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Xanthine Oxidase during Human Fetal Development[1]

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ABSTRACT. Through oxygen free radical production, xanthine oxidase (XOD, E.C.1.2.3.2) has been implicated in the pathogenesis of postischemic and hyperoxic tissue injuries among newborn. We measured the activity and evaluated the kinetic characteristics of XOD in human fetal liver, intestine, brain, and myocardium. Both the fetal liver and intestine contain a high XOD activity through gestation. The activity increases in the liver and decreases in the intestine with advancing gestation. The apparent Km for hypoxanthine is 4.8–5.5 μM in the intestine throughout gestation and in the liver at term but higher than 30 μM in the liver during the first half of pregnancy. The activity is undetectable both in the fetal brain and myocardium throughout gestation. Thus, XOD activity is present at least in the liver and intestine to account for the oxidation of hypoxanthine and xanthine. However, direct evidence for adenine nucleotide catabolism, followed by oxidation of the accumulated hypoxanthine during tissue reoxygenation in the human liver or intestine is not available. (*Pediatr Res* 27: 286–288, 1990)

Abbreviations

XOD, xanthine dehydrogenase/oxidase
HGPRT, hypoxanthine/guanine phosphoribosyltransferase

XOD (E.C. 1.2.1.37/1.2.3.2) catalyses the conversion of hypoxanthine to xanthine and on to uric acid. The enzyme exists *in vivo* predominantly as a NAD^+-dependent dehydrogenase but is converted to an oxygen-dependent oxidase reversibly by sulfhydryl oxidation or irreversibly by proteolysis. The human enzyme has an apparent Km of about 10–11 μM for hypoxanthine in the liver and jejunum, where the highest enzyme activity is found (1–8).

In the oxidation of hypoxanthine and xanthine, superoxide radicals are generated. These, and secondary radicals derived from them, are capable of initiating chain-reactions resulting in cell injury through lipid peroxidation, protein damage, etc. (9). XOD has been postulated to be the major source of superoxide radicals during reoxygenation of postischemic tissues. During ischemia, hypoxanthine accumulates as a result of adenine nucleotide catabolism, and simultaneously xanthine dehydrogenase is converted into oxidase (10). When oxygen is reintroduced to the tissue during postischemic reperfusion, hypoxanthine is oxidized to xanthine and uric acid by the oxidase. The result is a burst of superoxide radical and hydrogen peroxide production, and possibly tissue injury (2, 5, 11). This mechanism has been strongly implicated in the pathogenesis of ischemia-reperfusion injury of the small intestine (12–15).

Tissue damage in the CNS, lungs, myocardium, liver, and intestine is common among extremely premature infants. A role for the hypoxanthine-XOD system in the pathogenesis of several prematurity-related problems, including bronchopulmonary dysplasia, necrotizing enterocolitis, and retinopathy of prematurity has been suggested (16, 17). Yet, evidence for this hypothesis is mostly circumstantial, and essential data concerning the activity of XOD in fetal tissues as well as its kinetic characteristics during fetal development are lacking.

The aim of our study was to measure the activity and substrate affinity of XOD in the liver, brain, myocardium, and intestine during human fetal development.

MATERIALS AND METHODS

[8-^{14}C]hypoxanthine was obtained from Amersham International, Little Chalfont, UK; cellulose thin-layer plates from Eastman Kodak, Rochester, NY; NAD^+ from Boehringer Mannheim, Mannheim, FRG; pyruvic acid and lactic dehydrogenase from Sigma Chemical, St. Louis, MO; and Aquasol from New England Nuclear, Boston, MA.

Fetal tissues were obtained from elective terminations, and neonatal tissues from necropsies. The fetal samples were taken within 2 h after termination, and the neonatal samples within 20 h after death. The material consisted of samples from 45 fetuses (10–20 gestational wk), nine preterm babies (25–28 wk), and 15 term babies. The intestinal samples were taken from proximal jejunum. None of the neonates had signs of necrotizing enterocolitis or any other pathologic process of the intestine. All samples were quickly frozen and stored at −70°C. No decrease in enzyme activity took place under these conditions.

The tissue samples were homogenized with a Teflon pestle in 5 vol of 100 mM Tris-HCl, pH 8.0, containing 0.02% β-mercaptoethanol, and sonicated for 15 s at 50 W three times on ice. The sonicates were then centrifuged at 10 000 × *g* for 20 min at +4°C, and the supernatants dialyzed against the homogenization buffer at +4°C for 17 h.

