Metallothionein-I in the plasma and liver of neonatal rats

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The concentrations of metallothionein-I in the plasma and liver of neonatal rats were measured by radioimmunoassay. Plasma concentrations of the protein in male and female 4-day-old rats were 350 and 740 ng/ml respectively, and declined rapidly to only 3.5 ng/ml at 32 days of age. Concentrations in liver were also high in the newborn rats (200 µg/g), and declined from 12 days of age onwards.

Although metallothionein has generally been regarded as an intracellular protein, recently developed r.i.a. procedures have shown that small amounts of the protein also occur in the plasma of normal rats (Garvey & Chang, 1981; Mehra & Bremner, 1983). Concentrations in adult rats are only about 1–2 ng/ml of plasma, but are substantially increased in rats injected with Cd^{2+} , Cu^{2+} and, to a lesser extent, Zn^{2+} (Garvey & Chang, 1981; Mehra & Bremner, 1983). It has been suggested that the assay of metallothionein levels in plasma and urine may provide a useful index of exposure to Cd^{2+} in industrial workers (Shaikh & Hirayama, 1979; Chang *et al.*, 1980). However, before this view can be regarded as justifiable, information is required on the extent to which concentrations of metallothionein in the plasma are influenced by those in tissues such as liver and kidneys and by the physiological state of the subject.

It is known that metallothionein concentrations in the liver are kidneys are greatly influenced by factors such as age, sex, stress and hormonal status (see Kagi & Nordberg, 1979; Bremner, 1979). Since particularly high concentrations occur in the liver of foetal or neonatal animals (Bremner *et al.*, 1977; Mason *et al.*, 1981; Panemangalore *et al.*, 1983), it was of interest to establish whether this was reflected in newborn rats in increased concentrations of the protein in the plasma.

Experimental

Twenty female Hooded Lister rats (Rowett Institute strain), which had been maintained from weaning on a stock colony diet ('Oxoid'; H.C.

Styles, Bewdley, Worcs., U.K.), were used. When they weighed about 230 g they were mated to stock male rats. At 1 day after parturition, all litters were adjusted to contain 10 pups. Heparinized blood was collected from the pups and dams by cardiac puncture, and liver and kidneys were removed at 3–4-day intervals over a 32-day period.

Concentrations of metallothionein-I in plasma, liver and kidneys were measured by using the r.i.a. described by Mehra & Bremner (1983). Rat liver zinc-metlothionein-I, purified as described by Bremner & Davies (1975), was used as standard. The ^{125}I -labelled tracer was prepared from rat liver zinc-metlothionein-I by using the Bolton & Hunter method (Bolton, 1977). The specific radioactivity of the ^{125}I -zinc metallothionein was about 25 µCi/µg.

Binding of tracer to anti-metlothionein-I in the absence of added protein was about 40%. The final dilution of antiserum in the assay mixture was 1:50000.

Plasma was stored at +1°C and other tissues at –20°C before analysis. Liver and kidneys were homogenized with 10 vol. (v/w) of 50 mM-Tris/HCl buffer, pH 8.0, and centrifuged at 1500g for 15 min. The supernatants and the plasma samples were diluted as appropriate with 50 mM-Tris/HCl buffer, pH 8.0, containing 0.1% gelatin and 0.1% NaN_3 for the r.i.a. Typical dilutions of plasma ranged from 1:20 to 1:2 and of liver homogenates from 1:1000 to 1:10.

Results

A linear relationship between logity and log(concentration of metallothionein-I) existed over the range 100–25000 pg of protein, where:

$$\text{logit } y = \log[(100 \times b)/(b_0 - b)]$$

Abbreviation used: r.i.a., radioimmunoassay.

Table 1. Concentrations of metallothionein-I in the liver and plasma of neonatal rats
Concentrations are given as means $\pm$ S.E.M., with the number of samples in parentheses. See the text for details.

