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NADH-DEPENDENT PGE_2-9-KETOREDUCTASE ACTIVITY IN THE KIDNEY OF DIABETES INSIPIDUS BRATTLEBORO AND LONG EVANS RATS: RELATIONSHIP WITH URINARY PGE_2 AND $PGF_{2\alpha}$ EXCRETION

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Summary

Previous studies have suggested that the activity of the renal enzyme PGE_2-9-ketoreductase, catalysing the conversion of PGE_2 to $PGF_{2\alpha}$, might be altered in the diabetes insipidus Brattleboro rat.

Enzyme activity was therefore measured in cortical and papillary homogenates of diabetes insipidus (n=5), heterozygote (n=5) and normal (n=5) Long Evans rats. The urinary $PGE_2/PGF_{2\alpha}$ ratio was also measured in the three groups. Cortical PGE_2-9-ketoreductase activity was significantly lower ($p<0.01$) and papillary activity significantly higher ($p<0.01$) in diabetes insipidus than in control rats. This increased papillary activity corresponded to a decreased $PGE_2/PGF_{2\alpha}$ ratio in the diabetes insipidus rats.

The results suggest that ADH, in addition to its effects on arachidonic acid release, also influences renal PGE_2 catabolism.

Introduction

During the last 20 years knowledge of the relationships between the antidiuretic hormone (ADH) and several aspects of renal function has been gained from studies in the Brattleboro rat, which is devoid of endogenous ADH. Recently, a decreased urinary excretion of prostaglandins has been described in this strain [1,2] as well as decreased production of PGE by papillary homogenates [3].

In a previous study, we observed a different response of renal PGE_2 and $PGF_{2\alpha}$ in sodium-loaded diabetes insipidus Brattleboro and heterozygote control rats and a reduced $PGE_2/PGF_{2\alpha}$ ratio in the diabetes insipidus rats, suggesting an increased activity of the renal enzyme PGE_2-9-ketoreductase [4]. The present study was designed to measure the PGE_2-9-ketoreductase activity in the kidneys of diabetes insipidus rats, as compared to controls, and to correlate it with the urinary $PGE_2/PGF_{2\alpha}$ ratio.

Material and methods

Five diabetes insipidus rats, five heterozygote and five normal Long Evans rats, obtained from the Centraal Proefdierenbedrijf TNO (Zeist, Holland), were used in this study. They were fed a standard rat diet, containing 0.35g of sodium and 0.50g of potassium (K) per 100g (Labena, Assia Maabaroth, Israel) and drank distilled water ad libitum. Urine collections for prostaglandin measurement were obtained as previously described [4]. The animals were then anaesthetized with ether and a cannula inserted into the lower aorta. The kidneys were perfused with 50ml of heparinized saline (154mM) after clamping the aorta above the renal arteries and opening a renal vein for drainage. The kidneys were removed rapidly, immediately frozen on dry ice and kept at -70°C until prepared. Cortical and papillary homogenates were prepared in 9.2mM K-phosphate buffer (pH 7.3) containing 4.0mM MgCl and 1.4 dithithreitol 0.1mM. The homogenates were ultracentrifuged at 100,000G for 60 minutes and the supernatants used for enzyme assay by a modification of the method of Leslie and Levine [5] using PGE_2 as substrate at a final concentration of 0.5mM, and NADH as cofactor (1.0mM). $PGF_{2\alpha}$ was measured by radioimmunoassay (RIA) at times 0, 3 and 7 minutes. Urine prostaglandins were measured by RIA as previously described [6]. Protein was measured by the method of Macart and Gerbaut [7]. Statistical analysis was performed with the Student's 't' test for unpaired results.

Results

The PGE_2-9-ketoreductase activity in cortical homogenates of diabetes insipidus rats was 20.70±5.68 (SEM) pmoles $PGF_{2\alpha}$/min/mg protein. This was significantly lower ($p<0.01$) than the activity measured in both heterozygote and normal control rats. In contrast, the papillary enzyme activity in the diabetes insipidus group was 54.53±5.32 pmoles $PGF_{2\alpha}$/min/mg protein, which was significantly higher ($p<0.01$) than in controls. The urinary $PGE_2/PGF_{2\alpha}$ ratio was 0.32±0.03 in the diabetes insipidus rats. Values in heterozygote rats (1.11± 0.10) and in normal Long Evans rats (1.64±0.19) were significantly higher ($p<0.01$) (Table I).

TABLE I. Prostaglandin E_2-9-ketoreductase activity and urinary $PGE_2/PGF_{2\alpha}$ ratio in diabetic insipidus (DI), heterozygote (HE) and normal (N) rats

	PGE_2-9-ketoreductase activity pmoles $PGF_{2\alpha}$/min/mg protein		$PGE_2/PGF_{2\alpha}$ ratio
	cortex	papilla	
DI rats (n=5)	20.70±5.68*	54.53±5.32*	0.32±0.03*
HE rats (n=5)	43.31±6.05	35.78±6.77	1.11±0.10
N rats (n=5)	38.62±2.53	32.66±8.16	1.64±0.19

Results are expressed as mean ± SEM. *=$p<0.01$ compared with control groups

Discussion

The presence in the kidney of an enzyme with PGE_2-9-ketoreductase activity is of considerable interest, since Weber et al demonstrated changes in its activity in response to changes of dietary sodium intake in the rabbit [8]. In addition, Chaumet-Riffaud et al have observed an increased glomerular PGE_2-9-ketoreductase activity in sodium depleted rats [9]. Previous studies in the Brattleboro rat have demonstrated a decrease in the urinary excretion of PGE_2 [1,2], as well as a decrease of its production by papillary homogenates [3]. These changes are apparently reversible after treatment with ADH [10].

The known effect of ADH on prostaglandin synthesis is related to stimulation of phospholipase activity, leading to increased arachidonic acid release. However, we have previously observed a selective decrease in PGE_2 excretion, $PGF_{2\alpha}$ being quite similar in diabetes insipidus and control rats [2,4]. This fact, resulting in a decreased $PGE_2/PGF_{2\alpha}$ ratio, could be related to increased papillary PGE_2-9-ketoreductase activity. The results of the present study are in agreement with the above hypothesis, suggesting that ADH could influence PGE_2-9-ketoreductase activity.

It is also interesting to note the contrasting changes in the cortex and papilla. In this respect, Bankir et al have described an increased production of PGE_2 by glomeruli of Brattleboro rats, while papillae show a decreased production of PGE_2 [3]. In addition, prostaglandin production by glomeruli and papillae change in opposite directions in response to changes in sodium balance [9].

Finally, the information provided by the present work, indirectly confirms that the urinary $PGE_2/PGF_{2\alpha}$ ratio may be used as an approximate assessment of renal papillary PGE_2-9-ketoreductase activity, while urinary prostaglandins are probably unrelated to glomerular production.

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