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Factors affecting haem degradation in rat brain

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A functional deficiency of brain haemoproteins has been postulated as a cause of the neural manifestations of acute porphyria [1]. This deficiency could result either from impaired haem synthesis or, alternatively, from increased haem degradation in neural tissue. Factors that are known to precipitate acute attacks might conceivably enhance haemoprotein turnover in brain and thereby deplete the neural haem pool. The object of the present study was to investigate the rate of haem metabolism in normal mammalian brain and in the brains of animals subjected to treatments known to influence this process in liver tissue.

Female Wistar rats (150–230 g) were injected with [4-^{14}C]ALA* (5 μCi/30 μl) intraventricularly [2] and [G-^{3}H]ALA (5 μCi/100 g body wt) intraperitoneally. The radiochemicals were obtained from the Radiochemical Centre, Amersham, U.K. At the times indicated, rats were heparinized and killed by cardiac excision under light ether anaesthesia. Samples of blood were collected and frozen in liquid nitrogen. Initially, the rats were perfused through the ascending aorta with ice-cold physiological saline containing heparin [3] in order to eliminate any contribution from blood to tissue haem radioactivity. However, extremely low levels of haem radioactivity were found in blood, as has been reported previously [4], and in later experiments animals were not perfused. Similar results were obtained with and without perfusion.

The brain ventricles were opened and brain and liver samples were weighed, washed in chilled saline and frozen in liquid nitrogen. Tissues were maintained at −20° until analysis. Samples were thawed and homogenized in 3 vol. saline. Haem was extracted from the homogenate into ethyl acetate:glacial acetic acid (4:1, v/v) [5] and crystallized from the extract with the aid of carrier haemin [6]. Dried haem samples were prepared for determination of radioactivity by combustion in a Packard Sample Oxidizer.

Haem oxygenase activity in rat brain was measured by the method of Tenhunen *et al.* [7]. Tissue was homogenized in 4 vol. 0.1 M potassium phosphate buffer (pH 7.4) and centrifuged at 18,000 *g*. The reaction mixture (3.0 ml) contained 18,000 *g* tissue supernatant (6–9 mg protein), 17 μM haemin, 180 μM NADPH and 0.1 M potassium phosphate buffer (pH 7.4). In the control cuvette, NADPH was replaced by 0.1 M potassium phosphate buffer (pH 7.4). Hepatic haem oxygenase activity was assayed similarly, except that the NADPH concentration was 0.5 mM. The formation of bilirubin, determined from the increase in optical density at 468 nm, was linear for 10 min. Protein was determined by the method of Lowry *et al.* [8]. An extinction coefficient for bilirubin of 40 $mM^{-1}cm^{-1}$ was used [9]. Enzyme activity was expressed as nmoles bilirubin formed/10 mg supernatant protein/min.

Radioactivity incorporated into haem in rat brain fell rapidly between 6 and 16 hr after intraventricular injection of [4-^{14}C]ALA (Fig. 1). After 16 hr the decline was less rapid and by 48 hr the specific activity of brain haem had reached a plateau. The data of Schwartz [10] for the degradation of hepatic haem in dogs following intravenous injection of [4-^{14}C]ALA are also shown in Fig. 1 for comparison. Haem degradation in brain and liver apparently follow a similar time course up to about 24 hr after injection of [4-^{14}C]ALA. After 24 hr, radioactivity in hepatic haem continues to decline, whereas brain haem radioactivity remains relatively constant. The rates of brain and hepatic haem degradation in the 6–24 hr period following intra-

* ALA, δ-aminolaevulinic acid.

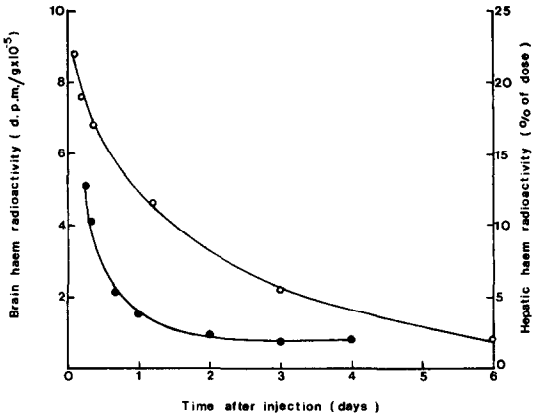

Fig. 1. Disappearance of radioactivity from haem after injection of [4-^{14}C]ALA. ●——●, Curve for rat brain following intraventricular injection of 5 μCi in 30 μl. Haem was isolated with the aid of carrier according to Labbe and Nishida [6]. ○——○, Data of Schwartz [10] for radioactivity of hepatic haem following intravenous injection in dogs.

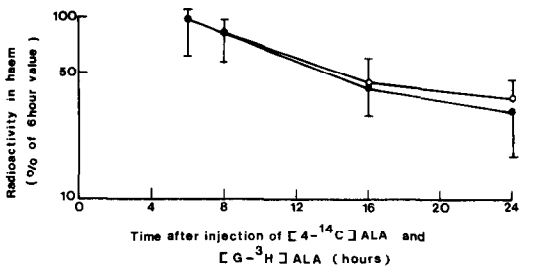

Fig. 2. Disappearance of radioactivity in rat brain and liver haem following injection of [4-^{14}C]ALA (5 μCi in 30 μl, intraventricularly) and [G-^{3}H]ALA (5 μCi/100 g body wt, intraperitoneally). Haem was isolated with the aid of carrier according to Labbe and Nishida [6]. Results expressed as means ± S.D. (N = 6–16).

ventricular administration of [4-^{14}C]ALA and intraperitoneal injection of [G-^{3}H]ALA in normal rats were found to be identical (Fig. 2). In both tissues, haem degradation appeared to be biphasic, with a rapid component up to 16 hr after injection and a slower phase thereafter.

