In Vivo Incorporation of ^{14}C-Leucine into Brain Protein of Methylmercury Treated Rats

by FRED F. FARRIS* and J. CRISPIN SMITH
Department of Pharmacology and Toxicology
University of Rochester
School of Medicine and Dentistry
Rochester, N.Y. 14620

The toxicity of methylmercury is due in large part to its actions on the central nervous system. Those features described by HUNTER et al. (1940), KURLAND et al. (1960) and BAKIR et al. (1973) are dominated by symptoms indicative of CNS disorders. Also characteristic of methylmercury intoxication is a latent period of several days or more between the accumulation of the compound in the brain and the onset of symptoms.

In an attempt to explain the sensitivity of the brain to methylmercury, investigators have monitored various biochemical parameters in systems poisoned with this compound. YOSHINO et al. (1966) measured changes in brain cortex slices from rats severely intoxicated with methylmercury. Although several alterations were observed in animals showing neurological symptoms, only protein synthesis decreased during both the latent period and after the onset of symptoms. More recently, CAVANAGH and CHEN (1971) observed that in vivo brain protein synthesis tended to decrease in rats mildly intoxicated with methylmercury.

No investigations have been reported concerning the effects of low or extremely high doses of methylmercury on in vivo protein synthesis. We, therefore, measured in vivo ^{14}C-leucine incorporation into brain protein in 48 hour old rats which received low levels of the mercurial during the fetal stage, and also in severely poisoned adult female rats showing neurological symptoms.

MATERIALS and METHODS

Materials: Methylmercury chloride was obtained from K and K Lab., Inc., Plainview, N. Y. Soluene tissue solubilizer from Packard Instr., Downers Grove, Ill. Aquasol and (U)-^{14}C-L-leucine (specific activity of 280 mCi/mmole) from New England Nuclear, Boston, Mass. Sprague-Dawley rats from Holtzman Farms. Other chemicals were reagent grade from various manufacturers.

General Methods: (a) Measurement of Methylmercury: Methylmercury concentrations were measured using the method of VON BERG et al. (1974). (b) Isolation of the Protein Fraction: The brain protein was isolated by the method of LAJTHA et al. (1957). (c) Counting

*Present address and to whom correspondence should be sent. College of Pharmacy, North Dakota State University, Fargo, N.D. 58102

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C-14 Labeled Samples: The protein or whole brain tissue was accurately weighed and dissolved in 1.0 or 2.0 ml Soluene. A 200 µl aliquot of the solubilized tissue was placed in a counting vial with 20 µl 38% hydrochloric acid, 3.5 ml water and 11.5 ml Aquasol. The vial was capped, shaken and counted with a Packard Model 3380 Liquid Scintillation Counter (Packard Instr., Downers Grove, Ill.).

The protein isolation and counting methods were tested by performing a simple preliminary experiment. Incorporation of ^{14}C-leucine was measured *in vivo* into brain protein of saline treated rats and in rats given a compound known to inhibit protein synthesis (cycloheximide). The 7 fold decrease in incorporation, in the cycloheximide treated rats, indicates that the methods are effective in measuring differences in protein synthesis when they exist.

Experiment A: Eight female rats were obtained on the 15th day of pregnancy. Five animals were given a subcutaneous injection of 1.0 mg methylmercury chloride in 1.0 ml 0.9% saline on various days during the remainder of pregnancy (Table 1 gives a detailed dosing regimen). The controls were similarly injected with 1.0 ml 0.9% saline on the 18 and 19th days of gestation. Forty-eight hrs after birth, 2 offspring from each female were sacrificed and their brains used for methylmercury analysis. The remaining offspring were intraperitoneally injected with 2 µCi ^{14}C-leucine and 30 min later each was decapitated, its brain quickly removed, and the protein fraction isolated and counted.

Experiment B: Fourteen female rats weighing 175 to 195 g were used in this experiment. For the first 3 days of the experiment, 8 rats were given a daily subcutaneous injection of 5.0 mg methylmercury chloride and 10.0 mg cysteine in 1.0 ml distilled water. The 6 controls received an equal volume of distilled water containing only the cysteine. On the 7th day, all rats (2 mercury treated had died) were placed under ether anesthesia and 13.9 µCi ^{14}C-leucine in 0.25 ml 0.9% saline injected into the Femoral vein. After 90 min the rats were decapitated, and their brains removed and sectioned into hemispheres, mid-brain, occipital and cerebellum. One hemisphere from each rat was used for measurement of brain methylmercury and for the determination of ^{14}C-leucine content of whole brain tissue. The remaining hemisphere and other sections were homogenized and the protein isolated and counted.

RESULTS and DISCUSSION

The dosing regimen for the pregnant female rats, and the brain mercury levels and leucine incorporation values for the 48 hour old offspring in Experiment A are given in Table 1. The results show that no significant difference existed in brain protein synthesis between the offspring of saline and methylmercury treated females. The absence of a significant reduction in this function, in the mercury treated animals, may be due to the relatively low brain mercury levels (4.5-9.7 ppm), or it may be that the lag of 7 days between commencement of dosing and measurement of leucine incorporation was innsufficient to observe inhibition. By compari-

TABLE 1

Dosing regimen for pregnant female rats, and brain mercury levels and ^{14}C-leucine incorporation values for 48 hour old offspring in Experiment A.

