PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **187**, 2–6 (1988)

Toxic Effect of Lithium in Mouse Brain (42628)

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Abstract. The effect of lithium ion on glucose oxidation in the cerebrum and cerebellum of mice was measured *in vitro* by the conversion of isotopic glucose into $^{14}CO_2$/mg wet weight. Glucose utilization is unaffected by lowest lithium dosage but is inhibited by high lithium concentrations (197–295 mM). Chronic administration of lithium to adult mice decreased the DNA content of the cerebrum and cerebellum at concentrations of 80 and 108 mM. The DNA content of selected postnatal stages of cerebrum and cerebellum was measured starting on Day 1 or 2. This served as another parameter to evaluate glucose oxidation studies at these ages. On the basis of wet weight, both brain parts of neonates of ages 1 and 10 days were approximately one-half that of the adult counterparts. On the basis of DNA content, the cerebrum enhanced its glucose utilization twofold from Day 1 to Day 10 and tripled its utilization from Day 10 to Day 20. The glucose utilization by cerebrum at Day 20 is similar to adult values. In contrast, glucose oxidation in the cerebellum remained relatively constant throughout the postnatal growth. The relative susceptibility of the two brain parts is discussed. © 1988 Society for Experimental Biology and Medicine.

In manic depressive illness, the use of lithium has led to studies related to its effect on adult and embryonic organisms (1, 2). However, the mechanism of action of lithium has remained elusive.

Our interest has centered on the effect of lithium on the developing organisms both prenatally and postnatally in the mouse (2, 3). We have reported that lithium is teratogenic when given at concentrations higher than corresponding therapeutic dosages administered to humans. Therapeutic dosages, however, may still be toxic to the embryo (2).

The present study tested the effect of lithium on the metabolism of glucose in mouse brain, particularly the cerebrum and cerebellum. In addition, we determined the effect of the drug on DNA content in these brain portions. Basically, we were trying to determine the concentration at which lithium affects glucose utilization and its effect on growing and adult brain.

Materials and Methods. All the experiments were performed on mice of inbred strain 129 Sv/SL which originated in the Jackson Laboratory and were maintained in our colony for several years. The adult mice ranged in age from 4 to 6 months.

Virgin females were kept together with adult males. Each morning females were checked for copulatory plugs and when found that day was designated as Day 0 of pregnancy.

Lithium as carbonate or chloride dissolved in deionized water was the sole source of drinking water given *ad libitum* for 21 days. The four lithium concentrations tested were 27, 54, 80, and 108 mM/liter. Control animal received deionized water. Purina chow was available at all times.

Lithium was periodically determined in plasma from blood drawn from the orbital sinus and measured by means of atomic absorption spectrophotometry (2).

DNA determination in brain of lithium-administered adult animals. Lithium-fed animals were decapitated, the calvaria was removed, and the cerebral hemisphere as well as the entire cerebellum was placed in ice-cold media. The brain tissue pieces were blotted, weighed, and placed in small glass homogenizers into which 0.5 ml of 0.5 N perchloric acid ($HClO_4$) was added and left overnight in the refrigerator. The tissue was homogenized thoroughly and transferred into test tubes to a total volume of 2.0 ml with 0.5 N $HClO_4$. DNA was determined by the method of Burton (4) as modified by Giles and Myers (5).

Glucose oxidation studies in vitro. Glucose utilization was determined by measuring its oxidation to carbon dioxide. Brain tissue was

0037-9727/88 $1.50
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recovered as indicated above, diced with sharp razor blade into fine pieces, and placed in special metabolism vials (6). All vials containing tissues in buffer solutions were kept in an ice bath until ready for incubation.

The incubation medium consisted of Kreb's Ringer bicarbonate buffer, pH 7.4 (7). Medium (1 ml) contained 1.0 mg glucose, and 0.2 μCi of glucose, D[^{14}C(U)] (sp act 14 mCi/mmole, New England Nuclear, Boston, MA). After gassing with 95% O_2 + 5% CO_2, the vials were incubated for 1 hr at 37°C in a shaker water bath (80–90 oscillations/min). At the end of the hour, 0.5 ml of hyamine was introduced into a small suspending container. The tissue metabolism was stopped with 2 ml of 2 *N* sulfuric acid, and the vials were agitated on the shaker bath for another 45 min to capture CO_2 in the hyamine. The hyamine in the beakers was transferred quantitatively into scintillation vials with three washes of 1.0 ml scintillation cocktail (3a70B purchased from RPI) and made to a total volume of 10 ml; the radioactivity was determined in a beta scintillation counter. The tissue was rinsed in several changes of distilled water, blotted, and weighed. Radioactivity of $^{14}CO_2$ (DPM) was calculated per milligram of tissue (wet wt).

