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BRIEF COMMUNICATION

Pre-Natal Amino Acid Transport Inhibition: Long Term Influences on Behavior and Protein Metabolism[1]

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SAMUELS, S., C. T. RANDT, I. FISH AND S. A. SCHWARTZ. *Pre-natal amino acid transport inhibition: Long term influences on behavior and protein metabolism.* PHARMACOL BIOCHEM BEHAV **24**(1) 143–146, 1986.—DBA/2J mice were exposed in utero, between days 15–18 of gestation, to either of two enzyme inhibitors, previously shown to decrease blood-brain, large-neutral amino acid transport in adults: L-methionine-RS-sulfoximine and 2-imidazolidone-4-carboxylic acid. The young mice demonstrated persistently altered motor behavior relative to saline controls when 40–42 days old and evidence of differences in the entry and incorporation of 14C-valine in brain at up to 80 days of age. The findings suggest that interference with blood-brain amino acid transport in utero has long term consequences. This may be related to some human conditions such as maternal phenylketonuria.

Amino acid transport Blood-brain Prenatal Long term effects Congenital Behavior

DISRUPTION of amino acid transport from blood to brain in early life has been implicated in several clinical conditions associated with mental retardation. Among these are phenylketonuria (PKU) [6] including maternal PKU [9], maple syrup urine disease [4], and early life protein malnutrition [5]. Adult mice, given either of two enzyme inhibitors, showed behavioral changes coincident with decreased blood-brain amino acid transport in acute experiments [13, 16, 17]. The present study was designed to assess the long term behavioral and biochemical effects on the offspring of dams given either of these two transport inhibitors during the last few days of pregnancy. The young mice were observed with regard to their food intake and two behavioral measures (at 40–42 days of age), and their brain weights and estimated blood-brain amino acid transport and brain protein incorporation at 20 or 80 days of age.

METHOD

Two different enzyme inhibitors were used: 2-imidazolidone-4-carboxylic acid (ICA; Aldrich Chemical Co., Milwaukee, WI), a competitive inhibitor of 5-oxoprolinase [19]; and L-methionine-RS-sulfoximine (Sigma Chemical Co., St. Louis, MO), a non-competitive inhibitor of the closely related enzymes, γ-glutamylcysteine synthetase [15] and glutamine synthetase [10].

Timed pregnant DBA/2J mice (Jackson Laboratory, Bar Harbor, ME) were given subcutaneous, interscapular injections of either of the two enzyme inhibitors: ICA at 1,480 mg/kg body weight on days 15 through 18 of gestation or MSO at 10 mg/kg on days 15 and 17 only. This protocol was based on our previous experience, giving the two inhibitors acutely to adult mice [13]. The schedule of frequent injections of the pregnant DBA/2J mice could be expected to cause rather severe stress with possible adverse consequences for the offspring. In order to distinguish between the effects of the enzyme inhibitors and those of the physical manipulation itself, two saline injected control groups were included in the experimental design, one in parallel with each of the drug injection schedules. Since the stress effect per se was not the focus of this study, an uninjected control group was not included in the experiment. Cannibalism during the first three days postnatally was high, presumably due, in part, to the stress of the injection schedule. Survival of ICA or MSO exposed litters was approximately 14% and for saline controls about 25%. On the fourth day postnatally, all litters were culled to four pups so as to minimize differences in maternal attention and social effects.

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TABLE 1

FOOD CONSUMPTION OF DAMS AND PROGENY*

Treatment	N	15–21 days gestation (dams) mean (g) SEM	5–10 days pre-weaning (dams) mean (g) SEM	N	43–48 days single caged (progeny) mean (g) SEM
Saline	8	3.82 ± 0.33	8.95 ± 0.41	24	2.85 ± 0.10
MSO	3	3.90 ± 0.36	9.63 ± 0.48	22	2.98 ± 0.26
ICA	3	5.92† ± 0.78	10.58‡ ± 0.43	28	3.20§ ± 0.05

*MSO administered SC to dams, 10 mg/kg on days 15 and 17 of gestation. ICA administered SC to dams, 1.48 g/kg on days 15, 16, 17, and 18 of gestation. Saline administered SC to dams, 0.3 ml, on days 15, 16, 17, and 18 of gestation.

†(ICA vs. Saline) $t=2.85$, $p<0.02$ (two-tailed).

‡(ICA vs. Saline) $t=2.58$, $p<0.05$ (two-tailed).

§(ICA vs. Saline) $t=2.50$, $p<0.02$ (two-tailed).