The assay mixture contained 160 μM [8-^{14}C]hypoxanthine (sp act 50–60 mCi/mmol), 5 mM $MgCl_2$, 0.3 mM NAD^+, 2 mM pyruvic acid, and 18 IU of lactic dehydrogenase (18), and 25 μL of sample in a total volume of 50 μL. The incubation was carried out at +37°C in air on an oscillating water bath, and terminated with 5 μL of 4.2 N perchloric acid on ice. The mixture was subsequently neutralized with cold 4.42 N KOH, and centrifuged at 800 × *g* for 10 min. A 10-μL aliquot of the supernatant was spotted on a cellulose chromatogram sheet, and developed in 95% ethanol: 1 M ammonium acetate, pH 7.5 (75:30) for about 2.5 h. The spots containing hypoxanthine and xanthine plus uric acid were scraped off and counted in Aquasol using liquid scintillation. The production of xanthine and uric acid from hypoxanthine was linear for at least 30 min. The kinetic param-

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eters were assayed at +37°C and pH 8.0 in the presence of 4.1–79.5 μM [8-^{14}C]hypoxanthine (3, 19, 20).

Possible changes in the enzyme activity during the first 24 h after death were evaluated using samples of rabbit liver, and identical storage conditions.

Blank values, obtained by adding perchloric acid and placing the tubes on ice before adding the isotope, were subtracted from the corresponding experimental values in all experiments.

All assays were performed in duplicate with three time points within the linear range of each assay. The Km and V_{max} values were estimated from double-reciprocal plots. Protein was measured according to Peterson (21). In statistical analysis, Student's *t* test (two-sided) was used. Results are given as mean + SD.

RESULTS

The activity of XOD increased significantly in the fetal liver (Fig. 1*A*) but decreased in the intestine (Fig. 1*B*) toward term. The intestinal activity exceeded that in the liver 4- to 37-fold during the first two trimesters but settled at a similar level near term.

The hepatic enzyme had an apparent Km of more than 30 μM (V_{max} 0.14 ± 0.10 nmol/mg protein/min) for hypoxanthine during the first half of pregnancy but less than 6 μM (V_{max} 1.6 ± 0.3 nmol/mg protein/min) at term (Fig. 2). The intestinal enzyme had a Km of 4.8 ± 1.6 μM (V_{max} 3.3 ± 1.4 nmol/mg protein/min) for hypoxanthine throughout gestation.

In both the fetal brain and myocardium XOD activity was less than the limit of detection of the assay (0.02 nmol/mg protein/min) throughout gestation. The enzyme activity did not change in samples of rabbit liver during storage at +4°C for up to 24 h after death.

DISCUSSION

Although the conversion of xanthine dehydrogenase to oxidase most likely was completed in our samples before assay, we decided to measure the combined activity, which does not seem to change during ischemia (10).

Even though the activity of XOD in the fetal liver appears to increase with gestation, it remains roughly of the same magnitude as that reported in adult liver (4, 22). The kinetic parameters of fetal hepatic XOD also appear to change. Its Km for hypoxanthine decreases as a function of gestational age, and at term is of the same magnitude as that in adult liver (3).

In the human fetal intestine, the activity of XOD appears to decrease from a high level during early fetal life to adult levels (4) by term, and its Km for hypoxanthine is of the same magnitude as that in the adult intestine (3). Both the fetal hepatic and intestinal activities exceed that of the human trophoblast throughout gestation (19).

The kinetic characteristics of XOD, and the other major cytoplasmic hypoxanthine-metabolizing enzyme, HGPRT (E.C. 2.4.2.8), determine the fate of intracellular hypoxanthine. In adult human tissues, both HGPRT and XOD appear to have a Km of about 4–11 μM for hypoxanthine (3, 23). The Km for hypoxanthine of hepatic HGPRT (about 59 μM) (Vettenranta K, Raivio KO, unpublished data) seems to be of the same

