Activity Changes of Molybdenum Hydroxylases in Liver, Kidney and Jejunum of Streptozotocin-Induced Diabetic Rats

OMAR A.M. AL-BAR

Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ABSTRACT. The aim of this study is to investigate the effect of diabetes induction on the specific activity of two molybdenum hydroxylases, namely aldehyde oxidase and xanthine oxidase. Streptozotocin in a single i.p. injection (65 mg/kg) induced diabetes in male Wistar rats as indicated by a sharp and significant increase of plasma glucose level after 5 days of the treatment. Three tissues (liver, kidney and jejunum) were excised and assayed for the activity of aldehyde oxidase and xanthine oxidase. Two substrates (phthalazine and 3-methylisoquinoline) were used to measure aldehyde oxidase activity, while xanthine was used for xanthine oxidase. Hepatic aldehyde oxidase activity was increased significantly (P < 0.025) in diabetic rats as compared to controls when phthalazine was used as a substrate. The other two tissues (kidney, jejunum) did not show any significant variation in aldehyde oxidase activity when diabetic and control rats were compared. No significant change of this enzymic activity was noted in the three tissues (liver, kidney and jejunum) when 3-methylisoquinoline was used as a substrate. On the other hand, xanthine oxidase activity in liver and jejunum was markedly decreased (P < 0.005 and P < 0.0005, respectively) in diabetic rats while the enzymic activity of kidney remained unchanged. These results suggest the presence of multiple isomeric forms of molybdenum hydroxylases and some of these forms can be induced or suppressed by the diabetic inducing agent streptozotocin.

Introduction

The role of microsomal monooxygenases or mixed-function oxidases in the biotransformation of a wide range of drugs and other foreign substances (xenobiotics) was investigated by several studies\textsuperscript{1-3}. On the other hand, the vital role
of two cytosolic molybdenum hydroxylases, aldehyde oxidase (E.C.1.2.3.1) and xanthine oxidase (E.C.1.2.3.2) in the metabolism of N-heterocyclic compounds and some drugs had been observed increasingly\[4-7\].

The induction of molybdenum hydroxylases in rats is rarely described in the literature. However, the activity of hepatic molybdenum hydroxylases in rabbits and guinea pigs is induced by pre-treatment with phthalazine and melatonin\[8,9\]. Also, an increase in the activity of xanthine oxidase was reported when xanthine was given to mice\[10\]. Whether these enzymes inducing properties are related to diseases or not is still unknown.

In view of the limited information regarding whether disease may affect the activity of molybdenum hydroxylases, this study was conducted to investigate the relationship between diabetes and molybdenum hydroxylase activity.

**Materials and Methods**

**Chemicals**

Phthalazine was supplied by Aldrich Chemical Company. 3-Methylisouquinoline was purchased from ICN Pharmaceuticals Inc. Xanthine and streptozotocin were bought from Sigma Chemical Company.

**Animals**

Male Wistar rats weighing 200-240 g were obtained from King Fahd Medical Research Center, Faculty of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were housed in groups of 3, allowed to feed *ad lib* and kept in constant temperature at 24°C and on a 12 hr dark-light cycle. Diabetes was induced in rats by a single *i.p.* injection of streptozotocin at a dose of 65 mg per kg body weight dissolved in 50 mM citrate buffer, pH 4.5 just before use. Controls were injected with saline alone. All animals were killed by cervical dislocation after 5 days of treatment. Three tissues (liver, kidney and jejunum) were excised and directly frozen in liquid nitrogen and stored in a deep freezer for one week at –80°C. Blood samples were collected from jugular vein in polypropylene centrifuge tubes, and plasma was separated for glucose determination.

