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Glutathione-related enzyme activities in human fetal adrenal, liver, and kidney[1]

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KEY WORDS glutathione transferases; glutathione reductase; glutathione peroxidase; adrenal glands; liver; kidney; fetus; microsomes; mitochondria

AIM: To understand the capacity of fetal adrenal to catalyze reaction metabolites. **METHODS**: Subcellular fractions were prepared by differential centrifugation in fetal adrenal and liver. Glutathione (GSH)-transferase, reductase, and peroxidase were measured. **RESULTS**: The mean values (μmol·min^{-1}/g protein) of GSH-transferase activities in adrenal microsome (112 ± 34), mitochondria (62 ± 35), and cytosol (191 ± 89) were 373 %, 270 %, and 167 %, respectively, higher than those in the corresponding fractions of fetal liver. Adrenal microsomal GSH-transferase was positively correlated with adrenal microsomal P-450 (r = 0.821, P < 0.01), and with adrenal microsomal aminopyrine *N*-demethylase (r = 0.829, P < 0.01). The GSH reductase contents (μmol·min^{-1}/g protein) in adrenal mitochondria (24 ± 14), and in S_9 (36 ± 15) were almost 5 times higher, compared with that in liver. Selenium-dependent GSH peroxidase was present in all the adrenal. **CONCLUSION**: Fetal adrenal, with greater capacities than those of liver in detoxifying reaction, may act as a drug-metabolizing organ during development.

The catalytic activities of monooxygenase and the extent of xenobiotic metabolism in human tissues have been the object of considerable study, because it still seems to be important with respect to the possible toxicological significance in human fetus[1,2]. We previously reported the existence of components of P-450 enzyme system and the capacity of this system in metabolizing xenobiotics in fetal liver[3,4], and the active role of glutathione (GSH) *S*-transferase in fetal hepatic metabolism of toxic electrophiles[5]. The fetal adrenal monooxygenase activties were even greater than that of fetal liver[6]. The GSH *S*-transferase and other GSH-related enzyme activities (GSH reductase and GSH peroxidase) were involved in detoxification of xenobiotics[7]. GSH peroxidase may play an important role in protective effect against cell injury by lipid peroxidase induced in the processes of the steroid hormone synthesis[8]. Thus, the purpose of this study was to understand the ability and the scope of fetal adrenal to handle metabolic compounds.

MATERIALS AND METHODS

Fetus Human fetal specimens were obtained by therapeutic abortion and legal abortion at 16 - 24 wk of gestation, approved by the Academic Committee and the Ethics Committee of Hubei Medical University. Clinical data see Tab 1.

Tab 1. Clinical data.

Fetus	Maternal age/a	Gestation/wk	Sex
1	24	16	M
2	30	24	F
3	28	17	F
4	25	20	F
5	25	21	F
6	26	24	M
7	27	24	M
8	25	24	M
9	33	19	M
10	23	24	M

Subcellular fractions After sedimentation of the nuclear fraction (200 × *g* for 10 min), supernatant was spun at 9000 × *g* for 20 min to get mitochondria. The mitochondrial pellets were washed with wash buffer (edetic acid 10 mmol·L^{-1}, 1.15 % KCl, pH 7.4), and spun again at 9000 × *g* for 20 min, to avoid the contamination of mitochondria fraction by soluble enzymes. The postmitochondria supernatant (S_9) was spun at 105 000 × *g* for 60 min to get microsomes.

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The microsomal pellets were suspended in sucrose 0.25 mol·L^{-1} corresponding to 1 g of adrenal or 2 g of liver (or kidney) per mL. The postmicrosomal fraction (cytosol) was used for the determination of GSH *S*-transferase.

Assay techniques GSH *S*-transferase activity was assayed[5]. Determination of GSH reductase, in the reaction system of 1 mL contained: oxidized glutathione (GSSG) 1.0, NADPH 0.05, edetic acid 0.5, sodium phosphate buffer 0.1 mmol·L^{-1} (pH 7.6) and a suitable amount of the GSH reductase sample to give a change in absorbance of 0.05 – 0.3 ·min^{-1}[9]. GSH peroxidase was measured spectrophotometrically. The enzymatic reaction was initiated by addition of 0.1 mL of H_2O_2 2 mmol·L^{-1}. The conversion of NADPH to NADP was followed by continuous recording of the change in absorbance of the system at 340 nm between 2 and 4 min after initiation of the reaction[10]. The concentration of P-450 was based on the use of the extinction coefficient of 105 mmol·L^{-1}·cm^{-1} for reduced cytochrome P-450 minus oxidized cytochrome P-450. Aminopyrine *N*-demethylase was determined by measuring the production of formaldehyde[4] (using 8 mmol·L^{-1} for 0.5 – 1.0 mg microsomal protein).

