NADH-DEPENDENT PGE₂-9-KETOREDUCTASE ACTIVITY IN
THE KIDNEY OF DIABETES INSIPIDUS BRATTLEBORO AND
LONG EVANS RATS: RELATIONSHIP WITH URINARY PGE₂
AND PGF₂α EXCRETION

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Summary

Previous studies have suggested that the activity of the renal enzyme PGE₂-9-
ketoreductase, catalysing the conversion of PGE₂ to PGF₂α, might be altered in
the diabetes insipidus Brattleboro rat.

Enzyme activity was therefore measured in cortical and papillary homo-
genates of diabetes insipidus (n=5), heterozygote (n=5) and normal (n=5) Long
Evans rats. The urinary PGE₂/PGF₂α ratio was also measured in the three
groups. Cortical PGE₂-9-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
PGE₂/PGF₂α ratio in the diabetes insipidus rats.

The results suggest that ADH, in addition to its effects on arachidonic acid
release, also influences renal PGE₂ 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 PGE₂ and
PGF₂α in sodium-loaded diabetes insipidus Brattleboro and heterozygote
control rats and a reduced PGE₂/PGF₂α ratio in the diabetes insipidus rats,
suggesting an increased activity of the renal enzyme PGE₂-9-ketoreductase [4].
The present study was designed to measure the PGE₂-9-ketoreductase activity
in the kidneys of diabetes insipidus rats, as compared to controls, and to corre-
late it with the urinary PGE₂/PGF₂α 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°С 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 PGE₂ as substrate at a final concentration of 0.5mM, and NADH as cofactor (1.0mM). PGF₂α 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 PGE₂-9-ketoreductase activity in cortical homogenates of diabetes insipidus rats was 20.70±5.68 (SEM) pmoles PGF₂α/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 PGF₂α/min/mg protein, which was significantly higher (p<0.01) than in controls. The urinary PGE₂/PGF₂α 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).

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<thead>
<tr>
<th></th>
<th>PGE₂-9-ketoreductase activity</th>
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<tr>
<td></td>
<td>pmoles PGF₂α/min/mg protein</td>
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<td>papilla</td>
<td></td>
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<td>38.62±2.53</td>
<td>32.66±8.16</td>
<td>1.64±0.19</td>
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</table>

TABLE 1. Prostaglandin E₂-9-ketoreductase activity and urinary PGF₂/PGF₂α ratio in diabetic insipidus (DI), heterozygote (HE) and normal (N) rats

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

The presence in the kidney of an enzyme with PGE₂-9-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 PGE₂-9-ketoreductase activity in sodium depleted rats [9]. Previous studies in the Brattleboro rat have demonstrated a decrease in the urinary excretion of PGE₂ [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 PGE₂ excretion, PGF₂α being quite similar in diabetes insipidus and control rats [2,4]. This fact, resulting in a decreased PGE₂/PGF₂α ratio, could be related to increased papillary PGE₂-9-ketoreductase activity. The results of the present study are in agreement with the above hypothesis, suggesting that ADH could influence PGE₂-9-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 PGE₂ by glomeruli of Brattleboro rats, while papillae show a decreased production of PGE₂ [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 PGE₂/PGF₂α ratio may be used as an approximate assessment of renal papillary PGE₂-9-ketoreductase activity, while urinary prostaglandins are probably unrelated to glomerular production.

Acknowledgments

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References