**Preparation of Enzymes**

Partially purified aldehyde oxidase and xanthine oxidase were prepared from liver, kidney and jejunum homogenates of both streptozotocin-treated and control rats according to the method of Johnson *et al.*\[11\]. Each tissue was weighed and transferred to a beaker containing two to three times by volume ice-cold
potassium chloride solution (1.15 w/v) and 0.1 mM EDTA. The tissues were
finely chopped using scissors and aliquots were transferred to a glass ho-
mogenizer. The homogenate was kept on ice, then was subjected to a steam
water bath in 500 ml Erlenmyer flasks at 50-55ºC for 10 minutes followed by
immediate cooling to 10ºC in ice. The homogenate was then centrifuged at 4ºC
for 45 minutes at 15,000 g. The resulting supernatant was filtered through glass
wool into a measuring cylinder, and solid ammonium sulfate was added to
reach 50% saturation (35.4 g/100 ml at 4ºC). The mixture was stirred on an ice-
bath using a magnetic stirrer. After completing the precipitation, the suspension
was centrifuged at 6000 g for 20 minutes at 4ºC, and the supernatant was dis-
carded. The precipitate was gently rinsed with distilled water and dissolved in a
minimum amount of 0.1 mM EDTA solution (approximately 3-5 ml). The par-
tially purified enzyme was then stored in a deep-freezer at –80ºC until used.

**Enzyme Assays**

The activity of partially purified aldehyde oxidase was determined spectro-
photometrically at 420 nm using the method described by Johnson et al.[9] with
both phthalazine (1 mM) and 3-methylisoquinoline (1 mM). The activity of xan-
thine oxidase was estimated with xanthine (50 µM) at 295 nm[8,12]. Initial
oxidation rates were followed using a Varian DMS 80 UV/VIS spectrophotometer at 37ºC in 67 mM phosphate buffer pH 7. The concentration of pro-
tein was determined using the Biuret method.

**Determination of Plasma Glucose**

The concentration of plasma glucose was determined spectrophotometrically
according to Trinder method[13].

**Results and Discussion**

**Plasma Glucose Levels**

The levels of plasma glucose in streptozotocin-induced diabetic and control
rats were found to be 29.0 ± 0.4 and 10.37 ± 2.05 mM respectively. The elevation of plasma glucose levels in treated animals was significant (P < 0.0005) as
compared to control. These results are similar to those reported by Khoja and
Salem[14].

**Aldehyde Oxidase and Xanthine Oxidase Activities**

The specific activity of partially purified aldehyde oxidase prepared from
different tissues (liver, kidney and jejunum) of both control and diabetic rats
was measured spectrophotometrically using two substrates (phthalazine, and 3-methylisoquinoline). Table 1 shows that using phthalazine as a substrate, the activity of hepatic aldehyde oxidase was significantly higher in streptozotocin-induced diabetic rats than in control animals (P < 0.025), whereas kidney and jejunal aldehyde oxidase activity was not significantly affected by the same treatment.

TABLE 1. Effect of streptozotocin treatment on rat aldehyde oxidase activity using phthalazine.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity* (µmol/min/mg protein)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 6)</td>
<td>Treated (n = 6)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.0039 ± 0.0009</td>
<td>0.0081 ± 0.0043</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0182 ± 0.0083</td>
<td>0.0191 ± 0.0047</td>
</tr>
</tbody>
</table>

*Values are given as means ± S.D.
*n.s.: not significant.

Furthermore, it is noticed from Table 1 that the activity of hepatic aldehyde oxidase was lower than kidney and jejunal enzyme. Using 3-methylisoquinoline as a substrate for aldehyde oxidase, no significant changes were shown in the enzymatic activity among the three tissues of diabetic rats compared to the controls (Table 2). Table 3 shows that the activity of xanthine oxidase of both liver and jejunum of streptozotocin-treated rats was significantly suppressed (P < 0.005 and P < 0.0005 respectively), while the enzyme activity from kidney was not affected by this treatment.

TABLE 2. Effect of streptozotocin treatment on rat aldehyde oxidase activity using 3-methylisoquinoline.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity* (µmol/min/mg protein)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 6)</td>
<td>Treated (n = 6)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.0023 ± 0.0006</td>
<td>0.0024 ± 0.0012</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0141 ± 0.0025</td>
<td>0.0117 ± 0.0023</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.0110 ± 0.0017</td>
<td>0.0112 ± 0.0004</td>
</tr>
</tbody>
</table>

*Values are given as means ± S.D.
*n.s.: not significant.
TABLE 3. Effect of streptozotocin treatment on rat xanthine oxidase activity using xanthine.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity* (µmol/min/mg protein)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 6)</td>
<td>Treated (n = 6)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.0089 ± 0.0018</td>
<td>0.0048 ± 0.0019</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0023 ± 0.0011</td>
<td>0.0023 ± 0.0010</td>
</tr>
<tr>
<td>Jejunum</td>
<td>0.0206 ± 0.0027</td>
<td>0.0066 ± 0.0002</td>
</tr>
</tbody>
</table>

*Values are given as means ± S.D.

