	1.05 ± 0.03	23.1 ± 1.6	4.41 ± 0.15	3.68 ± 0.24	1.51 ± 0.06
Castrated males + testosterone	0.96 ± 0.03	27.4 ± 1.8	4.05 ± 0.15	3.85 ± 0.23	1.87 ± 0.13
Females	0.89 ± 0.05	77.5 ± 2.8	5.01 ± 0.24	2.60 ± 0.22	1.63 ± 0.15
Castrated females	1.04 ± 0.01	47.1 ± 1.5	5.61 ± 0.01	2.25 ± 0.01	0.93 ± 0.02
Females + testosterone	0.90 ± 0.06	48.1 ± 3.6	3.81 ± 0.07	2.94 ± 0.23	2.43 ± 0.18

[a] Determinations are assayed on eight animals in duplicate. Results are expressed as means ± SEM and activities (μmol and nmol) are related to 100 mg of tissue.

On the contrary, testosterone exerts a permanent positive control on proximal brush border enzymes such as GGT and, in particular ALP, confirming previous research on GGT.[2] The significant decrease in ALP activity in both sexes and GGT activity in males caused by castration is difficult to explain. Hypophysary control by gonadotrophins induced by low levels of sexual hormones is an attractive hypothesis.

CONCLUSIONS

The hormone status of rats appears to play a pivotal role in the regulation of some renal enzyme activities. Thus, while AST has no sexual hormone-related activity, the cytosolic ALT and LDH enzymes show oestrogen regulation and the brush border GGT and ALP enzymes show testosterone-related control.

Acknowledgements

During 1992, Dr A. Odinecs was a fellow of the European Science Foundation. The research was supported by grants from the Board of Scientific and Technological Research (Rome).

REFERENCES

1. R. G. Price, Urinary enzymes, nephrotoxicity and renal disease. *Toxicology* **23**, 99–134 (1982).
2. J. P. Girolami, J. L. Bascands, D. Prevot, C. Pecher and Y. Manuel, Urinary output modulation of alanine aminopeptidase, γ-glutamyl transpeptidase and *N*-acetyl-β-D-glucosaminidase by castration and testosterone in male normal rat. *Enzyme* **41**, 181–186 (1989).
3. M. Nakamura, K. Miyata and T. Tamara, Sex difference in urinary excretion of alanine aminopeptidase in the rat. *Jpn. J. Nephrol.* **23**, 209–212 (1981).
4. T. Tanaka, A. Naganuma, N. Miura and N. Imura, Role of testosterone in γ-glutamyltranspeptidase-dependent renal methylmercury uptake in mice. *Toxicol. Appl. Pharmacol.* **112**, 58–63 (1992).
5. H. Koenig, A. Goldstone, G. Blume and Y. L. Chung, Testosterone-mediated sexual dimorphism of mitochondria and lysosomes in mouse kidney proximal tubules. *Science* **209**, 1023–1026 (1980).
6. M. Malyusz and H. J. Ehrens, Effect of castration and testosterone substitution on the urinary output of gamma-glutamyl transpeptidase and *N*-acetyl-beta-D-glucosaminidase of male rats with renovascular hypertension. *Enzyme* **29**, 93–99 (1983).
7. W. O. Berndt, Use of tissue slice technique for evaluation of renal transport process. *Environ. Health Perspect.* **15**, 73–88 (1976).
8. J. H. Smith, The use of renal cortical slices from the Fischer 344 rat as an *in vitro* model to evaluate nephrotoxicity. *Fundam. Appl. Toxicol.* **11**, 132–142 (1988).
9. A. Trevisan, S. Maso and P. Meneghetti, Renal cortical slices: an *in vitro* model for kidney metabolism and toxicity. *ATLA* **20**, 71–76 (1992).
10. A. Trevisan, P. Meneghetti, S. Maso, L. Secondin and G. Nicoletto, Sex- and age-related nephrotoxicity due to 1, 2-dichoropropane in vitro. *Arch. Toxicol.* **66**, 641–645 (1992).
11. A. Trevisan, P. Meneghetti, S. Maso and O. Troso, *In vitro* mechanisms of 1,2-dichloropropane nephrotoxicity using renal cortical slice model. *Hum. Exp. Toxicol.* **12**, 117–121 (1993).