Regional distribution of the enzymes of haem biosynthesis and the inhibition of 5-aminolaevulinate synthase by manganese in the rat brain

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The activity of 5-aminolaevulinate synthase, the rate-limiting enzyme of haem biosynthesis, is differentially distributed in various regions of the rat brain. The cerebellum possessed the highest enzyme activity of the eight regions studied. The cerebral cortex and the midbrain also exhibited high 5-aminolaevulinate synthase activity; the septum, hypothalamus, thalamus, amygdala and the hippocampus possessed much lower enzyme activity. However, the total porphyrin and haem contents of the different brain segments did not vary greatly. Mn^{2+} , when administered subcutaneously to rats, effectively inhibited the activity of 5-aminolaevulinate synthase in the cerebellum, midbrain and cerebral cortex; however, repeated injections of the metal ion neither decreased the haem and porphyrin contents of the brain nor induced haem oxygenase activity. Mn^{2+} was not an effective inhibitor of 5-aminolaevulinate synthase activity *in vitro*. On the other hand, studies carried out with the liver *in vivo* suggested that Mn^{2+} may alter the turnover rate of cellular haem and haemoproteins. In that event, it is likely that the inhibition of 5-aminolaevulinate synthase by Mn^{2+} was in part a result of the inhibition of protein synthesis by the metal ion. It is postulated that the haem and porphyrin contents of the brain are maintained at a steady-state level, due in part to the refractoriness to inducers of the regulatory mechanism for haem catabolic enzymes and in part to the ability of the organ to utilize haem precursors derived from extraneuronal sources.

Several investigators have reported the presence of 5-aminolaevulinate synthase (EC 2.3.1.37) activity and other enzymes of haem biosynthesis in the brain (Gibson & Goldberg, 1970; Barnes *et al.*, 1971; Paterniti *et al.*, 1978) as well as the ability of brain to carry out haemoprotein-dependent mixed-function oxidative activities (Paul *et al.*, 1977; Sasame *et al.*, 1977; Cohn *et al.*, 1977). Moreover, the differential ability of different areas of the brain to carry out haemoprotein-dependent mixed-function oxidase activity was shown by Fishman *et al.* (1976). Since it is generally assumed that the haem required for the formation of nerve-cell haemoproteins is formed in the cell itself, the findings of Fishman *et al.* could be interpreted as suggesting a differential distribution of haem biosynthetic activity in neural tissue.

It is well known that in the liver the activities of 5-aminolaevulinate synthase and haem oxygenase (EC 1.14.99.3), the rate-limiting enzymes of the haem biosynthesis and degradation pathways, respectively, are readily responsive to a variety of

factors (Granick & Urata, 1963; Bakken *et al.*, 1972) including metal ions (Maines & Sinclair, 1977). However, virtually nothing is known about the effect of such agents on the brain enzymes. In the liver, a number of divalent metal ions, such as Mn^{2+} and Co^{2+} , have been reported to be effective inhibitors of 5-aminolaevulinate synthase (Tephly & Hibbein, 1971; Maines & Kappas, 1975; Nakamura *et al.*, 1975; Maines *et al.*, 1976) and inducers of haem oxygenase (Maines & Kappas, 1974; Correia & Schmid, 1975; De Matteis & Gibbs, 1976; Maines & Kappas, 1977; Krasny & Holbrook, 1977; Woods & Carver, 1977; Legrum *et al.*, 1979; Maines, 1979). Neff *et al.* (1969) have provided evidence showing that Mn^{2+} directly causes abnormalities in the metabolism of certain biogenic amines in the brain. It therefore can be hypothesized that metal ions may also be capable of altering haem metabolism in the brain.

This investigation sought to determine whether haem biosynthesis and degradation activities in the brain are differentially distributed in various

regions, and to examine the effects of metal ions (Mn^{2+} and Co^{2+}) on the activities of the enzymes of haem metabolism in this organ.

