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REGULATION OF HEME AND DRUG METABOLISM ACTIVITIES IN THE BRAIN BY MANGANESE

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A novel effect of metal ions in the brain is described. Mn was found to alter heme metabolism and the cytochrome P-450-dependent mixed-function oxidase activities in rat brain. A more than 2-fold increase in benzo(α)pyrene hydroxylase and 7-ethoxycoumarin deethylase activities were observed in the brain of rats treated for 7 days with Mn. The increases were regionally distributed; the highest elevations were observed in the hippocampus, pons and the caudate putamen. Moreover, in rats treated with Mn for 1 or 7 days a marked depression in the activity of the mitochondrial ALA synthetase was observed. The activity of the microsomal heme oxygenase was also inhibited at 7 days, but not 1 day, after Mn treatment. These inhibitions were reflected in an initial decrease, followed by a rebound return to normal, in the concentration of cytochrome P-450 in the brain. Mn was ineffective in vitro in altering heme and drug metabolism activities. It is suggested that Mn-mediated alterations in heme metabolic activities promote changes in the composition of cytochrome P-450 species in the brain microsomal fractions, such that the relative concentrations of the molecular species which catalyse aryl hydrocarbon hydroxylase activity become selectively increased.

In the liver and in certain extrahepatic tissues the activities of ALAS and HO, the rate-limiting enzymes in heme biosynthesis and degradation pathways, respectively, are readily altered by a variety of factors, including metal ions (1-3). In the liver metal ions promote a biphasic response in the activity of ALAS which consists of an inhibition followed by a rebound increase. These agents invariably cause a marked increase in the activity of HO in this organ. Frequently alterations mediated by metal ions in heme

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Abbreviations: ALAS, δ-aminolevulinate synthetase; HO, heme oxygenase; BP, benzo(α)pyrene; MFO, mixed-function oxidase; cyt, cytochrome; 7-EC, 7-ethoxycoumarin.

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metabolic activities in the liver are reflected in drug metabolism processes (3).

However, with the exception of isolated reports (4,5), nothing is known about the effect of metal ions on cyt P-450-dependent MFO and the activities of ALAS and HO in the brain. Nonetheless, it may be postulated that certain metal ions which gain access to the brain may be capable of regulating heme and drug metabolism activities in this tissue. It follows, on the basis of reported finding (6) that Mn causes abnormalities in the metabolism of certain biogenic amines in the brain, and the observation that in the brain of Mn-treated rats the activity of ALAS is inhibited (4,5), it can be postulated that Mn treatment may also perturb drug biotransformation processes in this organ. Moreover, although in the brain heme biosynthesis activity is non-responsive (7) to factors which readily induce this activity in the liver, curiously, the cyt P-450-dependent MFO activity (8-10) is inducible (11) in this organ.

The present investigation was undertaken to determine whether heme biosynthesis and degradation activities, and the cyt P-450-catalyzed reactions in the brain are subject to alterations by Mn. Also, the interrelation between heme metabolic activity and MFO activity in the brain was examined.

Materials and Tissue Preparation: Male Sprague-Dawley rats (200-250 g) were purchased from Harlan Industries, Madison, WI. The animals were allowed access to food and water ad libitum. Rats were treated either with $MnSO_4.H_2O$, 500 µmol/kg, sc, and killed 24 h later or for 7 days with Mn, 1750 µmol/kg (total dose),by means of Mini-Osmotic pumps (Alza) implanted sc. The control animals received saline. The animals were killed at time intervals indicated in the legend of appropriate tables. Other chemicals were products of Sigma Chemical Co. St. Louis, MO. At indicated time intervals rats were heparinized, anaesthesized by ether, and the brain was completely perfused through the left ventricle with ice cold saline. Thereafter, the cerebral hemisphere was removed, and was dissected into various regions (12). Subcellular fractions were prepared as described before (4) using Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose and 0.05mM EDTA.

Assay Procedure: The activity of the mitochondrial ALAS was measured as described earlier (4). The microsomal fraction was used for the measurements of HO (13), BP-hydroxylase (14) and 7-EC deethylase (15) activities. The following procedure was developed for the measurement of the brain cyt P-450. The microsomal fractions were suspended in 0.05 M potassium phosphate buffer (pH 7.4) containing 0.2 percent Emulgen 911 and 20 percent glycerol. Sodium dithionite was added to the suspension prior to dividing between the reference and the test cuvette, the test cuvette was bubbled with CO. An extinction

coefficient of 91 mM-1 cm-1 was used (16). Biliverdin reductase activity was measured as previously described (17). The microsomal NADPH-cyt c (P-450) reductase activity was assessed as described by Yasukochi and Masters (18). The protein concentration was measured by the method of Lowry et al (19). All spectral studies were carried out using an SLM Aminco DW-2C spectrophotometer. The experimental results are expressed as the mean ± SD of 4 determinations. The data were analyzed using Student's t-test; a p value of ≤ 0.05 was considered significant.

