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Developmental changes in enzymatic systems involved in protection against peroxidation in isolated rat brain microvessels

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Activities of 3 enzymes involved in the major detoxification pathway for peroxides were assessed in rat brain microvessels. Between the 7th and 60th day after birth, glutathione peroxidase specific activity remained constant in microvessels, while glutathione reductase specific activity increased from day 14 to day 60. On the other hand, the specific activity of these two enzymes evolved similarly in total brain homogenate: they increased between day 7 and day 30, and then reached a plateau. In contrast, catalase specific activity in microvessels was markedly decreased from day 7 to day 60. A significant decrease in this enzyme specific activity was also observed in brain homogenate during development. However, in microvessels, catalase specific activity remained higher than that of brain homogenate throughout the time period studied. Our results support the idea that enzymatic mechanisms against peroxidative damage are required in early age, and could be potent at the level of the blood–brain barrier.

Free radical reactions lead to peroxidation of membrane lipids and disturb cellular function. The high concentrations of polyunsaturated fatty acids in the brain as well as the intensity of oxygen metabolism render this tissue particularly sensitive to peroxidative damages by reactive oxygen species such as superoxide, hydrogen peroxide and organic peroxides [13]. Protective systems against peroxidative damage could be an important factor for preserving cerebral integrity.

These systems include superoxide dismutases, glutathione peroxidase (destroying H_2O_2 in cytosol and mitochondria) and catalase (destroying H_2O_2 in peroxysomes) and some non-enzymatic elements such as vitamin E, vitamin C or glutathione (being mainly free radical scavengers). The blood–brain barrier, which anatomically consists mainly of capillary endothelial cells [5, 12], physically protects the central nervous system by regulating movements of molecules between plasma and brain extracellular space [5, 7, 12]. So, alterations in the capillary endothelium could lead to dysfunction or pathological consequences for cerebral tissue. It has been demonstrated that adult rat brain contains enzymes able to prevent free radical damages [4, 14].

In studies on microvessels isolated from adult rat brain, specific activities of catalase, glutathione peroxidase (selenium dependent) and glutathione reductase were found to be significantly higher than in total brain homogenate [15]. The activity of these enzymes in brain microvessels during development, however, has not been reported so far. The present experiments were undertaken in order to determine the developmental evolution of specific activities of catalase, glutathione peroxidase and glutathione reductase in isolated rat brain microvessels versus cerebral homogenate.

Male Sprague–Dawley rats aged 7, 14, 30 and 60 days were used. Capillaries from perfused brains were isolated according to the method developed by Goldstein et al. [10], with minor modifications previously reported by Tayarani et al. [15, 16]. Cortical shells, dissected free of choroid plexus, were weighed, minced in a buffered solution (in mM: NaCl 35, KCl 4, $CaCl_2$ 3.2, $MgCl_2$ 1.2, HEPES 15, glucose 5, pH 7.4 and 1% bovine serum albumin), and homogenized. The number of brains pooled at various ages was for each experiment: 26–29 at day 7, 16 at day 14, 11 at day 30, and 9 at day 60.

Microvessels were further purified according to the method previously reported by Tayarani et al. [15, 16]. As previously described [11, 15], the purity of each microvessel fraction was confirmed by phase contrast mi-

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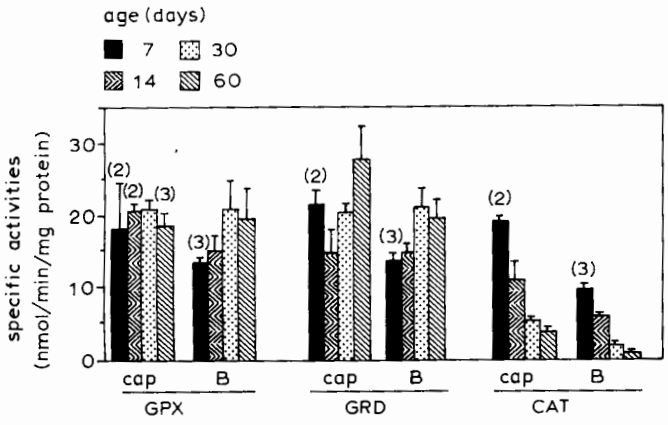

Fig. 1. Developmental changes in specific activities of glutathione peroxidase (GPX), glutathione reductase (GRD) and catalase (CAT) in isolated rat brain microvessels (cap) and total brain homogenate (B). Results are expressed as mean ± S.D. ($n \geq 4$ unless otherwise indicated in parentheses). Glutathione peroxidase activity is expressed in nmol NADPH oxidized/min/mg protein, at pH 7.6, 37°C and 0.5 mM glutathione reduced. Glutathione reductase activity is expressed in nmol NADPH oxidized/min/mg protein, at pH 7.4, 37°C and 0.2 mM glutathione disulfide. Catalase activity is expressed in nmol hydrogen peroxide consumed/min/mg protein, at pH 7.4 and 37°C.

croscopy, and the enrichment in marker enzyme γ-glutamyl transpeptidase was determined in a limited number of samples. The final pellet of highly purified brain microvessels was suspended in 1.4 ml of 50 mM Tris-HCl buffer, pH 7.6, containing 0.1 mM EDTA and 0.1% lubrol (incubation buffer). The suspension was sonicated, frozen, thawed and resonicated. After centrifugation, enzymatic activities were measured spectrophotometrically in the supernatant, according to the methods described previously [15].