Experimental

Materials

Sprague-Dawley rats (150–180g) were used throughout the study. All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Porphyrins were purchased from Porphyrin Products, Salt Lake City, UT, U.S.A. Coproporphyrin III was used as the standard for porphyrin measurements. Solutions of haematin and biliverdin were prepared as described by Maines & Kappas (1976a). Rats were injected subcutaneously in the loose skin of the neck with 25 μ mol of $CoCl_2 \cdot 6H_2O$ /100g body wt. (Co^{2+}) or 50 μ mol of $MnSO_4 \cdot H_2O$ /100g body wt. (Mn^{2+}) once every 24h for 2 days. The animals were killed 24h after the last injection. For certain experiments Mn^{2+} was also administered at a dose of 100 and 200 μ mol/100g body wt.

Tissue preparations

The following procedures were carried out for the preparation of the brain tissue. Rat brains were immediately removed after decapitation, transferred to a Petri dish placed on ice, and dissected at 4°C under a microscope. The brain was sectioned into the septum, hypothalamus, thalamus, amygdala, midbrain, cerebellum, hippocampus and cerebral cortex. Sections obtained from five to ten rats were pooled and used for each measurement. In other experiments only three areas of the brain (the cerebral cortex, midbrain and the cerebellum) were dissected. All brain tissue was homogenized (tissue:buffer 1:2, w/v) in 0.05 M-Tris/HCl buffer, pH 7.4, containing 0.25 M-sucrose. The whole brain homogenate, the 9000g fraction, and the microsomal fraction were prepared as described earlier (Maines & Kappas, 1975). The mitochondrial fraction was prepared by homogenizing the tissue in 5 vol. of the buffer, followed by centrifugation at 1000g for 10 min, and then by centrifugation of the supernatant at 8000g for 10 min.

The following procedure was used to prepare partially purified rat liver biliverdin reductase (EC 1.3.1.24) (O'Carra & Colleran, 1971). The liver was perfused *in situ* with 0.9% NaCl until fully blanched, and then was removed and homogenized in 4 vol. of the buffer described above. The cytosol (105 000g supernatant) then was prepared. The 40–60%-satd. $(NH_4)_2SO_4$ fraction was obtained and dialysed overnight against the buffer. The enzyme activity of the dialysate was measured with biliverdin-IX α as the substrate; one unit of activity was defined as the amount of the enzyme (mg of protein) necessary for the formation of 1 μ mol of bili-

rubin/min using the NADPH generating system described below. The microsomal and the mitochondrial fractions were prepared from perfused (0.9% NaCl) livers, utilizing the buffer system described above. The smooth and rough endoplasmic reticulum of the liver was prepared by sucrose-density-gradient centrifugation in the presence of CsCl as described by Dallner (1974).

Enzyme assays

The activity of brain 5-aminolaevulinate synthase was measured by modifying the procedure described for the liver enzyme by Marver *et al.* (1966) and Granick (1966). The following procedural modifications were used to optimize the system for the brain assay. The incubation medium (0.3 ml) contained 4–6 mg of protein, 50 mM-glycine, 50 mM-sodium citrate, 25 mM- Na_2HPO_4 , 10 mM- $MgCl_2$, 5 mM-pyridoxal 5-phosphate and 10 mM-disodium EDTA. The duration of incubation (37°C) was 45 min, in the total absence of light. All subsequent experimental processes were carried out in subdued lighting at 4°C. The aminoketones formed were converted into pyrroles by condensation with acetylacetone. Aminoacetone pyrrole was removed by extracting twice with 2 vol. of dichloromethane; the duration of each extraction was exactly 10 s. The activity of the enzyme was detected spectrophotometrically by measuring $A_{552} - A_{575}$. An absorbance coefficient of 58 $mm^{-1} \cdot cm^{-1}$ was used. For each measurement, the absorption spectrum between 500–600 nm of the colour salt formed after the addition of Ehrlich–mercury reagent (Mauzerall & Granick, 1956) to 5-aminolaevulinate pyrrole was recorded. The recording of the absorption spectrum was essential for the accurate assessment of 5-aminolaevulinate synthase activity, since it was observed that minor procedural inaccuracies would lead to distortion of the absorption spectrum of the Ehrlich-colour salt of 5-aminolaevulinate pyrrole. The presence of EDTA in the assay medium was not an absolute requirement for enzyme activity; however, its inclusion enhanced the quality of the absorption spectrum of the colour salt by decreasing its absorption in the 525 nm region. The exact mechanism by which EDTA mediated this effect is not clear, although the chelation of contaminating trace elements present in reagents could be in part responsible for the observation.