Glucose utilization was measured in the cerebrum and cerebellum at selected days of age, namely, Postnatal Days 1–2, 10–11, 20–21, and in adults (4 to 6 months). In order to obtain adequate amounts of tissue, brain parts from several newborns were pooled.

For *in vitro* studies, various concentrations of lithium solutions were added to the incubation medium. Lithium carbonate was used for the lower concentrations, but due to poor solubility characteristics of the carbonate salt, lithium chloride was substituted in the higher concentrations. The concentrations of lithium solutions actually used ranged from 0.63 to 393 moles. Control vials contained no lithium.

Statistical analysis. The data were analyzed using Student's *t* test.

Results. *Glucose oxidation in adult brain: cerebrum vs cerebellum.* The results of *in vitro* glucose utilization in adult brain are presented in Fig. 1. Addition of lithium in various concentrations to the medium affected cerebrum and cerebellum somewhat differently. Cerebrum did not exhibit any effect on glucose uptake even when lithium concentration was raised to 98 m*M*/liter. Significant decrease in glucose metabolism by cerebrum occurred at a concentration of 197 m*M*/liter ($P < 0.05$), and at still higher lithium concentrations the decrease was highly significant ($P < 0.001$).

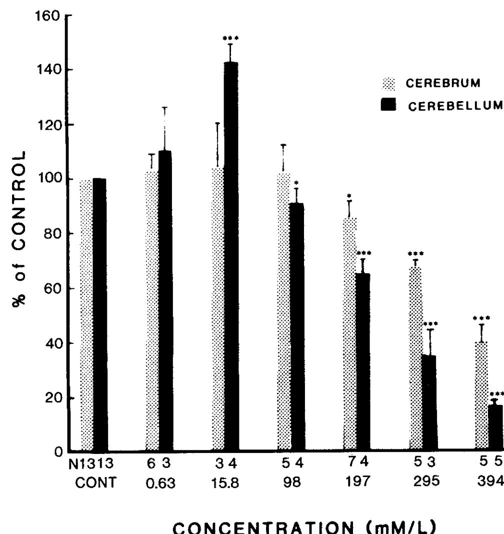

FIG. 1. Effect of varying concentrations of lithium ion on glucose oxidation of adult cerebrum and cerebellum *in vitro* as expressed as percentage of control. Note: Vertical lines on bars are standard errors of the mean (SEM). Asterisks indicate significance as compared to control (CONT): * $P < 0.05$; ** <0.01; *** <0.001. *N* = sample size.

In the cerebellum, a concentration of 15.8 m*M*/liter of lithium significantly stimulated glucose uptake as compared to controls ($P < 0.001$), but at 98 m*M*/liter concentration, the uptake of glucose was decreased ($P < 0.05$). At still higher concentrations, glucose uptake was markedly decreased ($P < 0.001$). Analysis of variance clearly indicated that this decreased trend was dose related and highly significant ($P < 0.0001$) both in the cerebrum and in the cerebellum at the above-stated concentrations.

Glucose oxidation in brain during postnatal growth. Figure 2A depicts *in vivo* uptake of glucose by the brain parts at various stages of normal postnatal development. When calculated on the basis of unit weight of the tissue, both the cerebrum and cerebellum of 1- to 2-day-old newborns utilized glucose ap-