RESULTS: A rather impressive levels of heme biosynthesis and degradation activities (Table I) were detected in the rat brain. Indeed, the activities of ALAS and HO in the brain were comparable to those previously reported for the liver (2,3). As shown, 24h and 7 days after Mn treatment marked decreases in ALAS activity were detected. The activity of HO was not altered 24h after Mn treatment, however, the enzyme activity was significantly decreased after 7 days. Biliverdin reductase activity was not altered by Mn treatments.

Measurable levels of cyt P-450 were detected in the brain microsoms (Fig 1). Unlike the previous report (10), the spectrum obtained using the presently described procedure was devoid of interference by hemoglobin at 430 nm and exhibited a symmetrical absorption peak at 450nm. This permitted an accurate measurement of the brain cyt P-450 concentration.

As shown in Table II, 24h after treatment of rats with Mn a significant decrease in brain microsomal cyt P-450 concentration was observed. This decrease, however, was not accompanied by depressions in rates of BP-hydroxylase and 7-EC deethylase activities. Conversely, in rats exposed to Mn for 7 days cyt P-450 content was comparable to the control value, and at the same

TABLE I - Effect in vivo of Mn treatment on activities of δ-aminolevulinate synthetase, heme oxygenase and biliverdin reductase in rat brain

Treatment (days)	ALA Synthetase (pmol ALA/mg/h)	Heme Oxygenase (nmol bilirubin/mg/h)	Biliverdin Reductase (nmol bilirubin/mg/h)
Control	185.0 ± 21.2	2.86 ± 0.24	38.4 ± 1.2
Mn (1)	107.0* ± 21.8	2.63* ± 0.05	40.2 ± 3.0
Mn (7)	67.2* ± 16.8	2.06 ± 0.30	34.2 ± 1.8

Rats were treated with Mn and the indicated parameters were assesed. Experimental details are described in the text. *P ≤ 0.05 when compared with control.

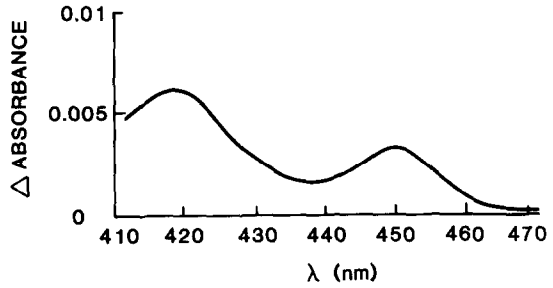

Fig. 1: Co-reduced difference spectrum of rat brain microsomal cytochrome P-450. The microsomal fractions (0.2 mg protein/ml) were prepared from the perfused brain of rats. The spectrum was obtained as described in the text.

time, a nearly 2-fold increase in hydroxylase and deethylase activities were observed. As noted, the activity of NADPH-cyt c (P-450) reductase did not respond to Mn treatment.

As shown in Table III, MFO activity is differentially distributed, and affected by Mn, in different regions of the brain. BP-hydroxylase activity in the hypothalamus, thalamus, and caudate putamen, exceeded that of the other regions. Moreover, a nearly 2-fold increase in the hydroxylase activity was detected in the hippocampus. Also, a significant increase in enzyme activity in the caudate putamen and pons region was detected. The hydroxylase activity in other regions was essentially unaltered.

The *in vitro* effects of Mn on the listed enzyme activities were examined. The presence of Mn at final concentrations of 50 - 400 μM in assay systems for

TABLE II - Effect *in vivo* of Mn treatment on cytochrome P-450 concentration and activities of NADPH-cytochrome c (P-450) reductase, benzo(α)pyrene hydroxylase and 7-ethoxycoumarin deethylase in rat brain microsomal fraction

Treatment (days)	Cytochrome P-450 (pmol/mg)	NADPH-Cytochrome c (P-450) Reductase (μmol/mg/h)	Benzo(α)pyene Hydroxylase (pmol/mg/h)	7-Ethoxycoumarin Deethylase (pmol/mg/h)
Control	5.1 ± 0.9	8.02 ± 0.57	0.9 ± 0.1	53.3 ± 6.7
Mn (1)	3.5 ± 0.4 *	7.48 ± 0.53	0.9 ± 0.2 *	61.5 ± 5.1 *
Mn (7)	6.0 ± 0.7	8.57 ± 1.07	1.9 ± 0.4	103.6 ± 25.4

Male Sprague-Dawley rats were treated with Mn, and assays were carried out as decribed in detail in the text. *$P \leq 0.05$ when compared with the control.