TABLE 2

BODY AND BRAIN WEIGHTS OF MICE EXPOSED *IN UTERO* TO MSO, ICA OR SALINE

	N		Body Weight at 21 days mean±SEM	N		Body Weight at 43 days mean±SEM	N		Body Weight at 80 days mean±SEM	Brain Weight 80 days		Ratio brain wt / body wt
	Litters	mice	(g)	Litters	mice	(g)	Litters	mice	(g)	N mice	mean±SEM (g)	
Saline	3	10	8.00 ±0.20	6	24	18.75±0.60	3	10	23.50 ±1.00	10	0.360 ±0.005	0.011
MSO	6	18	8.03 ±0.36	6	22	19.15±0.38	4	14	25.88 ±0.81	14	0.362 ±0.007	0.014
ICA	8	28	6.77*±0.27	7	28	17.33±0.53	3	11	21.15†±0.57	11	0.339‡±0.003	0.012

*$t=2.61$, $p<0.02$ (two-tailed).

†$t=2.09$, $p<0.05$ (two-tailed).

‡$t=3.68$, $p<0.002$ (two-tailed).

TABLE 3

OPEN FIELD ACTIVITY OF PROGENY AT 40 DAYS OF AGE

	N (Litters)	Activity (mean) SEM
Control (saline)	13	939 ± 40.1
MSO	8	791 ± 60.9*
ICA	8	792 ± 57.4†
Running Wheel (at 42 days of age)		
Control (saline)	13	4900 ± 604.0
MSO	8	8274 ± 905.5‡
ICA	8	8611 ± 665.8§

*MSO vs. Saline, $t=2.12$, $p<0.05$ (two-tailed).

†ICA vs. Saline, $t=2.14$, $p<0.05$ (two-tailed).

‡MSO vs. Saline, $t=3.22$, $p<0.005$ (two-tailed).

§ICA vs. Saline, $t=3.98$, $p<0.001$ (two-tailed).

Food intake of the dams was measured on days 15 through 21 of gestation and during the fifth through tenth days of the suckling period. At 43 days the offspring were caged singly and the daily food intake measured through 48 days of age. Body weights of the offspring were recorded when 21, 43, and 80 days old.

Open field testing was performed at 40 days of age in a circular metal enclosure, 40 cm in diameter by 38 cm high. The walls were black and the white floor was marked off into 12 cm squares by means of 1 cm wide black lines. An overhead cold-white fluorescent lamp projected 30 lumens at the floor. Four crossed infrared photocell circuits 1 cm above the floor measured an animal's activity which was recorded on a digital counter in an adjacent room. Prior to testing, between 1200 and 1600 hours, each mouse was placed in a ventilated 1 liter cardboard container one third filled with fresh cotton. After 30 min an animal was gently lowered to the floor in the center of the open field and activity counts were recorded for three consecutive five minute periods. In addition, fecal boli were counted. The enclosure was wiped clean with a mildly scented liquid after each mouse to eliminate any residual odor.

Running activity over a 24 hour period was measured at 42 days of age in a vertically oriented perforated metal wheel 15 cm in diameter by 4 cm wide. A 3 cm square aperture permitted access to an adjacent 11×6×5 cm wire mesh cage where food and water were available ad lib. Testing was

TABLE 4

^{14}C-VALINE IN PROGENY AT 20 DAYS OF AGE

Treatment	N (mice)	dpm/g brain soluble (free) SEM	dpm/g brain insoluble (protein) SEM	dpm/g brain total SEM
Saline	9	10,700 ± 633	18,200 ± 667	28,900 ± 920
MSO	13	9,600 ± 388	15,200* ± 721	24,800 ± 819
ICA	12	10,100 ± 318	15,000† ± 664	25,100 ± 736

*MSO vs. Saline, $t=2.91$, $p<0.01$ (two-tailed).
†ICA vs. Saline, $t=3.33$, $p<0.005$ (two-tailed).

TABLE 5

^{14}C-VALINE IN PROGENY AT 80 DAYS OF AGE

Treatment	N (mice)	dpm/g brain soluble (free) SEM	dpm/g brain insoluble (protein) SEM	dpm/g brain total SEM
Saline	30	10,200 ± 274	7,410 ± 297	17,610 ± 404
MSO	19	8,840* ± 589	6,960 ± 333	15,800‡ ± 677
ICA	24	9,090† ± 334	6,850 ± 367	15,940§ ± 496

*MSO vs. Saline, $t=2.22$, $p<0.05$ (two-tailed).
†ICA vs. Saline, $t=2.59$, $p<0.02$ (two-tailed).
‡MSO vs. Saline, $t=2.45$, $p<0.02$ (two-tailed).
§ICA vs. Saline, $t=2.64$, $p<0.02$ (two-tailed).

begun at 1400 hours and the number of revolutions was recorded at 1, 3, and 24 hr.