	Experimental Group			
	Saline Treated	CH_3HgCl Treated	CH_3HgCl Treated	CH_3HgCl Treated
No. of Pregnant Females	3	1	2	2
Days of Treatment During Pregnancy	18,19	16,17	16-18	16-19
Brain Methylmercury µg Hg^0/mg Brain (No. of Offspring)	0.0 (3)	4.5-4.9 (2)	6.1-8.4 (4)	8.4-9.7 (4)
Mean DPM ^{14}C / mg Protein ± S.E.M. (No. of Offspring)	2294±97 (17)	2189±139 (9)	2189±166 (11)	2005±74 (9)
p [a]	-	<0.6	<0.6	<0.1

[a]Data from methylmercury and saline treated groups were compared for statistical significance by "Student's t" test.

son, CAVANAGH and CHEN (1971) measured in vivo brain protein synthesis in male rats given 5 mg/Kg/day methylmercury dicyandiamide for up to 8 consecutive days. Their results suggest that this function tended to decrease in the treated animals. Seven days after commencement of dosing, and before onset of symptoms, ^{14}C-glycine incorporation had decreased by 16%. After onset of mild symptoms it had dropped by 23%. No mention was made of brain mercury content but a reasonable estimate might place it at about 15-25 ppm. This is about twice as high as in our rats (Table 1).

The methylmercury treated adult rats in Experiment B all showed signs of severe intoxication. The earliest sign was reddening or bleeding around the eyes and nose. Other common signs were extensive weight loss, sluggishness and hunched posture. As the experiment progressed, the animals often showed a tendency to walk backwards, diarrhea and a complete loss of function of the hind legs. On the 7th day, some of the rats were unable to move about their cage. Of the 8 poisoned rats, 2 expired on the 6th day and another died under ether anesthesia on the 7th.

Table 2 shows that, on the final day of the experiment, the mean body weight of the poisoned animals was 63% that of the controls. Since all animals received the same amount of leucine, it is reasonable to expect a larger percentage to be distributed to the brains of the intoxicated rats due to their greater brain to body weight ratio. The concentraions of ^{14}C-leucine found in whole brain tissue (Table 2) support this assumption. Therefore, all

TABLE 2

Body weight, brain mercury, whole brain ^{14}C-leucine, and ^{14}C-leucine incorporation values for adult female rats in Experiment B.

	Control Animals (Mean ± S.E.M.)	Treated Animals (Mean ± S.E.M.)	Treated as % of Controls	p [a]
Body Weight on 7th Day	202.3 ± 3.8	127.2 ± 2.1	63	-
Brain Methylmercury as Hg^0 (μg/g Brain)	< 0.1	53.6 ± 4.4	-	-
Whole Brain Leucine (DPM ^{14}C/mg Brain)	78.7 ± 2.4	131.7 ± 6.5	167	-
Cerebellum Protein (DPM ^{14}C/mg Protein)	746.2 ± 24.5 [b]	554.3 ± 54.2 [b]	74	< 0.01
Hemisphere Protein (DPM ^{14}C/mg Protein)	711.3 ± 23.5 [b]	512.1 ± 59.6 [b]	72	< 0.01
Mid-Brain Protein (DPM ^{14}C/mg Protein)	658.8 ± 30.1 [b]	495.2 ± 44.8 [b]	75	< 0.02
Occipital Protein (DPM ^{14}C/mg Protein)	770.9 ± 30.8 [b]	600.3 ± 60.1 [b]	78	< 0.05

[a] Values from treated and control animals were compared for statistical significance by "Student's t" test. All tabulated data are based on values from 6 controls and 5 treated rats.
[b] All values are corrected for variations in body weight. Raw data were normalized to correspond to a ^{14}C-leucine dose of 100 μCi per Kg of body weight for each animal.

leucine incorporation data were corrected for differences in body weight. It is the corrected data that are given in Table 2.

A comparison of the leucine incorporation results (Table 2) shows that a significant inhibition of this function occurred in at least three brain regions in the methylmercury treated rats. Only the difference measured in the occipital region is of questionable significance. On the average, protein synthesis in the poisoned animals was about 75% of that found in the controls. Brain mercury levels were approximately 54 ppm (Table 2) in our rats. This is probably slightly higher than the levels in the adult male rats given a single 75 mg/Kg dose of methylmercury thioacetamide by YOSHINO et al. (1966). They measured ^{14}C-leucine uptake into protein of brain cortex slices, and found 57% of normal incorporation in animals during the latent period and 42% of normal in rats after onset of symptoms. Our results confirm the observation that brain protein synthesis is significantly depressed in rats showing symptoms due to methylmercury intoxication (YOSHINO et al., 1966). Although our measurements suggest a less intense depression of this parameter than do those of YOSHINO et al. (1966), it should be emphasized that the two experiments were considerably different in design.

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