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

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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[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-Methylisoquinoline 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 24ºC 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 –80ºC. 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 homogenizer. 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 discarded. 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 partially purified enzyme was then stored in a deep-freezer at –80ºC until used.

Enzyme Assays

The activity of partially purified aldehyde oxidase was determined spectrophotometrically 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 xanthine 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 protein 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.

Tissue	Specific activity* (μmol/min/mg protein)		P
	Control (n = 6)	Treated (n = 6)	
Liver	0.0039 ± 0.0009	0.0081 ± 0.0043	< 0.025
Kidney	0.0182 ± 0.0083	0.0191 ± 0.0047	n.s.

*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-methyl-isoquinoline.

Tissue	Specific activity* (μmol/min/mg protein)		P
	Control (n = 6)	Treated (n = 6)	
Liver	0.0023 ± 0.0006	0.0024 ± 0.0012	n.s.
Kidney	0.0141 ± 0.0025	0.0117 ± 0.0023	n.s.
Jejunum	0.0110 ± 0.0017	0.0112 ± 0.0004	n.s.

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

TABLE 3. Effect of streptozotocin treatment on rat xanthine oxidase activity using xanthine.

Tissue	Specific activity* (μmol/min/mg protein)		P
	Control (n = 6)	Treated (n = 6)	
Liver	0.0089 ± 0.0018	0.0048 ± 0.0019	< 0.005
Kidney	0.0023 ± 0.0011	0.0023 ± 0.0010	n.s.
Jejunum	0.0206 ± 0.0027	0.0066 ± 0.0002	< 0.0005

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

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 (O^{2-}) 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

destruction by necrosis[23]. 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.

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تغيرات نشاطية إنزيمات الموليبيدنوم هيدروكسيلزز فى كبد و كلية وصائم الجرذان المصابة بالسكري المستحث بالاستربتوزوتوسين

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المستخلص. هدف الدراسة هو التحقق من تأثير السكري المستحث على نشاطية إنزيمات الموليبيدنوم هيدروكسيلزز . تسبب استعمال الاستربتوزوتوسين بارتفاع معنوي في جلوكوز مصل الدم في الجرذان ($P<0.0005$). تم تحضير اثنين من إنزيمات الموليبيدنوم هيدروكسيلزز (ألدهايد أوكسيديز و زانثين أوكسيديز) بنقاوة جزئية من ثلاثة أنسجة مختلفة (الكبد والكلية والصائم) في ذكور الجرذان البالغة و استخدم اثنان من مواد تفاعل ألدهايد أوكسيديز (٣- ميثايل أيزوكينولين و الثالازين). أعطى الثالازين نشاطية ألدهايد أوكسيديز الكبدى مرتفعة معنويًا ($P<0.025$) في الجرذان المصابة بالسكري مقارنة مع نشاطية إنزيمات الكلية والصائم . بينما مع ٣-ميثايل أيزوكينولين لم تكن هناك اختلافات معنوية في نشاطية هذا الإنزيم.

أظهر زانثين أوكسيديز نشاطية منخفضة باستخدام الزانثين كمادة تفاعل فى كل من الكبد و الصائم ($P<0.005$ و $P<0.0005$) على التوالي في الجرذان المصابة بالسكري مقارنة بالجرذان الضابطة. أشارت النتائج إلى وجود أشكال جزيئية أو أيزوميرية عديدة لهذه الإنزيمات وأن تأثير الاستربتوزوتوسين على هذه الهيئات الجزيئية متفاوت .