Pergamon

Biochemical Pharmacology, Vol. 50, No. 3, pp. 421–423, 1995.
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0006-2952/95 $9.50 + 0.00

0006-2952(95)00149-2

Short Communication

ONTOGENESIS OF RAT LIVER MICROSOMAL GLUTATHIONE TRANSFERASE

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(*Received* 16 *December* 1994; *accepted* 15 *March* 1995)

Abstract—The ontogenesis of rat liver microsomal glutathione transferase was investigated by activity measurements and immunochemical methods. The activity rises from a very low level (3% of adults) at day 8 pre-partum to adult levels at days 50–150. Increases are associated with the neonatal and late-suckling clusters. Interestingly the capacity to become activated by *N*-ethylmaleimide is much lower in females early and late in life (days 35–100 and 300–550). After the initial increases (from 10% of adult levels at day 8 pre-partum), protein levels determined immunochemically remain constant throughout life with no apparent sex differences. The developmental pattern of microsomal glutathione transferase resembles those of other drug-metabolizing enzymes indicating that the function of the enzyme is required in adult life.

Key words: microsomal glutathione transferase; ontogeny

Glutathione transferases serve in the detoxication of a vast number of substances [1]. In some cases however, more reactive products can be formed [2]. Microsomal glutathione transferase [3, 4] is a membrane protein present at high levels in liver microsomes and outer mitochondrial membranes [5]. This enzyme catalyses many reactions involving xenobiotics as well as the reduction of phospholipid hydroperoxides and the conjugation of hydroxy-alkenals [6]. As a consequence, it has been proposed that microsomal glutathione transferase can protect membranes against lipid peroxidation [6, 7]. The subcellular, extrahepatic and phylogenetic distribution of this protein has been studied [5]. However, the ontogenesis as well as activity during the life time of a species is not known.

Methods and materials

Rat liver microsomes were prepared from male and female Sprague-Dawley rats as described [8]. Three to five animals were included in each age group and for each sex, except for foetuses where livers from 3–15 animals were pooled.

Enzyme activity was measured spectrophotometrically at 340 nm in a reaction system (30°) containing 5 mM glutathione, 0.5 mM CDNB, 2.5% (v/v) ethanol (as solvent for CDNB), 0.1 M potassium phosphate, pH 6.5, and 0.1% Triton X-100 [9, 10].

Activation of microsomal glutathione transferase with NEM was performed as described [11]. Briefly, 90 μL of the microsome fraction was mixed with 10 μL NEM (10 mM) at room temperature. After 30 sec aliquots were withdrawn for assay.

SDS-PAGE was performed according to Laemmli [12], except that the concentration of Tris-HCl in the separation gel was doubled in order to improve resolution.

Western blotting and immunodecoration followed by peroxidase anti-peroxidase staining employed standard procedures [13]. Quantitation was performed using a Shimadzu scanner in the reflectance mode at 450 nm.

Protein concentration was determined by the method of Peterson [14].

All chemicals were of analytical purity and obtained from common commercial sources.

Results and discussion

The activity and NEM-activation of the CDNB conjugating capacity of rat liver microsomes was followed from day 8 pre-partum to day 550 of adult life. As can be seen in Fig. 1(a) the enzyme activity rises from a very low (but significant) level *in utero* to reach a maximum at day 9 post-partum followed by a decrease and a stabilization at close to adult levels at day 50 and 150 for males and females, respectively. Since it is known that there is a contribution from tightly bound cytosolic glutathione transferases to the CDNB conjugating activity in microsomes (70% in 40-day-old males [15]) it is also necessary to study the activity after NEM-treatment (which specifically increases the activity of microsomal glutathione transferase [10]). The developmental pattern of the activity of NEM-treated microsomes (Fig. 1b) resembles the unactivated activity with two notable differences. There is no overshoot at day 9 and there is a large sex difference early and late in life with female microsomal CDNB

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† Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; NEM, *N*-ethylmaleimide.