The total porphyrin contents of the brain sections were fluorimetrically assayed by the procedure described by Granick *et al.* (1975) using the solvent mixture aqueous 1 M- $HClO_4$ /methanol (1:1, v/v) in a Hitachi Perkin–Elmer fluorescence spectrophotometer. The excitation wavelength was 400 nm with a slit width of 20 nm, and the emission spectrum was scanned from 580 to 680 nm with a 12 nm slit.

Haem oxygenase activity was measured in the

brain sections using essentially the same assay system described earlier for the purified liver enzyme (Maines *et al.*, 1977). The enzyme source was the microsomal fraction, and the protein concentration was adjusted to 1–2 mg/ml of the incubation mixture. The assay system contained 0.01 unit of biliverdin reductase, and the concentration of haematin was 20 μ M. The duration of incubation was 15 min at 37°C and a NADPH-generating system (0.8 mM-glucose 6-phosphate, 1 unit of glucose-6-phosphate dehydrogenase and 0.8 mM-NADP⁺) was used. The reaction was initiated by the addition of NADP⁺. The enzyme activity was calculated from the amount of bilirubin formed by using an absorbance coefficient of 40 mM⁻¹.cm⁻¹ for $A_{464} - A_{530}$ (Maines & Kappas, 1974).

5-Aminolaevulinate dehydratase activity (EC 4.2.1.24) was measured by the procedure described by Mauzerall & Granick (1956). The amount of protein in the assay medium (0.225 ml) was 2–3 mg and the concentrations of 5-aminolaevulinate and dithiothreitol were 150 and 20 mM, respectively.

The haem content of the brain sections was measured in the microsomal fraction. The cytochrome *P*-450 concentration in the rough and smooth endoplasmic reticuli of liver was measured from the reduced–CO difference spectrum by using sodium dithionite as the reducing agent and an absorbance coefficient of 91 mM⁻¹.cm⁻¹ for $A_{450} - A_{490}$ (Omura & Sato, 1964). The concentration of haem was measured by the pyridine haemochromogen method of Paul *et al.* (1953) using the reduced–oxidized difference spectrum, $A_{557} - A_{575}$, and an absorbance coefficient of 32.4 mM⁻¹.cm⁻¹. Protein was measured by using the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

All spectral studies were carried out using an Aminco–Chance DW-2 spectrophotometer. All experiments were repeated at least three times and the brain sections of five to ten rats were pooled for each measurement. The results are expressed as the means $\pm$ s.d. for three experiments. The data were analysed by using the Students' *t* test and a *P* value of ≤ 0.05 was regarded as denoting significance.

Results

Distribution of 5-aminolaevulinate synthase and dehydratase, and the total porphyrin content in different regions of the rat brain

In the studies shown in Fig. 1, the rat brain was dissected into eight different regions, the septum, hypothalamus, thalamus, amygdala, cerebellum, midbrain, hippocampus, cortex; the 5-aminolaevulinate synthase activity and the total porphyrin content were measured. These studies were carried out using whole tissue homogenates, because the

Fig. 1. Distribution pattern of 5-aminolaevulinate synthase activity in rat brain

Male Sprague–Dawley rats (150–200 g) were killed. The brain was immediately removed and dissected as described by Glowinski & Iversen (1966), and the tissues obtained from ten rats were pooled. The tissue homogenate was prepared (tissue:buffer, 1:1, w/v) using 0.05 M-Tris/HCl buffer, pH 7.4, containing 0.25 M-sucrose. The activity of 5-aminolaevulinate synthase was measured as detailed in the Experimental section. Each assay was carried out in triplicate and the experiment was repeated three times. The values represent means $\pm$ s.d.

amount of tissue recovered from many brain sections was very small (Table 1) and the 5-aminolaevulinate synthase activity deteriorated to a certain extent during the rather lengthy procedure of dissection. However, the subcellular distribution of the enzyme activity was established in preliminary experiments; the specific 5-aminolaevulinate synthase activity of the whole homogenate of the brain was approx. 40% of that of the mitochondrial fraction.