Briefly, glutathione peroxidase measurements were done at pH 7.6 and 37°C and in the presence of 0.5 mM reduced glutathione. The assay mixture in incubation buffer consisted of 0.12 mM NADPH, 0.5 mM, reduced glutathione, 1 unit/ml of glutathione reductase and 0.2 mM of either cumene hydroperoxide or hydrogen peroxide (initial concentrations). NADPH disappearance was monitored at a wavelength of 340 nm. Glutathione reductase activity was measured in incubation buffer at pH 7.4 and 37°C by monitoring NADPH disappearance at 0.2 mM glutathione disulfide and 0.12 mM NADPH (initial concentrations). Catalase activity was measured at 37°C in incubation buffer at pH 7.4 using 10 mM hydrogen peroxide (initial concentration) as substrate. The consumption of H_2O_2 was recorded at an absorption wavelength of 240 nm. Corrections for non-enzymatic activities were made in all measurements.

Total cerebral cortices were homogenized in the incubation buffer and were treated as microvessel samples. Protein content was determined using the dye-binding assay of Bradford [3].

As shown in Table I, marked enrichment in γ-glutamyl transpeptidase was observed in the microvessel fraction compared to total brain homogenate (ratio microvessels/brain). This result is in agreement with previously reported data [10]. A rapid increase in this enzyme specific activity was noticed in microvessels between day 14 and 30, followed by a slight increase up to day 60. Similar changes were observed using a histochemical method [6], and suggested a correlation between γ-glutamyl transpeptidase activity and differentiation in the day 14–30 period, following a period of growth (days 2–12 after birth). Therefore, it seems that γ-glutamyl transpeptidase might be considered an element involved in maturation of the blood–brain barrier.

Fig. 1 shows the changes in specific activities of glutathione peroxidase, glutathione reductase and catalase in isolated rat brain microvessels and in cerebral homogenates during development. Glutathione peroxidase specific activity in microvessels was nearly constant during the whole development, while for glutathione reductase there was a fall at day 14, followed by a continuous increase from day 14 to 60. At this stage, glutathione reductase specific activity was 1.5-fold higher than glutathione peroxidase.

The developmental pattern of these enzymatic activities was quite different in total brain homogenate compared to capillaries. Glutathione peroxidase specific activity showed a progressive increase from day 7 to day 30, then reached a plateau.

Glutathione peroxidase and glutathione reductase specific activities were similar during the first postnatal pe-

TABLE I

SPECIFIC ACTIVITY OF γ-GLUTAMYL TRANSPEPTIDASE IN ISOLATED RAT BRAIN MICROVESSELS AND TOTAL BRAIN HOMOGENATE

*Units of gamma-glutamyl transpeptidase are defined as nmol of amino-5-nitro-2 benzoate formed per min determined spectrophotometrically at 405 nm and 37°C. Results are expressed as mean ± S.D. Number of determinations is shown in parentheses.

Age (days)	Units */mg protein		
	Microvessels	Brain	Ratio microvessels/brain
7	28.9±0.4 (2)	1.8±0.2 (3)	17.1
14	44.6 (1)	3.6±0.6 (2)	12.2
30	367.0 (1)	12.4±1.2 (2)	29.6
60	408.0±7.1 (2)	18.8±0.8 (4)	21.7

riod. These results are in agreement with previous studies [4, 8]. There was a marked time-dependent decrease in catalase specific activity in isolated microvessels and brain homogenate during development. However, the specific activity of this enzyme in microvessels was 2–3 times higher than in brain homogenate at all ages studied. These results suggest a significant role for catalase during the neonatal period, becoming progressively weaker with age. However, the real function of catalase during this period remains unclear. Therefore, it seems that enzymatic systems involved in the protection against peroxidative damages during early postnatal development and adulthood are different. So, immediately after birth, catalase may be one of the major protective factors against peroxidative damage, particularly in brain microvessels where its specific activity was found to be 2-fold higher than in cerebrum. Vitamin E, which is known to prevent accumulation of lipid peroxides, could also contribute to providing efficient protection in newborn rat. Catalase is principally located in microperoxisomes [9]. A significant decrease in the microperoxisome population after the period of active myelination in cerebrum and cerebellum was observed by Arnold and Holtzman [1], and may be correlated to the decrease in catalase specific activity that we found in the same tissues (data not shown for cerebellum homogenate). Catalase may act either in certain metabolic functions (e.g. myelination), or as an antioxidant, or in both ways. Del Maestro and Mc Donald proposed that a change may also occur in localization of hydrogen peroxide-generating sites in the brain [8]. However, we observed a similar decrease in catalase specific activity in endothelial cells themselves. At the end of the second week of life, after the complete maturation of blood–brain barrier and brain, at the onset of an increasing aerobic metabolism [2], the required protection may involve newly activated mechanisms such as glutathione peroxidase (to eliminate H_2O_2), or superoxide dismutase (to control $O_2^{\cdot-}$). Indeed, specific activity of superoxide dismutase has been shown to be increased up to 10 days after birth in cerebrum [8].

This pattern is conserved during adulthood, since glutathione peroxidase and glutathione reductase specific activities were found to be constant, while catalase declined, and superoxide dismutase was increased in brain capillaries during aging [16]. These observations and our present results suggest that the major protecting elements against peroxidation are glutathione peroxidase and superoxide dismutase; and at least one of them, glutathione peroxidase, is active throughout the early postnatal period in microvessels.

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