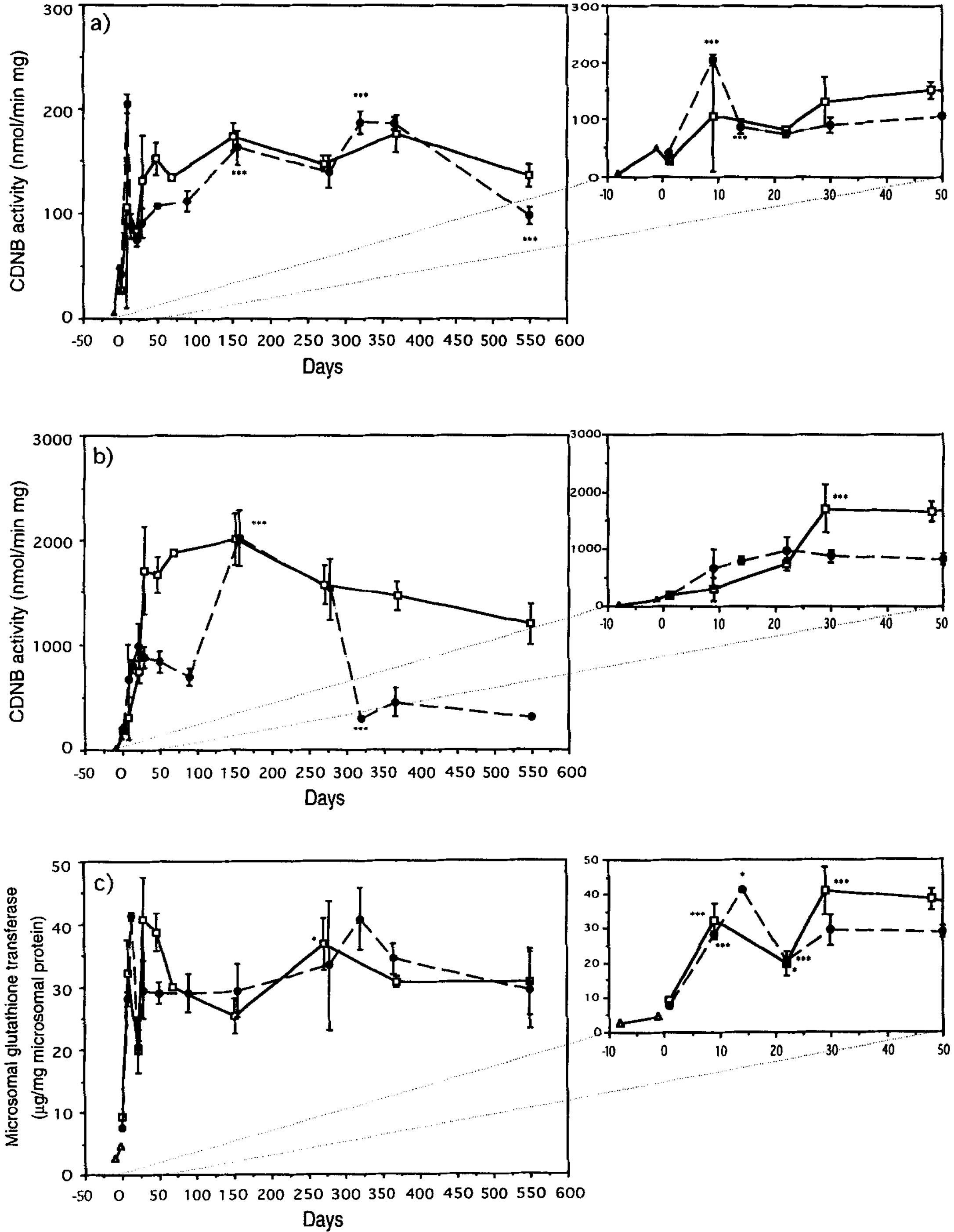

Fig. 1. (a) Developmental profile and life span distribution of glutathione transferase activity in rat liver microsomes. (b) Developmental profile and life span distribution of NEM-activated glutathione transferase activity in rat liver microsomes. (c) Developmental profile and life span distribution of microsomal glutathione transferase protein in rat liver microsomes determined by immunochemistry. ●, Females; □, males; △, sex not determined. Values are means ± SD (N = 3–5). Statistically significant differences between consecutive samples are indicated on the latter (* = $P < 0.05$, *** $P < 0.001$) (Tukey–Kramer multiple comparisons test).

activity being less responsive to NEM treatment. Although NEM activation is a specific way to study the activity of microsomal glutathione transferase, the degree of activation might vary during life. Therefore, the amount of microsomal glutathione transferase protein was determined immunochemically by Western blots (Fig. 1c). The data clearly show that the enzyme is detectable *in utero* and rises to a peak at days 9–14, followed by a decrease, rising again to adult levels at day 30. Thereafter the amounts are constant. Only a slight sex difference is observed between days 30 and 50, which is in accord with the activity measurements. However, the differences in NEM activation observed between males and females cannot be explained by differences in the amount of microsomal glutathione transferase protein. Therefore other factors must determine the capacity of activation. For instance the amount of endogenous inhibitor(s) (for which there is experimental evidence [16]) might vary during the life span of the female. The variation in the capacity for activation might have physiological consequences. It is known that oxidative stress can activate the microsomal glutathione transferase *in vivo* [17]. Since the enzyme has a proposed role in protection against oxidative stress [7] modulation of the capacity to be activated can be considered an interesting area for further research.

Microsomal glutathione transferase develops rapidly with the neonatal cluster and after a decrease rises again with the late-suckling cluster. No changes are apparent at puberty and protein levels then remain constant up to 550 days. This developmental profile resembles those of other drug metabolizing enzymes including representatives of cytosolic glutathione transferase and UDP-glucuronyltransferase as well as NADPH cytochrome P-450 reductase [18]. Since the foetus is protected by the drug metabolizing capacity of the mother (and the placenta) this developmental profile appears logical. It has often been proposed (and indeed shown) that drug metabolizing enzymes can also perform important endogenous functions. For microsomal glutathione transferase it can be concluded that such functions should be related to adult life.

Acknowledgements—This work was supported by the Swedish Cancer Society and Funds from the Karolinska Institute.

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