The enzyme activities in adrenal, liver, and/or kidney were measured in duplicate and simultaneously.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Difference between two groups of the adrenal and liver fractions was detected using the paired t test. Correlation was examined by linear regression analysis.

RESULTS

The fetal adrenal bore the characteristics with small tissue weight and higher microsomal recovery rate (by determination from the proportion of the weight of 105 000 × g pellet per gram of tissue) when compared with fetal liver and kidney (Tab 2).

GSH *S*-transferase The "aryl transferase" substrate 1-chloro-2, 4-dinitrobenzene (CDNB) was selected because examination was limited by the small amount of adrenal gland available. In the present study, GSH *S*-transferase activity was found in S_9, and the mitochondrial, microsomal, and cytosolic fractions in adrenal, liver, and kidney. The higher activities of mitochondrial and microsomal GSH *S*-transferases in adrenal were about 270 % and 373 %, respectively, of the corresponding activity in liver (Tab 3).

Tab 2. Tissue weight, microsomal recovery, P-450 content, and aminopyrine *N*-demethylase activity in fetus. $n = 10$ fetuses. $\bar{x} \pm s$.

	Adrenal	Liver	Kidney
Tissue weight, g	2.8 ± 1.0	35 ± 14	8.6 ± 2.4
Microsome			
Recovery, %	20 ± 13	14 ± 6	7 ± 4
Protein, mg/g tissue	5.0 ± 2.2	10.7 ± 4.7	5.8 ± 1.6
P-450, μmol/g protein	0.38 ± 0.24	0.15 ± 0.04	–
Aminopyrine *N*-demethylase, μmol·min^{-1}/g protein	0.33 ± 0.12	0.26 ± 0.14	–

Tab 3. GSH *S*-transferase activity in cellular fractions of human fetus. $n = 10$ fetuses. $\bar{x} \pm s$.

	GSH *S*-transferase, μmol·min^{-1}/g protein			
	Mitochondria	S_9	Cytosol	Microsome
Adrenal	62 ± 35	147 ± 84	191 ± 89	112 ± 34
Liver	23 ± 15	73 ± 40	114 ± 66	30 ± 28
Kidney	34 ± 32	167 ± 69	86 ± 37	55 ± 44

Adrenal microsomal GSH *S*-transferase was highly correlated with adrenal microsomal P-450 content. However, in microsomal fraction of liver, poor relationship exhibited between the rate of CDNB catalyzation and P-450 content ($n = 10$, $r = 0.448$, $P > 0.05$). Moreover, in adrenal microsomes, high positive correlation was observed between GSH *S*-transferase activity and aminopyrine *N*-demethylase activity, which was catalyzed via cytochrome P-450 enzyme (Fig 1).

Though unchanged GSH *S*-transferase activity in developing process of fetal liver was proved by determination using CDNB as a substrate, adrenal GSH *S*-transferase in microsomes declined with advancing fetal development from 4 – 6 months ($n = 10$, $r = -0.66$, $P < 0.05$).

Glutathione reductase (GSH reductase) and glutathione peroxidase (GSH peroxidase) With one case showing no detectable activity, GSH reductase contents in the other 9 cases, were below 10 μmol·min^{-1}/g protein in liver mitochondria. GSH

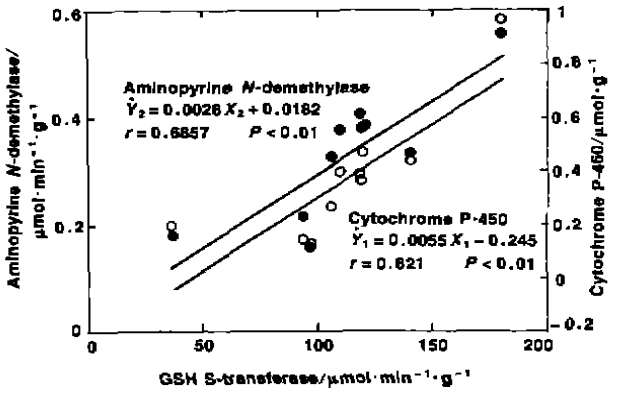

Fig 1. Correlations between GSH *S*-transferase activity and cytochrome P-450 content (○), or and aminopyrine *N*-demethylase activity (●) in fetal adrenal microsomes.

reductase in liver S_9 was almost in the same range, but slightly higher than that in mitochondria. Compared with the corresponding fraction of liver, higher content of GSH reductase was shown in adrenal mitochondria and S_9 at 24 ± 14 and 36 ± 15 $\mu mol \cdot min^{-1} \cdot g^{-1}$, respectively. The interindividual variance of GSH reductase was 4.5 – 6.4 fold in these two subcellular fractions (Fig 2).