As shown in Fig. 1, 5-aminolaevulinate synthase activity, generally accepted as reflecting cellular haem biosynthetic activity, was differentially distributed in the indicated brain sections. The enzyme activity in the cerebellum significantly exceeded that in other areas of the brain, and was followed by the activities of the midbrain and the cortex. On the other hand, the 5-aminolaevulinate synthase activity in the thalamus was extremely low. Moreover, the pattern of 5-aminolaevulinate synthase activity was not in keeping with the porphyrin content of the various brain sections (Table 1). In contrast with the differential 5-aminolaevulinate synthase activity, the porphyrin content of the indicated brain areas did not vary greatly. It was unexpectedly found that the

porphyrin content of the thalamus was not lower than that of the regions of high 5-aminolaevulinate synthase activity.

In addition, the activity of 5-aminolaevulinate dehydratase was measured in various brain regions. This enzyme, which constitutes the second enzyme in porphyrin biosynthetic pathway, was also most active in the cerebellum (Table 1).

Distribution of haem oxygenase and total microsomal haem in the brain

The remainder of the brain studies were carried out utilizing cellular fractions obtained from the cerebellum, the cerebral cortex and the midbrain, since haem biosynthetic activity, as assessed by the activity of 5-aminolaevulinate synthase, was found to be the most prevalent in these regions.

As shown in Table 2, the distribution of the haem catabolic enzyme, haem oxygenase, in various brain regions did not closely follow that of the anabolic enzymes, the synthase and the dehydratase (Fig. 1 and Table 1). Unlike the synthase, great variations in the tissue distribution of haem oxygenase were not observed, although the extent of haem oxidation activity, as determined by the amount of bilirubin formed, was somewhat lower in the cerebellum than in the cortex and the midbrain. The microsomal content of haem in the cortex and the midbrain did not substantially vary. However, the haem content of the cerebellum was significantly lower than that of the other regions.

Effect of manganese and cobalt on haem biosynthesis and degradative activities of the rat brain

The cellular regulatory mechanisms for the production of the enzymes of haem biosynthesis and degradation in the liver are extremely responsive to the effect of various metal ions, including Co^{2+} and

Mn^{2+} . The most striking effects, however, are exerted on the activities of 5-aminolaevulinate synthase and haem oxygenase, and result in a substantial decrease in the cellular haem content. Therefore, the effects of Co^{2+} and Mn^{2+} on these enzymes in the brain were investigated. It was found that the treatment of animals with rather large doses of Co^{2+} (two doses of $25 \mu\text{mol}/100 \text{g}$ body wt.), which approaches the LD_{50} of the agent, neither inhibited the 5-aminolaevulinate synthase activity nor induced the activity of haem oxygenase (data not shown). In contrast with Co^{2+} , as shown in Table 3, Mn^{2+} effectively inhibited the activity of 5-aminolaevulinate synthase in the cerebellum, the cerebral cortex, and the midbrain, when administered at a much less toxic dose. Moreover, the extent of the inhibition of 5-aminolaevulinate synthase was essentially the same in all the regions studied. In contrast with the effect of Mn^{2+} on the synthase activity, this agent did not alter the cellular content of haem or porphyrins in any regions of the brain studied (data not shown). Curiously, Mn^{2+} did not exert an inductive effect on the activity of haem oxygenase in the brain. The effect of Mn^{2+} treatment on the haem oxygenase activity of the brain was further investigated. Rats were treated once with 100 or $200 \mu\text{mol}$ of $\text{Mn}^{2+}/100 \text{g}$ body wt. In these studies also, the enzyme activity was found to be unaltered by the metal ion (data not shown).