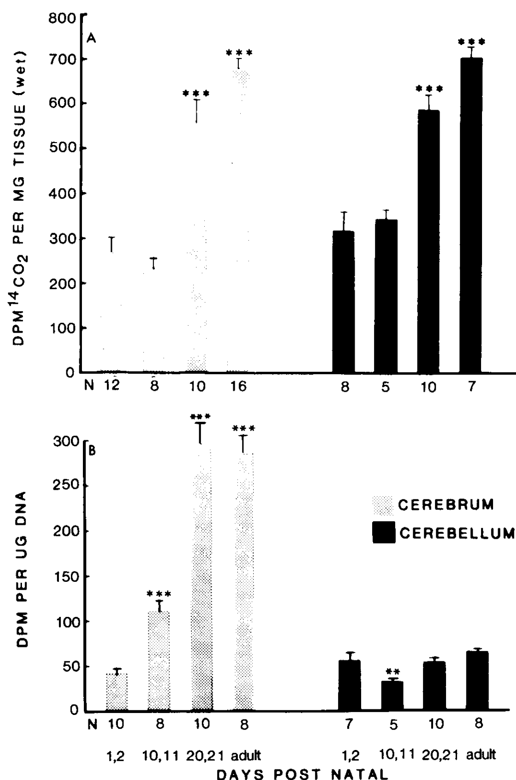

FIG. 2. (A) Measurement of glucose oxidation in cerebrum and cerebellum of selected postnatal ages expressed on the basis of milligrams wet weight. Note: Vertical lines are SEM. Asterisks denote significant differences from adult values as stated in legend for Fig. 1. N = sample size. (B) Same data as (A) expressed on the basis of DNA content of the respective brain part (see Fig. 3).

proximately to the same extent as those at age 10–11 days. Between Days 10–11 and 20–21, there was an abrupt twofold increase in glucose uptake by both cerebrum and cerebellum. Even at Days 20–21, the cerebrum and cerebellum utilized less glucose ($P < 0.05$) than the adult brain.

Glucose oxidation in brain recalculated on the basis of DNA content. Since the DNA content is a better measure of cell number, it seemed appropriate to recalculate the results on glucose oxidation per DNA content. These calculations are depicted in Fig. 2B.

On the basis of microgram DNA content of tissue, quite a different pattern of glucose uptake for cerebrum and cerebellum emerges. In the neonate cerebrum, the glucose uptake was increased twofold between 1–2 and 10–11 days ($P < 0.001$). A further threefold increase occurred between 10–11 and 20–21 days neonatal brain tissue ($P < 0.001$), but no further increase in glucose uptake occurred at the adult stage. By contrast, in the cerebellum the glucose uptake per DNA basis was significantly decreased from 1–2 days to 10–11 days but remained unaltered up to the adult stage. Not to be overlooked is the observation that the cerebrum of the 20- to 21-day neonate and the adult animals utilized five times the amount of glucose used by the corresponding cerebellum on the basis of DNA content.

DNA content of brain. DNA content of cerebrum and cerebellum of selected postnatal stages including the 4- to 6-month adult are shown in Fig. 3. The amount of DNA in cerebrum was significantly different from that of the cerebellum at every age measured. The least yet significant ($P < 0.01$) difference between the brain parts was found on Days 1–2. The cerebrum contained higher DNA content per unit wet weight at this time than the cerebellum; however, on Days 10–11 and thereafter, the cerebellum contained about five times that contained in the cerebrum.

In the cerebrum the quantity of DNA decreased significantly ($P < 0.001$) from days 1–2 as compared to days 10–11. There was

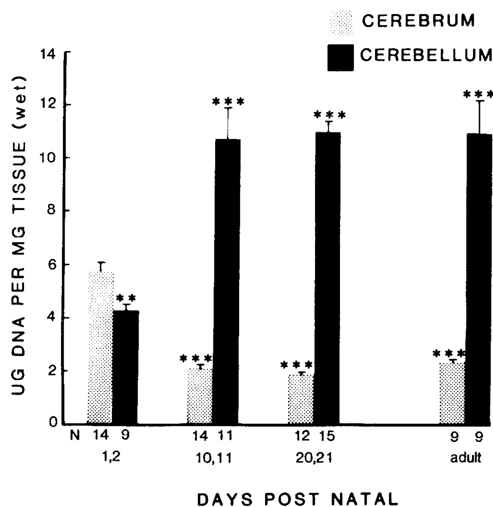

FIG. 3. Measurement of DNA content in cerebrum and cerebellum of selected postnatal ages. Note: Vertical lines are SEM. Asterisks denote significant differences from controls as stated in legend to Fig. 1. N = sample size.

no further change in the DNA content of the cerebrum, even up to adult stage. However, DNA content in the cerebellum showed more than a twofold increase in DNA content from Days 1–2 to Days 10–11 ($P < 0.001$). From this day onward, the DNA content remained fairly uniform in the cerebellum.

Effect of lithium on DNA content in brain. As a background for lithium effects on DNA content in adult brain, we determined morning blood plasma levels of lithium. The two highest concentrations of lithium, 80 and 108 m*M*, administered to pregnant mice produced plasma levels of 1.8 ± 0.4 and 2.7 ± 0.6 mEq/liter of lithium, respectively. These plasma levels are higher than those found when therapeutic dosages are given to humans. The lower two concentrations, 27 and 54 m*M*, resulted in plasma levels of 0.4 ± 0.1 and 1.2 ± 0.2 mEq/liter of lithium, respectively; both levels are within the therapeutic range.