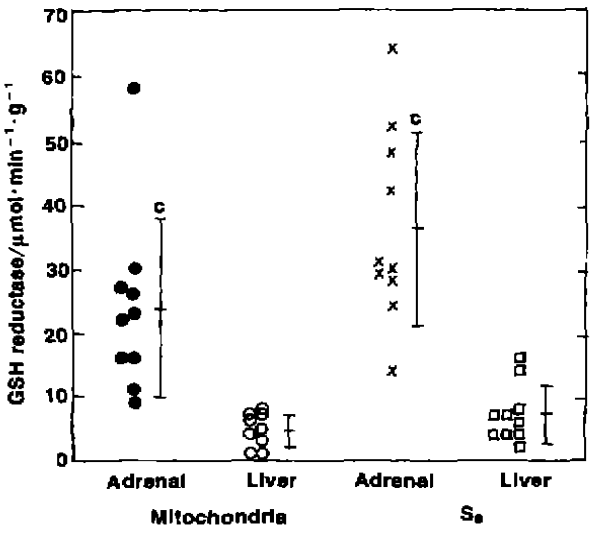

Fig 2. Distribution of GSH reductase in fetal adrenal mitochondria (●), S_9 (×), and in fetal liver mitochondria (○), S_9 (□). $n = 9 - 10$ fetuses. $\bar{x} \pm s$. $^cP < 0.01$ *vs* corresponding fraction of liver.

The activity of selenium-dependent GSH peroxidase which utilized hydrogen peroxides as substrate was present in adrenal mitochondria and S_9 in all 10 cases, at 482 % and 179 %, respectively, of the corresponding fraction of liver. Six out of 10 cases showed no measurable GSH peroxidase activity in liver mitochondria, the reason is unknown (Fig 3).

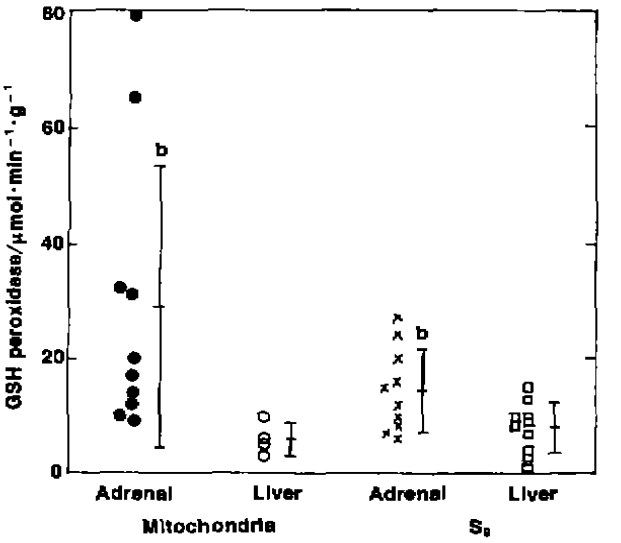

Fig 3. Distribution of GSH peroxidase in fetal adrenal mitochondria (●), S_9 (×), and in fetal liver mitochondria (○), S_9 (□). $n = 4 - 10$ fetuses. $\bar{x} \pm s$. $^bP < 0.05$ *vs* corresponding fraction of liver.

DISCUSSION

Evidences suggest that the level of expression of glutathione *S*-transferase is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals and that the biological control of glutathione *S*-transferase is complex as it exhibits sex-, age-, tissue-, species-specific patterns of expression[11]. However, adrenal gland is the organ that has not been extensively studied.

Microsomal GSH *S*-transferase, which is an enzyme distinct from the cytosolic[12], localized in the membrane of the endoplasmic reticulum might play a special role in drug metabolism, since the cytochrome P-450 system (which produces many of the reactive intermediates of xenobiotic metabolism which can serve as substrates for the GSH *S*-transferase) is localized in the same membrane. Such membrane-bound GSH *S*-transferase may be advantageous for the inactivation of reactive metabolites formed within the microsomal membrane, which are lipophilic and, consequently, preferentially remain within the membrane rather than diffusing into the cytoplasm. From the evidence that

microsomal GSH *S*-transferase is strongly enhanced by chemical compounds treatment, it has been suggested that the potentiation of microsomal GSH *S*-transferase activity might be an adaptive mechanism increasing GSH *S*-transferase activity when it is needed[13]. It is not clear whether this is true in fetal development, however, this speculation may explain the higher concentration of GSH *S*-transferase in fetal adrenals than in fetal liver. For it is supported by the evidence that fetal adrenal has more active P-450 system[6] and the correlation analysis of GSH *S*-transferase and P-450 content, and aminopyrine *N*-demethylase (Fig 1).