The molecular basis for the inhibition of brain 5-aminolaevulinate synthase activity by Mn^{2+} was explored. In one series of experiments the effects of addition *in vitro* of Mn^{2+} on the synthase activity was assessed. It was observed that low to moderate concentrations of Mn^{2+} ($\leq 200 \mu\text{M}$) did not alter the enzyme activity; at much higher concentrations (500 – $1000 \mu\text{M}$) Mn^{2+} moderately inhibited (10–20%) the enzyme activity in the mitochondrial

Table 1. *Distribution patterns of porphyrins and 5-aminolaevulinate dehydratase activity in rat brain*

Male Sprague-Dawley rats (150–200 g body wt.) were killed. The brains were removed and dissected as described in the legend to Fig. 1. The brain sections from ten animals were pooled and the tissue homogenate was prepared in 0.05 M-Tris/HCl buffer, pH 7.4. The total porphyrin content (uro-, copro-, and protoporphyrin) and the activity of 5-aminolaevulinate dehydratase were measured as described in the Experimental section. The tissue weight shown represents the mean average value for one rat. The S.D. values are those for three experiments. Abbreviation: n.d., not determined.

Brain section	Weight (mg)	Total porphyrin content (pmol/mg of protein)	5-Aminolaevulinate dehydratase activity (nmol of porphobilinogen formed/h per mg of protein)
Septum	24 ± 4	2.12 ± 0.23	n.d.
Hypothalamus	42 ± 7	3.00 ± 0.50	n.d.
Thalamus	63 ± 10	2.66 ± 0.32	0.49 ± 0.08
Amygdala	70 ± 7	2.50 ± 0.27	0.36 ± 0.10
Cerebellum	110 ± 8	2.45 ± 0.22	0.70 ± 0.11
Midbrain	104 ± 15	3.10 ± 0.34	0.44 ± 0.07
Hippocampus	85 ± 9	2.45 ± 0.22	0.49 ± 0.09
Cortex	73 ± 11	2.52 ± 0.25	0.56 ± 0.07

Table 2. *Distribution pattern of haem oxygenase activity and microsomal haem in rat brain*

The brains of rats were dissected and the tissues obtained from five rats were pooled. The microsomal fraction was prepared using a 0.1M-potassium phosphate buffer, pH 7.4, and was used for the measurements of haem oxygenase activity and the haem content. The procedures used for the measurement of these values are described in the Experimental section. The data presented are means $\pm$ S.D. for six experiments.

Brain section	Haem oxygenase activity (nmol of bilirubin/h per mg of protein)	Microsomal haem content (nmol/mg of protein)
Cerebellum	1.39 $\pm$ 0.15	1.01 $\pm$ 0.13
Midbrain	1.84 $\pm$ 0.22	1.62 $\pm$ 0.15
Cortex	1.59 $\pm$ 0.21	1.33 $\pm$ 0.15

Table 3. *Effect of Mn²⁺ in vivo on 5-aminolaevulinate synthase activity in rat brain*

Male Sprague-Dawley rats were injected with MnSO₄·H₂O (50 μ mol/100g body wt., subcutaneously). This treatment was repeated twice at 24h intervals. The animals were killed 24h after the last injection. The brains were removed and dissected (Glowinski & Iversen, 1966), and the tissues obtained from five rats were pooled. The tissue preparation and the determination of 5-aminolaevulinate synthase activity were carried out as described in the Experimental section and in the legend to Fig. 1. The values presented are the means $\pm$ S.D. for eight experiments. The values obtained for the enzyme activity in Mn²⁺-treated animals were significantly different (**P* $\leq$ 0.05) from the control values.

Treatment	5-Aminolaevulinate synthase activity (pmol/h per mg of protein) in:		
	Cerebellum	Midbrain	Cortex
Control	56.18 $\pm$ 7.91	35.29 $\pm$ 7.06	34.51 $\pm$ 6.28
Mn ²⁺	33.33* $\pm$ 4.70	20.78* $\pm$ 4.71	23.53* $\pm$ 4.0

fraction as well as in the cellular homogenate. The response *in vitro* of the brain enzyme to Mn²⁺ did not differ from that of the hepatic 5-aminolaevulinate synthase, i.e. Mn²⁺ exerted very little inhibitory effect on the enzyme activity in the liver. This finding suggests that there were no tissue-specific interactions between Mn²⁺ and the brain 5-aminolaevulinate synthase which caused the inhibition of the activity of this enzyme in the brain.