Age (days)	Concentration of metallothionein-I (ng/ml or μ g/g fresh wt.)			
	Plasma		Liver	Kidneys
	Male	Female		
1			192 $\pm$ 12.5 (25)	16.3 $\pm$ 0.7 (25)
4	354 $\pm$ 65 (11)	737 $\pm$ 161 (9)	184 $\pm$ 8.3 (25)	24.4 $\pm$ 1.8 (25)
8	62.8 $\pm$ 19.7 (10)	137 $\pm$ 56 (7)	200 $\pm$ 9.8 (25)	18.9 $\pm$ 1.9 (25)
12	44.3 $\pm$ 11.6 (11)	32.7 $\pm$ 5.6 (9)	182 $\pm$ 10.6 (25)	15.9 $\pm$ 1.1 (25)
15	24.5 $\pm$ 4.1 (12)	14.9 $\pm$ 3.3 (9)	111 $\pm$ 7.0 (25)	11.8 $\pm$ 1.2 (25)
18	12.1 $\pm$ 3.5 (5)		62.6 $\pm$ 7.3 (5)	10.0 $\pm$ 0.7 (5)
22	5.0 $\pm$ 1.4 (5)		22.5 $\pm$ 2.0 (5)	6.2 $\pm$ 0.6 (5)
26	5.1 $\pm$ 1.3 (5)		12.6 $\pm$ 1.1 (5)	7.0 $\pm$ 0.3 (5)
32	3.5 $\pm$ 0.4 (5)		8.5 $\pm$ 1.1 (5)	8.0 $\pm$ 1.5 (5)

Fig. 1. Cross-reactivity of rat liver Zn-metallothionein-I and of neonatal-rat plasma

Various amounts of rat liver metallothionein-I (●) and of neonatal-rat plasma (▲) were incorporated in the inhibition assays. See the text for details.

b_0 is the radioactivity (c.p.m.) precipitated in the absence of unlabelled antigen and b is the radioactivity (c.p.m.) precipitated in the presence of standard or unknown. Both b_0 and b were corrected for non-specific binding.

The average recovery of rat liver metallothionein-I added to samples of neonatal-rat plasma was $90 \pm 21\%$. Further validation of the assay procedure was obtained by comparing the cross-reactivities of the neonatal plasma and authentic rat liver zinc-metallothionein-I (Fig. 1). The parallel nature of the curves strongly suggests that the antigen in the plasma was identical with the hepatic protein.

The changes in metallothionein-I levels in the plasma, liver and kidneys of the neonatal rats are shown in Table 1. Concentrations in the plasma were particularly high in the immediate postnatal period and declined in an exponential manner over the first 3 weeks of life. In the 4-day-old female pups, mean concentrations were over 700 ng/ml and in some samples were as high as 1300 ng/ml. Mean values in 4-day-old male pups were not as great (350 ng/ml) and ranged from 35 to 590 ng/ml. Concentrations were reduced to 137 ng/ml in the female and 63 ng/ml in the male pups at 8 days of age and continued to decrease until 22 days, when they were 5 ng/ml in males. The apparent half-life for the disappearance of metallothionein-I from the plasma over the 4–15-day period for both sexes was 2.2 days, although this makes no allowance for changes in plasma volume.

Concentrations of metallothionein-I were also extremely high in the liver of the newborn rats (Table 1). These did not differ between sexes and only overall mean values are presented. In rats aged 1–12 days the livers contained about 200 μ g of metallothionein-I/g fresh wt., but concentrations declined gradually thereafter and were about 10 μ g/g after 32 days. There was no relationship between liver and plasma concentrations of the protein in the early post-natal samples, but in rats aged between 12 and 32 days it could be described by the equation:

$$y = 0.23x - 0.26 \text{ (S.E. of slope} = 0.034) \text{ (} P < 0.01)$$

where x and y are the concentrations of metallothionein-I in liver (μ g/g) and plasma (ng/ml) respectively.

Renal concentrations of metallothionein-I in the male pups, although slightly elevated in the immediate postnatal period (24 μ g/g at day 4), were only 10% of those in the liver. They decreased gradually to 6–8 μ g/g at 3–4 weeks of age.