The results of Table 3 show that jejunum has the highest xanthine oxidase activity followed by liver and then kidney of both diabetic and control rats. These findings confirm the same order of activity previously described by Stripe and Corte[15]. Several factors are known to regulate molybdenum hydroxylases activity in vivo including hormonal influences, genetic determinants and chemical induction. It has been shown that testosterone induced both aldehyde oxidase and xanthine oxidase in mice and rats[16-18]. The activity of molybdenum hydroxylases was shown to increase in rabbit liver using phthalazine or 1-phthalazinone[9].

The present study shows that the activity of hepatic aldehyde oxidase with both phthalazine and 3-methylisoquinoline was much lower than kidney or jejunum (Tables 1 and 2). This could be attributed to tissue distribution of the enzyme. In addition, there is a difference between hepatic, kidney and jejunal enzyme activity using these substrates. This variation may give an indication to the presence of multiple forms (isoenzymes) of aldehyde oxidase. At least two aldehyde oxidase isozymes were reported to be isolated from various tissues of mice and rats[17,19].

Streptozotocin is a N-nitroso-compound which acts as a nitric oxide donor in pancreatic islets, thus damaging insulin secreting cells and producing diabetes in animals[20]. This classical diabetogen was reported to enhance the generation of superoxide anion (O2–) by the xanthine oxidase system of pancreatic beta-cells[21]. Application of streptozotocin to isolated rat pancreatic islets produced a marked reduction of glucose stimulated insulin secretion and treatment of streptozotocin morphologically revealed that almost all beta-cells of the islets were destroyed[22]. Recently a plausible mechanism of streptozotocin action in β-cells of the rat pancreas was reported to enhance ATP dephosphorylation after streptozotocin treatment which in turn supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals and finally β-cells undergo the
In conclusion, this study confirmed earlier results that molybdenum hydroxylase activity can be altered by various physiological stress conditions such as diabetes, and these changes (inductive or suppressive) are tissue-related.

References


تغيرات نشاطية إنزيمات المولبيدينوم هيدروكسيغلز في كبد وكلية
وصائم الجرذان المصابة بالسكري المستثخت بالاستربوتوزوتونين

عمر عبد الرحمن مصطفى البار
قسم الكيمياء الحيوية، كلية العلوم، جامعة الملك عبد العزيز
جدة - المملكة العربية السعودية

المستخلص. هدف الدراسة هو التحقق من تأثير السكري المستثخت على نشاطية إنزيمات المولبيدينوم هيدروكسيغلز. تسبب استعمال الاستربوتوزوتونين ارتفاع معنوي في جلوكوز مصل الدم في الجرذان (P<0.005). تم تحضير اثنين من إنزيمات المولبيدينوم هيدروكسيغلز (ألدهايد أوكسيداز و زانثين أوكسيداز) بمقابلة جزئية من ثلاثة أنسجة مختلفة (الكبد والكلية والصائم) في ذكور الجرذان البالغة واستخدم اثنان من مواد تفاعل ألدهايد أوكسيداز (3- ميثابيل أيزوكينولين و النثالازين). أعطت النتائج نشاطية ألدهايد أوكسيداز الكبدى مرتفعة معنويًا (P<0.025) في الجرذان المصابة بالسكري مقارنة مع نشاطية إنزيمات الكلية والصائم. بينما مع 3- ميثابيل أيزوكينولين لم تكن هناك اختلافات معنوية في نشاطية هذا الإنزيم.

أظهر زانثين أوكسيداز نشاطية منخفضة باستخدام الزائنين كمادة تفاعل في كل من الكبد والصائم (P<0.005 و P<0.0005) على التوالي في الجرذان المصابة بالسكري مقارنة بالجرذان الضابطة. أسهمت النتائج إلى وجود أشكال جزيئية أو أيزوميرية عديدة لهذه الإنزيمات وأن تأثير الاستربوتوزوتونين على هذه الهيئات الجزيئية متفاوت.