It was speculated that interorgan GSH redox cycle may be operative wherein liver is the central to the reduction of GSSG[14]. In our results, both GSH reductase and GSH peroxidase were more abundant in fetal adrenal than that of liver. This is suggestive of greater capacity of adrenal than that of liver to reduce GSSG. This observation indicated that adrenal gland might play an important role in the maintaining the redox of GSH in the gestation period. Lower levels of GSH reductase/GSH peroxidase in fetal liver suggested the possibility of liver being more susceptible to toxic response to electrophilic compounds that require the GSH redox system for their detoxification.

On the whole, the present observations underline our previous suggestion[6], that adrenal gland not only synthesize gestational steroid hormones, but also serve as important drug-metabolizing organ in the fetus. However, these facts call for the attention to the possibility that there might be interactions and interference's between xenobiotic- and steroid-metabolizing activities[15].

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人胎肾上腺、肝和肾内谷胱甘肽相关酶活性[1]

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关键词 谷胱甘肽转移酶类; 谷胱甘肽还原酶类; 谷胱甘肽过氧化物酶类; 肾上腺; 肝; 肾; 胎儿; 微粒体; 线粒体

目的: 通过测定谷胱甘肽相关酶活性及其在亚细胞分布, 了解胎肾上腺在发育期间对活性代谢产

物的解毒处理能力. 方法: 制备肾上腺、肝亚细胞组分. 测定谷胱甘肽转移酶(GST)、还原酶、过氧化物酶. 结果: GST在胎肾上腺微粒体、线粒体、胞浆中含量分别是肝各亚细胞组分中的373 %、270 %和167 %. 肾上腺微粒体GST活性与细胞色素P-450、与氨基比林脱甲基酶活性皆呈正相关. 肾上腺线粒体谷胱甘肽还原酶、过氧化物酶分别是肝线粒体中的506 %和482 %. 结论: 胎肾上腺有比胎肝更大的解毒能力. 提示胎肾上腺兼有药物代谢器官的功能.

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Dual effects of 5-hydroxytryptamine on stable analogue of thromboxane A_2-induced aggregation and release reaction in rabbit platelets[1]

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KEY WORDS platelet aggregation; adenosine triphosphate; calcium; serotonin; thromboxane A_2

AIM: To study the effects of 5-hydroxytryptamine (5-HT) on stable analogue of thromboxane A_2 (STA_2)-induced platelet shape, aggregation, and release reaction. **METHODS**: Platelet shape change and aggregation were quantified by the light transmission through platelet-rich plasma (PRP). Release reaction was evaluated by the amount of ATP in the medium and cytosolic-free Ca^{2+} was measured by fluorescent imaging. **RESULTS**: (1) STA_2 0.3 - 3 $\mu mol \cdot L^{-1}$-induced shape change followed by aggregation. When STA_2 1 or 3 $\mu mol \cdot L^{-1}$ was added to PRP, the release reaction was occurred. Pretreatment of PRP with 5-HT 3 $\mu mol \cdot L^{-1}$, the shape change by STA_2 was abolished and the aggregation by STA_2 0.3 $\mu mol \cdot L^{-1}$ was enhanced ($P < 0.01$), STA_2 1 or 3 $\mu mol \cdot L^{-1}$-induced aggregation was not affected, but the release reaction was partially suppressed ($P < 0.01$). (2) STA_2 0.3 $\mu mol \cdot L^{-1}$-induced $[Ca^{2+}]_i$ elevation was further increased by 5-HT pretreatment, but the $[Ca^{2+}]_i$ mobilizations by STA_2 3 $\mu mol \cdot L^{-1}$ was decreased by 5-HT, especially the peak level. (3) The aggregation without release reaction was increased from 3.4 ± 2.1 to 25.6 ± 1.8 % ($P < 0.01$) with 10 s interval and the enhancement was declined with the prolongation of the intervals. The aggregation with release reaction was not affected by changing the intervals, but the release reaction was decreased in the same treatment. **CONCLUSION**: The dual effects of 5-HT on STA_2-induced aggregation and release reaction and the molecular mechanism of this effect was probably through the regulative action of 5-HT on $[Ca^{2+}]_i$ mobilization by STA_2.

Blood platelet plays an important role directly through its functions or indirectly due to some active substances or cytokines released during platelet activation, for example, adenosine diphosphate (ADP), 5-hydroxytryptamine (5-HT), calcium ion (Ca^{2+}), platelet activating factor (PAF), and some enzymes. In addition, the platelet is the most important store of 5-HT, as a local circulating regulator it can be released from dense granules of platelets upon suitable stimulation, and enhanced the aggregations by ADP and epinephrine[1-3] but the effects of 5-HT on release reaction are still uncleared. In the present research, a stable analogue of thromboxane A_2 (STA_2) was selected since it caused both aggregation and release reaction, which relied on the concentration of STA_2. Furthermore, the effects of 5-HT on STA_2-induced platelet shape change, aggregation and release reaction were observed separately, and cytosolic-free Ca^{2+} ($[Ca^{2+}]_i$) was also evaluated in washed single

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