The possibility that the inhibition of 5-aminolaevulinate synthase by Mn²⁺ was a result of the interference of the metal ion with the normal turnover of enzyme protein was examined. These studies were carried out using the liver as the model system, and changes in the distribution patterns of haem and cytochrome *P*-450 in the rough and smooth endoplasmic reticuli were used as indicators of alterations in the turnover rate of the enzymes of haem metabolism. As shown in Table 4, in rats treated with Mn²⁺ (50 μ mol/100g body wt.) the liver 5-aminolaevulinate synthase activity as well as the specific haem and cytochrome *P*-450 contents of both smooth and rough endoplasmic reticulum fractions were severely diminished. Moreover, when compared with the control rats, the distribution patterns of haem and cytochrome *P*-450 in the liver smooth and rough endoplasmic reticuli of the animals treated with Mn²⁺ were perturbed. In the control animals, the percentage distributions of haem and cytochrome *P*-450 in these two endoplasmic reticulum fractions were 40% in the rough

versus 60% in the smooth endoplasmic reticulum; in the Mn²⁺-treated animals, the distribution were 30% with the rough versus 70% with the smooth endoplasmic reticulum. However, the decreases in the specific contents of haem and cytochrome *P*-450 in the liver rough endoplasmic reticulum of the animals treated with Mn²⁺ were not accompanied by a disproportionate increase in haem oxygenase activity. The latter enzyme is the enzyme that degrades cellular haem, including that of cytochrome *P*-450 (Maines & Kappas, 1976b). Since the cellular contents of haem, proteins and other constituents are determined by the balance between synthesis and degradation, the above findings could be interpreted as a selective inhibition by Mn²⁺ of the rate of protein synthesis. Therefore, the inhibition of enzyme protein synthesis may in part represent the mechanism whereby Mn²⁺ inhibits 5-aminolaevulinate synthase activity. The finding that the total protein content of the rough and smooth endoplasmic reticuli was also decreased in response to Mn²⁺ treatment is in agreement with such an occurrence; however, the possibility that Mn²⁺ may alter the sedimentation property of the cellular organelles, and thus the extent of their recovery in the course of the isolation, cannot be ruled out.

Discussion

The present study reports the differential activity of 5-aminolaevulinate synthase in eight regions of the rat brain, and demonstrates that certain areas of

Table 4. *Effects of Mn²⁺ in vivo of 5-aminolaevulinate synthase activity and various properties of the endoplasmic reticulum in rat liver*

Rats (150–200g body wt.) were treated with MnSO₄·H₂O (50 µmol/100g body wt., subcutaneously, two injections at 24 h intervals) and were killed 24 h after the last injection. The livers were perfused, homogenized, and various subcellular fractions were prepared. The measurement of 5-aminolaevulinate synthase activity was carried out as described in the Experimental section. The rough and the smooth endoplasmic reticuli were prepared as described by Dallner (1974) and were assayed for the contents of haem, cytochrome *P*-450 and protein as well as for the haem oxygenase activity, as detailed in the Experimental section. **P* ≤ 0.05 when compared with the control. Abbreviation: n.d., not determined.

Treatment	Cell fraction	Total protein content (mg/g wet wt.)	5-Amino-laevulinate synthase activity (pmol/h per mg of protein)	Total haem content (nmol/mg of protein)	Cytochrome <i>P</i> -450 content (nmol/mg of protein)	Haem oxygenase activity (nmol/h per mg of protein)
Control	Homogenate	n.d.	75.0 ± 8.4	n.d.	n.d.	n.d.
	Mitochondria	n.d.	112.0 ± 12.0	n.d.	n.d.	n.d.
	Rough endoplasmic reticulum	4.20 ± 0.38	n.d.	0.98 ± 0.13	0.67 ± 0.12	1.70 ± 0.24
	Smooth endoplasmic reticulum	4.71 ± 0.28	n.d.	1.47 ± 0.12	0.99 ± 0.04	1.93 ± 0.14
Mn ²⁺	Homogenate	n.d.	50.4* ± 12.1	n.d.	n.d.	n.d.
	Mitochondria	n.d.	70.4* ± 9.0	n.d.	n.d.	n.d.
	Rough endoplasmic reticulum	2.92* ± 0.38	n.d.	0.47* ± 0.06	0.27* ± 0.06	5.88* ± 0.32
	Smooth endoplasmic reticulum	3.10* ± 0.29	n.d.	0.98* ± 0.13	0.62* ± 0.10	5.62* ± 0.39

the organ possess significantly greater activity than others. A novel finding is that of the ability of an exogenous agent, Mn²⁺, to alter the activity of 5-aminolaevulinate synthase in the brain.