The effect of these concentrations, given for 21 days, are illustrated in Fig. 4. The lower two concentrations did not significantly affect the DNA content in the cerebrum, but the two higher concentrations caused a significant decrease ($P < 0.01$) in the DNA content as compared to control levels.

The cerebellum showed no changes in DNA content at the lower two concentrations either. There was a decreasing trend at the 80 m*M* level but a statistically significant decrease ($P < 0.02$) from the control value was obtained only after treatment with 108 m*M* of lithium.

Discussion. Glucose oxidation of brain slices was measured *in vitro* and was expressed on the basis of (1) wet weight and (2) cell number. On a wet weight basis, the cerebrum and cerebellum of developing brain utilized glucose approximately to equal extent (Fig. 2A). Also, different concentrations of lithium affected *in vitro* glucose utilization by the two brain components of adult animals to the same extent with minor variations (Fig. 1). It is reported that lithium stimulates glucose metabolism in the brain of mice (8, 9), and rats (10–12); we also observed *in vitro* a significant increase in glucose metabolism in the cerebellum at lithium concentrations of 15.8 m*M* (Fig. 1). Administration of equivalent dose of lithium in humans produces plasma lithium levels within therapeutic range. *In vitro* incubation with much higher lithium concentrations produced a marked inhibition of glucose utilization by both the parts of brain tissue.

Since the DNA content is a reliable indicator of cell number because of its constancy per cell (13), we compared the differences in the cell numbers in the two brain parts. Our data (Fig. 3) indicate that the cerebellum contains approximately five times more cells

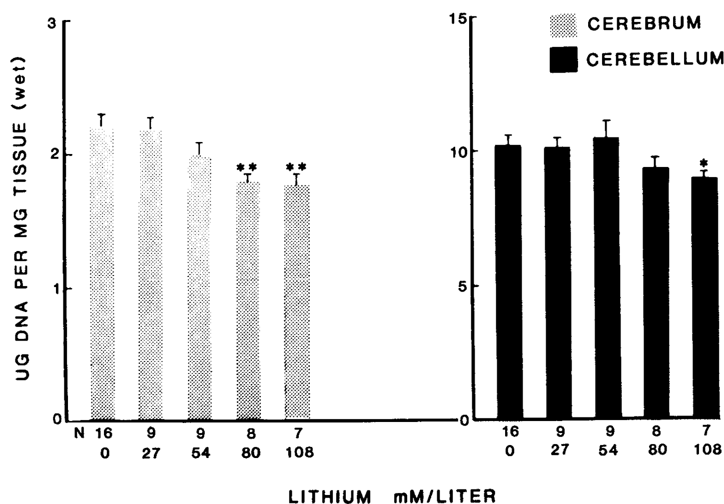

FIG. 4. Effect of varying concentrations of lithium ion given chronically for at least 3 weeks on DNA content in adult cerebrum and cerebellum. Note: Vertical lines are SEM. Asterisks indicate significant difference from control values as stated in legend to Fig. 1. N = sample size.

than the cerebrum from days 10–11 postnatal stage to adult age, an estimate which compares favorably with that reported by Howard (14). Based on per cell basis, however, the cerebrum appears to utilize five times more glucose than the cerebellum at 20–21 days postnatal and adult stages (Fig. 2B).

Effect of chronic lithium treatment on DNA content of adult mouse brain is dose related. Feeding higher lithium dosages (80 m*M*) resulted in lower DNA content in cerebrum. The cerebellum was also affected at very high lithium dosage (108 m*M*). The most dramatic change in the cell numbers as reflected in DNA content (Fig. 3) was found between newborn and Days 10–11 in both the cerebrum and cerebellum. It is well documented that the largest increase in DNA and a massive increase in granule cells occurred at approximately this time period in the cerebellum (15–18). These observations indicate that lithium causes brain cell loss at levels above therapeutic dosages. Although we have biochemical evidence of cortical brain damage at high doses of lithium, there is no morphologic evidence as to whether neurons and/or glial cells have been affected. There is a possibility that even therapeutic dosages of lithium alone (19, 20) or in combination with other psychotropic drugs (21, 22) can cause neurologic damage to humans.

We gratefully acknowledge the support of the Minnesota Medical Foundation.

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Received April 10, 1987. P.S.E.B.M. 1988, Vol. 187.
Accepted September 2, 1987.