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Lithium Distribution and Regional Serotonin Metabolism in the Brain of Dogs under Prolonged Lithium Intoxication

(lithium intoxication/serotonin/brain regions)

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The regional distribution of lithium (Li) and changes in serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were examined after dogs had been maintained under conditions of chronic Li intoxication for prolonged periods. The distributions of Li were uneven in the brain. The amygdala, thalamus and hippocampus showed the highest levels, the inner capsule, diencephalon, midbrain, cerebral gray matter, cerebral white matter, medulla and pons contained intermediate levels, while the lowest levels were detected in the cerebellum and spinal cord. There were considerable variations in these levels among the individual dogs. 5-HT levels were decreased significantly in the medulla (to 55% of the control), cerebral gray matter (to 63%) and cerebral white matter (to 75%), and 5-HIAA levels were also decreased significantly in the pons (to 17%), lenticular nucleus (to 42%), cerebellum (to 42%) and inner capsule (to 68%). Correlations between the Li ion levels and the extent of the regional 5-HT or 5-HIAA decrease were nil. The extent of the decrease in the levels of these compounds seems to be dependent not on the Li levels but rather on the vulnerability of each brain region.

Li is an effective therapeutic and prophylactic agent against affective disorders (for review see Ref. 1). However, the mechanism underlying these effects is not well understood. Since it has been postulated that alterations in monoamine metabolism may play a role in the pathogenesis of affective disorders, the effects of Li ion on the monoamine metabolism in the brain have been well documented. All the data, particularly findings related to serotonin (5-HT) metabolism show discrepancies.

As Li intoxication is sometimes clinically evident, we examined the effects of this ion on the regional 5-HT metabolism in the brain of dogs under conditions of an induced, prolonged and severe Li intoxication.

MATERIALS AND METHODS

Li carbonate was added to the food given to 9 mongrel dogs. Starting with the dose of 40 mg/kg body wt./day for the first week, the dose was increased every week by 10 mg/kg/day until the dogs showed anorexia,

vomiting, diarrhea, tremor and ataxia. After the occurrence of these symptoms, 50 to 70% of the doses were given to maintain the dogs under the conditions of Li toxicity. The durations of Li administration, durations of Li intoxication and the doses at sampling are shown in Table I.

TABLE I. *Duration of Lithium Administration, Duration of Intoxication and Lithium Dose at Sampling*

	Duration of administration*	Duration of intoxication*	Li_2CO_3 dose at sampling**
Dogs used for Li determination (4)***	39–77 (52)	10–48 (26)	50–110 (80)
Dogs used for 5-HT and 5-HIAA determination (5)***	38–140 (110)	25–110 (60)	110–140 (117)

*: Range with average number of days in parenthesis,
**: range with average in parenthesis (mg/kg body wt./day),
***: numbers of animals used.

The four dogs which died of intoxication were used for the determination of Li levels. The brains were divided into the regions shown in Table II. Li levels were determined by the method of Bond *et al.* (2) using a Hitachi 508 atomic absorption spectrophotometer.

The remaining 5 dogs and 4 control dogs were exsanguinated. The brain regions shown in Fig. 2 were excised, weighed immediately and then frozen at −75°C for no longer than 30 days. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined by the method of Curzon and Green (3) using an Aminco-Bowman spectrophotofluorometer. Recovery rates for 5-HT and 5-HIAA were 50 to 70% and 70 to 80%, respectively.

RESULTS

Distribution of Li in the Blood, Cerebrospinal Fluid and Brain (Table II and Fig. 1)

TABLE II. *Lithium Distribution in Blood, Cerebrospinal Fluid and Brain of Lithium Intoxicated Dogs*

Dog No.	1	2	3	4
Li_2CO_3 dose at death (mg/kg body wt./day)	50	80	80	110
Plasma	1.79	3.06	1.19	2.76
Red blood cells	2.04		1.32	2.59
Cerebrospinal fluid	0.86	2.64	—	1.39
Cerebral gray matter	2.86	2.82	3.75	4.33
Cerebral white matter	2.58	2.82	4.42	4.55
Inner capsule	2.49	3.35	5.39	4.51
Hippocampus	3.22	3.79	4.32	6.24
Amygdala	3.96	4.38	5.08	7.05
Thalamus	3.66	3.12	5.28	6.50
Diencephalon	2.32	2.36	4.80	6.67
Midbrain	2.59	3.33	4.42	5.25
Pons	2.22	2.89	3.24	3.93
Cerebellum	1.53	2.31	2.45	2.99
Medulla	2.46	3.67	3.14	4.36
Spinal cord	1.98	2.08	2.55	3.20

Values are expressed as meq/l or meq/kg fresh tissue.