Discussion

These results provide the most convincing demonstration so far of the occurrence of metallothionein in extracellular fluids under normal physiological conditions. The assay used for measurement of rat metallothionein-I has already been fully characterized and is specific for this isoprotein (Mehra & Bremner, 1983). Further evidence of its validity for the analysis of neonatal-rat plasma was obtained by the complete recovery of added metallothionein-I and the parallel nature of the dilution curves for the purified liver metallothionein-I and for the plasma. Small amounts of metallothionein ($< 2 \text{ ng/ml}$) occur in the plasma of normal adult rats (Garvey & Chang, 1981; Mehra & Bremner, 1983), and concentrations can be increased by up to 20-fold by injection of large amounts of Cd^{2+} or Cu^{2+} (Garvey & Chang, 1981; Mehra & Bremner, 1983). However, the concentrations in the 4-day-old rats exceeded these by at least an order of magnitude. Indeed, in some cases they were 1000 times greater than those in adult rats. Unfortunately no accurate values were obtained for plasma from 1-day-old rats, but it was apparent that they were substantially greater than those at 4 days. Concentrations decreased rapidly during the early postnatal period, but at 32 days were still greater than those in adult rats (Mehra & Bremner, 1983).

There was no evidence of increased concentrations of the protein in the plasma or liver of the dams (results not shown). It seems likely that the metallothionein in the plasma of the offspring originated in the neonatal liver, since extremely high concentrations of the protein were also found in this organ during the first week of life. It has previously been shown, using chromatographic and Cd^{2+} -binding assays, that concentrations of total metallothionein in the livers of newborn rats are about $1000 \mu\text{g/g}$ wet wt. and that metallothionein-I accounts for about one-third of the total metallothionein (Mason *et al.*, 1981; Panemangalore *et al.*, 1983). The current findings of about $200 \mu\text{g}$ of metallothionein-I/g of the 1-day-old rats, obtained by a specific assay, are therefore in good agreement with these earlier, less-direct estimates. There is, however, some conflict regarding the rate of decline in liver metallothionein concentrations with age. Mason *et al.* (1981) reported that the concentration of Zn bound to metallothionein declined continuously from day 1 onwards. In contrast, Panemangalore *et al.* (1983) found no marked change in hepatic metallothionein or Zn concentrations until after day 5, although the total metallothionein content increased slightly between 5 and 15 days, after which it rapidly declined. In the current study, no change in metallothionein-I

concentrations was found until after day 12, which suggests that this particular isoprotein is preferentially accumulated in the immediate postnatal period. However other reports indicate that metallothionein-I is normally degraded more rapidly than metallothionein-II (Andersen *et al.*, 1978; Bremner & Mehra, 1983).

Although there was a significant correlation between liver and plasma concentrations of the protein in the older rats, this was not the case in the immediate postnatal period, and there were large variations in plasma concentrations in pups within the same litter, even when liver concentrations of metallothionein-I were similar. Clearly a variety of factors must influence secretion of the protein into plasma, possible including the occurrence of any liver dysfunction (Mehra & Bremner, 1983). It may be relevant that liver function is not fully developed in the perinatal rat, and biliary secretion, for example, is abnormal (Little *et al.*, 1979).

At this stage it is unknown whether the occurrence of metallothionein-I in plasma is a result of active secretion of the protein or merely of its leakage from tissues. It is therefore uncertain whether the plasma protein has any real function, although there is evidence of its importance in the transport of Cd^{2+} in Cd^{2+} -exposed subjects (Nordberg *et al.*, 1982). Under normal circumstance, Cu^+ and Zn^{2+} are the main metals on metallothionein, but it seems unlikely that it plays a major role in their transport in adult rats because of the low concentrations of the plasma protein. However, this may not be so in the newborn rat, as about $0.3 \mu\text{g}$ of Cu and/or Zn could be present as metallothionein/ml of plasma. Studies are required to establish whether plasma metallothionein has any effect on the metabolism of either of these metals in the young rat.

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