It was found that treatment of rats with lead (20 mg/kg intravenously), allylisopropylacetamide (400 mg/kg intraperitoneally), phenobarbitone (100 mg/kg intraperitoneally) or lead in combination with phenobarbitone did not alter the rate of haem degradation in rat brain. Treatment with AIA has previously been shown to accelerate hepatic haem degradation [11–13], whereas treatment with phenobarbitone had no effect [11].

Mean brain and liver haem oxygenase activities were very similar (Table 1). Prior treatment of the animals with lead, phenobarbitone, lead in combination with phenobarbitone or haem administered intraperitoneally did not alter brain haem oxygenase activity. However, haem administered intraventricularly increased brain haem oxygenase activity to 154 per cent of control levels. This increase was unaffected by prior treatment of the animals with lead.

Hepatic haem oxygenase activity was found to be increased approximately 14-fold 16 hr after treatment with lead. Forty-eight hours after injection of lead it had fallen to five times the control value.

Intraperitoneal administration of haem caused a 9-fold increase in liver haem oxygenase activity while phenobarbitone was found to be without effect. Treatment with lead in combination with phenobarbitone yielded haem oxygenase activity which was not significantly different from that obtained with lead alone. These observations are in agreement with previously-reported data [7, 14].

Table 1. Effects of various treatments on haem oxygenase activity in rat brain and liver

Treatment	Haem oxygenase activity* (nmoles bilirubin/10 mg protein/min)	
	Brain	Liver
Control	0.17 ± 0.013	0.13 ± 0.014
Lead (20 mg/kg, 16 hr before death)	0.18 ± 0.013	1.8 ± 0.42†
Lead (20 mg/kg, 48 hr before death)	0.21 ± 0.067	0.6 ± 0.14†
Phenobarbitone (50 mg/kg subcutaneously for 4 days)	0.18 ± 0.006	0.16 ± 0.023
Phenobarbitone (50 mg/kg subcutaneously for 4 days) + lead (20 mg/kg, 16 hr before death)	0.18 ± 0.018	2.2 ± 0.38†
Heam (40 μmoles/kg intraperitoneally twice daily for 2 days prior to death)‡	0.19 ± 0.033	1.2 ± 0.29†
Haem (0.25 μmoles intraventricularly 16 hr prior to death)‡	0.26 ± 0.032†	
Haem (0.25 μmoles intraventricularly 16 hr before death)‡ and lead (20 mg/kg, 17.5 hr before death)	0.26 ± 0.014†	

* Results expressed at means ± S.D. (N = 5).
† Significantly different from control, $P < 0.001$.
‡ Injected as methaemalbumin prepared from crystalline haemin.

The initial fast phase of hepatic haem turnover has been attributed to degradation of a pool of free or unassigned haem with a short half-life [15]. The similarity in the rate of disappearance of radioactivity from the early-labelled haem pool in brain and liver (Fig. 1) suggests that such a pool of free haem also exists in neural tissue. Yannoni and Robinson [16] have proposed that a haem pool with a rapid turnover rate is characteristic of all tissues in which haem synthesis takes place. This pool is regarded as a regulatory one, controlling haem synthesis by repressing ALA synthetase and regulating haem degradation by stimulating haem oxygenase activity [13, 15].

Bissell and Hammaker [15] attribute the slow phase of hepatic haem degradation, i.e. 16–30 hr after [4-^{14}C]ALA administration, solely to the degradation of cytochrome P-450, a rapidly turning over microsomal haemoprotein. The concentration of this haemoprotein in brain has been shown to be very much lower than in liver (1:50) [17]. This could account for the early plateau found for radioactivity in brain haem in the present study (Fig. 1). Since radioactively labelled haem accumulates chiefly in the mitochondrial fraction of brain cells [17], it would appear that this latter part of the curve probably reflects turnover of mitochondrial haemoproteins. On this assumption, it would appear that their half-life is similar to that of hepatic mitochondrial haemoproteins [18].

Haem oxygenase activity in brain was found to be very similar to that of liver, which is in marked contrast to the relatively lower levels of haem-synthesizing enzymes found in brain tissue [17]. However, the lack of effect in brain of factors known to influence hepatic haem degradation and haem oxygenase activity suggests that the mechanisms which control hepatic haem turnover are not operative in neural tissue. Alternatively, these factors in their active forms fail to reach 'receptor' sites in the brain [9]. Substrate-mediated increase in brain haem oxygenase activity was observed. It is possible that macrophages rather than neurons or glial cells contribute to this haem-mediated increase in haem oxygenase activity following intraventricular administration of haem [19].

The results of the present study indicate that brain haemoproteins have a low turnover rate, which accords with the data previously obtained with respect to haem biosynthesis [17]. Destabilization of brain haemoproteins could lead to increased brain haem turnover as haem oxygenase is substrate inducible. However, it would appear unlikely that porphyrinogenic agents (such as AIA) or lead affect brain haemoprotein function in normal tissue in this way.

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