The dose of Li required to maintain a state of intoxication varied from 50 to 110 mg/kg body wt./day and the Li levels in the blood, cerebrospinal fluid and brain regions at death also varied considerably. Li levels in the cerebrospinal fluid were lower than the levels in the blood and in most areas

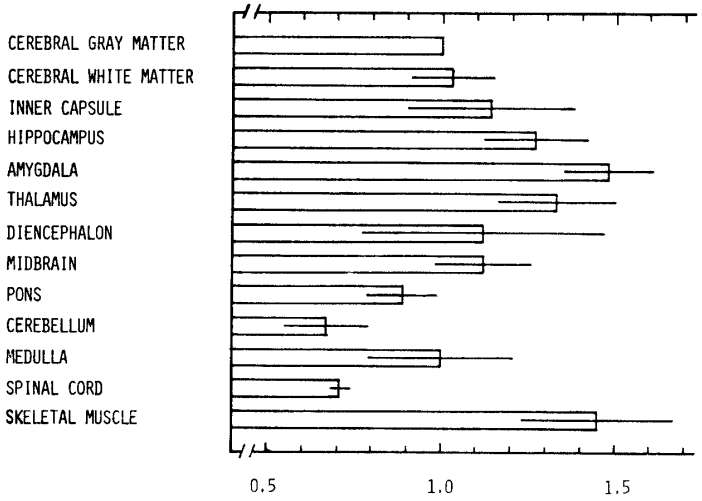

Fig. 1. Relative levels of lithium in various regions of dog brains after prolonged lithium intoxication. Mean±S. D. of 4 dogs.

of the brain. Relative ratios of Li levels in different brain regions were compared to findings in the cerebral gray matter and the averages and standard deviations in each region are shown in Fig. 1. Li levels were highest in the amygdala, thalamus and hippocampus. Levels in the inner capsule, diencephalon, midbrain, cerebral gray matter, cerebral white matter, medulla and pons were intermediate, and the lowest levels were found in the cerebellum and spinal cord.

5-HT and 5-HIAA Levels in the Brain Regions (Figs. 2 and 3)

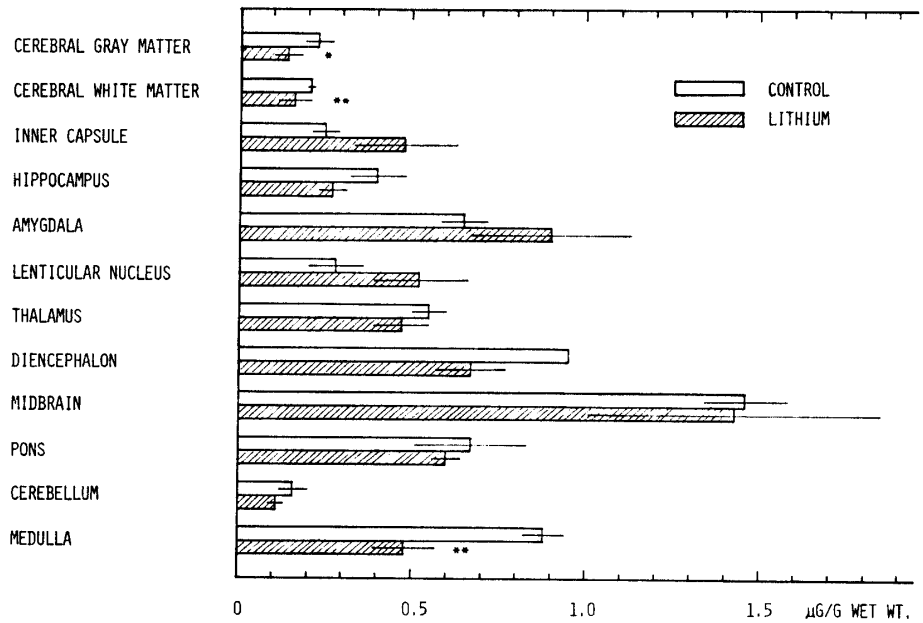

Fig. 2. Serotonin levels in various regions of dog brains after prolonged lithium intoxication. Mean±S. D. of 5 control or 4 lithium intoxicated dogs,
* : significantly different from control, $p<0.05$,
** : significantly different from control, $p<0.005$.