TABLE III - Effect in vivo of treatment for 7 days with Mn on benzo(α)pyrene hydroxylase activity in different regions of rat brain

Region	Treatment	Benzo(α) pyrene Hydroxylase (pmol/mg/h)
Amygdala	Control	1.59 ± 0.13
	Mn	1.56 ± 0.23
Hypothalamus	Control	*3.63 ± 0.25
	Mn	4.11 ± 0.28
Thalamus	Control	*1.82 ± 0.12
	Mn	2.08 ± 0.25
Hippocampus	Control	0.67 ± 0.06
	Mn	**1.16 ± 0.20
Caudate putamen	Control	*2.03 ± 0.05
	Mn	**2.80 ± 0.16
Pons	Control	0.71 ± 0.09
	Mn	**1.06 ± 0.17
Cerebellum	Control	0.85 ± 0.29
	Mn	1.36 ± 0.41
Cortex	Control	0.50 ± 0.15
	Mn	0.63 ± 0.12

The 9,000g supernatant fraction obtained from pooled brain sections from 3 rats was used for each determination. Experimental details are described in the text. *$P \leq 0.05$ when compared with other regions; **$P \leq 0.05$ when compared with the control.

ALAS, HO, 7-EC deethylase and BP-hydroxylase did not inhibit or stimulate rates of enzyme activities. Also, the treatment in vitro of the the microsomal fractions with Mn did not alter cyt P-450 spectrum.

DISCUSSION: The presently observed increase in cyt P-450-dependent MFO activity in the brain in response to Mn treatment represents an unprecedented finding. Moreover, the ability of Mn to inhibit both the activity of ALAS and that of HO in the brain constitutes an unusual tissue response (3). The finding that the in vitro treatment of enzyme preparations with Mn did not cause significant changes in ALAS and HO activities suggests that the in vivo inhibitory action of the metal ion did not represent a direct inhibition of the enzymes. In addition, the finding that biliverdin reductase activity was not inhibited in the brain of Mn-treated rats suggests that Mn did not exert a generalized inhibitory action on enzymes of heme metabolic pathway in this organ. Considering that ALAS and HO in the brain are known to be refractory to various factors which regulate the activity of these enzymes in the liver

(7), the present findings (Table I) may distinguish metal ions as a unique category of agents capable of regulating heme metabolic activities in the brain.

In the absence of an increase in heme biosynthesis activity, the Mn-mediated increases (Table II) in the cyt P-450-dependent biotransformations of BP and 7-EC does not appear to reflect an induction phenomenon; rather, it appears more likely that the increases may represent the effect of Mn on the isomeric composition of cyt P-450 in the brain. It may be postulated that Mn treatment promoted a selective decrease, perceivably, through an accelerated rate of destruction and/or an inhibited rate of production in the populations of those molecular species of cyt P-450 which are not involved in the biotransformation of BP and 7-EC. It may be further postulated that, as the result, the specific microsomal content of the active isozymes could be increased. This concept is consistent with the finding that 7 days after Mn-treatment the more than 2-fold increases in the rates of oxidative reactions were not accompanied by increases in either the cyt P-450 concentration or the NADPH cyt c (P-450) reductase activity. Also, the finding that 1 day after Mn treatment a significant decrease in the microsomal cyt P-450 concentration was not accompanied by decreases in BP hydroxylase and 7-EC deethylase activities is consistant with this suggestion.

Furthermore, the present study demonstrates that the hydroxylation of BP and deethylation of 7-EC in rat brain are not limited to specific regions. This finding contrasts the reported distribution pattern of cyt P-450-catalysed morphine N-demethylase activity which has been reported to be absent from various brain sites (8). These differences may reflect the differential distribution of isozymes of cyt P-450 in the brain. This possibility is plausible considering that, in the liver, the molecular species of cyt P-450 which catalyse the N-demethylation of morphine are different from those which catalyse aryl hydrocarbon hydroxylase reactions (20). It follows that the differential response of the various brain regions to Mn may reflect tissue properties, such as, the differential response of the regulatory mechanism for

cyt P-450 metabolism in different regions, or disparity in the accessibility of Mn to different brain sites.

Mn intoxication in man is not an uncommon occurance, and the central nervous system is the target tissue for Mn toxicity (21). The present finding that Mn caused profound perturbations in the activities of the rate-limiting enzymes of heme metabolism pathway, and the fact that catalase, a hemoprotein, exerts protective effects on several monoxygenases in the brain, including dopamine-β-hydroxylase, and phenylalanine, tyrosine and tryptophan hydroxylases (22) renders the present findings of potential toxicological relevance.

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