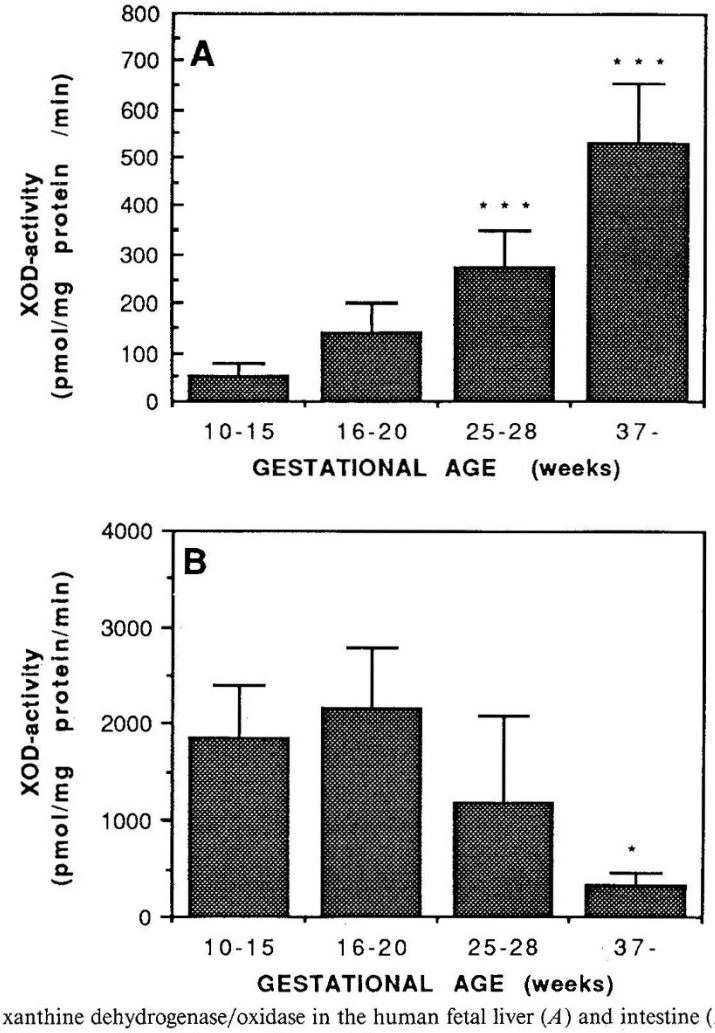

Fig. 1. The apparent activity of xanthine dehydrogenase/oxidase in the human fetal liver (*A*) and intestine (*B*) as a function of gestational age. *** indicates $p < 0.001$ and * $p < 0.05$ *versus* values of the preceding gestational age group.

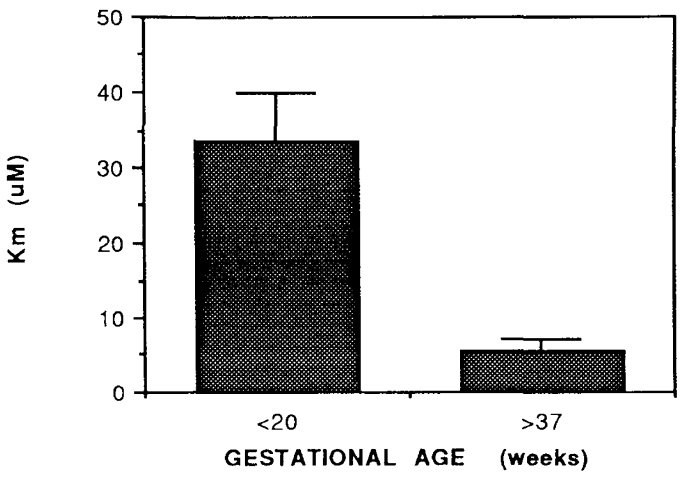

Fig. 2. The apparent Km of the human fetal hepatic XOD as a function of gestational age.

magnitude as that of XOD during early fetal life but substantially higher near term. The hepatic activities of HGPRT (Vettenranta K, Raivio KO, unpublished data) and XOD seem to increase in parallel with advancing gestation. Thus, in the human fetal liver near term, the capacity to metabolize hypoxanthine appears high and its catabolism at low concentrations kinetically favored over reutilization.

The gestational change observed in the kinetic characteristics of hepatic XOD might well be explained by different isoforms of the enzyme. However, any data on dissimilar isoforms appearing in adult or fetal tissues have not been reported. In addition, even though the effect of several inhibitors on XOD activity has been demonstrated, their physiologic role as well as possible changes in that role with gestation also remain to be established (4).

Conflicting data on the activity of XOD in the human adult brain have been reported (22, 25). Our data appear to agree with those of Krenitsky and co-workers (25) in suggesting a low XOD activity in the human cerebrum.

Animal experiments have provided evidence suggesting a role for XOD in the pathogenesis of postischemic myocardial injury and dysfunction (26–28). Differences in the myocardial activity of XOD between various mammalian species (29) have, however, prompted studies on its role in the pathogenesis of human myocardial injury. Our data on the human fetal heart are in agreement with those of the majority of previous investigators (30–34), who could not find any or only trace amounts of XOD activity in adult human myocardium. Although Wajner and Harkness (22) recently reported high XOD activity in adult human heart, it thus appears more likely that XOD is not a significant source of oxygen free radicals in the human myocardium.

Although our data do not contradict the concept of XOD as a source of oxygen-free radicals in the neonatal postischemic liver (35) or intestine (12) by verifying the presence of XOD activity in these tissues throughout gestation, conclusions concerning *in vivo* mechanisms are difficult to draw from studies using disrupted cells. Consequently, the putative role of XOD in neonatal postischemic tissue injuries as well as the pathways of fetal hepatic and intestinal hypoxanthine catabolism, essentially remain to be further studied using, *e.g.* cultured cells and/or perfusion of the liver and intestine.

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