It is curious that haem oxygenase, unlike 5-aminolaevulinate synthase, was totally non-responsive to all regimens of Mn²⁺ treatment employed in these experiments. The possibility of the coupling of the cellular regulatory mechanism for the production of haem oxygenase and 5-aminolaevulinate synthase has been suggested in studies with rat liver (Maines & Kappas, 1975) as well as in those with avian hepatocytes in culture system (Maines & Sinclair, 1977). Those studies showed a reciprocal response to various metal ions of the cellular regulatory mechanisms for the synthesis of 5-aminolaevulinate synthase and haem oxygenase. The metal-ion-mediated decreased activity of 5-aminolaevulinate synthase was accompanied by a concomitant increase of haem oxygenase activity. Such a reciprocal relationship for the production of these enzymes also occurs naturally, and was observed in the liver and the kidney during the neonatal period (Maines & Kappas, 1978).

The finding reported here, that the Mn²⁺-mediated inhibition of 5-aminolaevulinate synthase in the brain, unlike that in other organs, was not accompanied by an increase in haem oxygenase activity, suggests that the cellular regulatory mechanism for the synthesis of haem oxygenase in the brain is

buffered and/or refractory to metal ions, since there is little doubt that Mn²⁺ gains entry to the tissue (Neff *et al.*, 1969). Perhaps the haem metabolic pathway enzymes as a whole are non-inducible in the brain; the absence of such a response could be an important factor in maintaining the homeostasis of the brain. This suggestion is in keeping with the finding of Paterniti *et al.* (1978) that 5-aminolaevulinate synthase, when measured in whole brain, is not responsive to a number of agents and conditions that are normally potent inducers of the liver enzyme. These agents include chemicals such as allylisopropylacetamide (De Matteis *et al.*, 1961); 3,5-dicarbethoxy-4-dihydrocollidine (Granick & Urata, 1963); ethanol (Shanley *et al.*, 1968); allyl compounds (Ioannides & Parke, 1976); and the nutritional status (Condie *et al.*, 1976).

Another factor that may contribute to the maintenance of the homeostasis of haem metabolism in the brain could be the ability of the neural cells to utilize porphyrin synthesized extraneurally for the production of haem. It follows that such ability may be extended to include the utilization of extraneurally produced haem molecules. This hypothesis is supported by the findings that the total porphyrin content of various brain sites did not vary substantially and did not follow the pattern of 5-aminolaevulinate synthase activity, and that inhibition of 5-aminolaevulinate synthase activity was virtually ineffective in altering the cellular

content of haem and porphyrin complexes. Moreover, the observed pattern of 5-aminolaevulinate synthase activity in different brain segments did not correspond to the distribution pattern of *N*-demethylation activity reported by Fishman *et al.* (1976). They observed the highest morphine *N*-demethylation activity, believed to be a haemo-protein-dependent reaction, in the hypothalamus and the thalamus. These regions of the brain, particularly the thalamus, exhibited low 5-amino-laevulinate synthase activity in our experiments, although the porphyrin content of these areas was not lower than that of the other regions.

The inhibition of protein synthesis by metal ions via electrostatic interactions with the polyanionic phosphate backbone of DNA is known (Eichorn & Shin, 1968). Moreover, it has been demonstrated that Mn^{2+} can substitute for Mg^{2+} as the metal activator of DNA polymerase and increase the error frequency of nucleotide incorporation (Sirover & Loeb, 1976). On the basis of the studies *in vitro* as well as those with the liver *in vivo*, it can be proposed that the mechanism by which Mn^{2+} inhibits the activity of 5-aminolaevulinate synthase in the brain may involve in part the inhibition of enzyme-protein synthesis.

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