Blood-brain amino acid transport and incorporation into brain protein were estimated using (U) 14C-L-valine (232 μCi/μmole; New England Nuclear, Boston, MA) injected subcutaneously at 30.3 μCi/kg body weight. Exactly 30 min after injection each animal was killed by decapitation and the brain rapidly removed, frozen, and weighed. The brain specimens were repeatedly extracted with ethanolic trichloroacetic acid [16] to give soluble (free) and insoluble (protein) amino acid fractions. The radioactivity was measured in each fraction separately by liquid scintillation spectrometry and converted to disintegrations per minute (dpm) per gram brain by the channels ratio method. The brain total radioactivity (soluble + insoluble) was taken as an index of blood-brain transport while the radioactivity of just the insoluble fraction was used as an estimate of the rate of incorporation into brain protein.

RESULTS

Exposure of the dams and fetal mice to either of the two enzyme inhibitors during late pregnancy resulted in several long term effects. As can be seen in Table 1, food consumption in the dams and progeny, during the different periods of observation, was greater in the ICA treated animals than in the controls. By contrast, the measured food intake of the MSO exposed mice did not differ significantly from the controls.

Despite their increased food intake, the body weights of the ICA treated progeny were lower than those of matched saline controls at 21, 43, and 80 days of age while the brain weights were significantly lower at 80 days of age (Table 2). No such differences were seen in the comparable MSO exposed animals.

In a 15 min test period in the open field at 40 days of age, the offspring of both ICA and MSO injected dams were significantly less active than those of saline controls ($p<0.01$; Table 3). A significant increase in the number of fecal boli was observed for the MSO group only ($p<0.01$). Activity in a running wheel, measured on 42 day old mice, revealed significantly increased activity for both the ICA and MSO exposed progeny ($p<0.001$; Table 3). No differences were noted between males and females on the behavioral measures in any of the groups.

The tracer studies with (U) 14C-L-valine revealed a number of significant differences in both 20 and 80 day old animals. At 20 days of age, incorporation into brain protein appeared to be decreased in both the MSO and ICA exposed groups although transport seemed to be unaffected (Table 4). In contrast to this, at 80 days of age transport appeared to be more affected than incorporation by both enzyme inhibitors (Table 5). At both ages the same trends were evident even when statistically significant differences were lacking.

DISCUSSION

Our previous in vivo studies in adult mice with MSO and ICA have demonstrated that these enzyme inhibitors can markedly diminish blood-brain transport of large-neutral amino acids, including valine [16, 17, 18]. It is known that the blood-brain barrier is permeable to the acidic amino acids, aspartate and glutamate, at birth though these are largely excluded in the adult [12]. Our data indicate that the large-neutral amino acid transport system is operational before birth. The L-system of Christensen [3], which has been im-

plicated in blood-brain transport of the large-neutral amino acids in vivo [1], has been shown to be highly sensitive to concentration differences of competing large-neutral amino acids [3]. In the maternal-fetal system, it would be vulnerable to the amino acid concentration imbalances characteristic of the amino acidopathies [11].

Persistent behavioral and biochemical alterations have been demonstrated following prenatal exposure to either MSO or ICA: enzyme inhibitors that have previously been shown in vivo, in adult mice [16,17], to decrease transport across the blood-brain barrier of the amino acids valine and tyrosine and to reduce their incorporation into brain proteins.

The decreased activity of both experimental groups in the open field environment may be indicative of lack of motivation for exploration, or to motor inhibition due to fear, or to some other factor. The larger number of fecal boli of the MSO animals tends to support fear as an explanation. Increased running wheel activity of the MSO and ICA exposed progeny indicates that these mice did not suffer from general debilitation. The feeding data, which show normal or increased food intake in the MSO and ICA exposed dams and offspring, may be taken as evidence that undernutrition did not account for the results.

The tracer studies with (U) 14C-L-valine in the 20 and 80 day old mice demonstrate that there are persistent biochemical effects on blood-brain transport and amino acid incorporation into brain protein following exposure to either of the two enzyme inhibitors during late pregnancy. Although the duration of the effect of MSO in adult mice on blood-brain amino acid transport is about 24–48 hours [13], it has been shown that it binds to the enzyme glutamine synthetase, in vivo, for as long as 90 days [14]. Both ICA and MSO have been found to inhibit brain protein turnover in vivo by 40–50%, presumably due to their comparable effect on blood-brain amino acid transport [16,17]. The long term effects of inhibitors of amino acid transport, administered during fetal life, suggests a critical period of development for these neurochemical phenomena similar to that demonstrated for early life behavioral imprinting. This demonstration of a persistent decrease in brain amino acid metabolism may be comparable to the findings of Lee and Chow [7,8] in animals and Chow *et al.* [2] in man which showed life long increases in nitrogen excretion reflecting altered protein metabolism following early life undernutrition. A decrease of amino acid transport, imposed in fetal life with lasting biochemical and behavioral consequences, may have relevance for an improved understanding of numerous congenital conditions in humans.

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