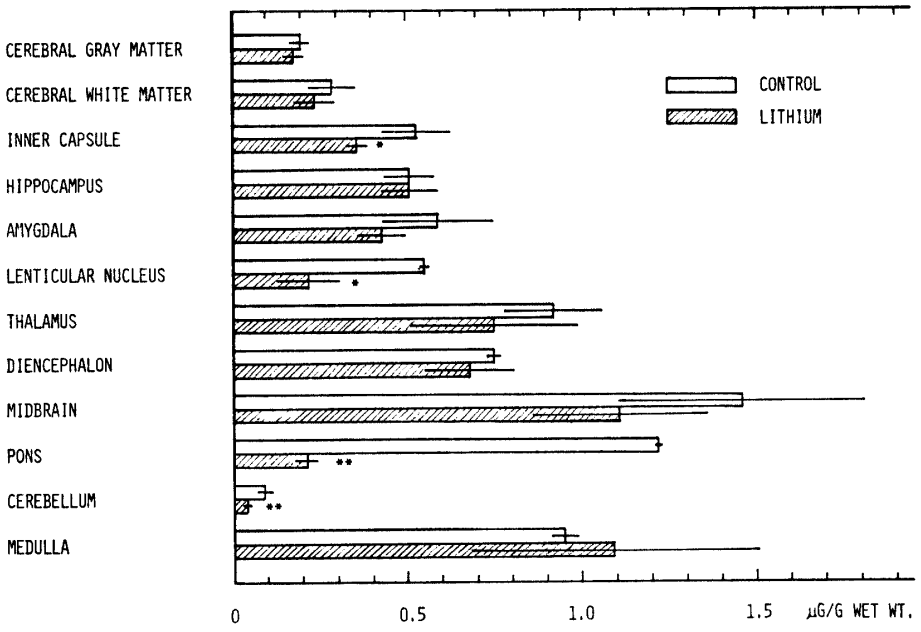

Fig. 3. 5-Hydroxyindoleacetic acid levels in various regions of dog brains after prolonged lithium intoxication. Mean±S. D. of 5 control or 4 lithium intoxicated dogs,
* : significantly different from control, $p<0.025$,
** : significantly different from control, $p<0.005$.

5-HT levels were decreased significantly in the medulla (to 55% of the control, $p<0.005$), cerebral gray matter (to 63%, $p<0.05$) and cerebral white matter (to 75%, $p<0.005$). 5-HIAA levels were also decreased significantly in the pons (to 17%, $p<0.005$), lenticular nucleus (to 42%, $p<0.025$), cerebellum (to 42%, $p<0.005$) and inner capsule (to 68%, $p<0.025$).

DISCUSSION

Following the ingestion of Li, this ion is then distributed unevenly throughout body organs (4—7). Regarding the distribution in various parts of the brain, different results have been reported in the literature.

In an acute experiment, Ichihashi *et al.* (8) reported no significant difference in Li distribution in the telencephalon, diencephalon+midbrain, pons+medulla and cerebellum of rats 2 hours after a single I. P. administration of 3.5 meq/kg of LiCl. Ikeda (7) also found no significant difference in Li levels in rat brain regions 3 or 9 hours after a single oral dose of 60 mg (1.6 meq)/kg of Li_2CO_3. Ebadi *et al.* (5), however, reported an uneven distribution ; 24 hours after a single I. V. administration of 7.2 meq/kg of LiCl to rats, the globus pallidus and caudate nucleus attained the highest and the spinal cord and pons showed the lowest levels.

In subacute or chronic studies, results have also been inconclusive. Ho *et al.* (6) found no significant difference in Li levels in rat brain regions after daily injections of 2.0 meq/kg of LiCl for 4 to 18 days. Bond *et al.* (2)

administered a non-toxic dose (30 meq/kg dry food) of LiCl to rats for 2 to 42 days and found a uniform distribution of this ion in the brain. Ikeda (7) administered per os 60 mg/kg/day of Li_2CO_3 to rats for 7 days and found no significant difference in the distribution. However, he observed significantly higher levels in the midbrain+diencephalon and cerebrum than in the spinal cord, cerebellum and medulla+pons when rats were severely intoxicated following increasing doses up to 300 mg (8.1 meq)/kg/day for 3 to 7 days.

One case of Li intoxication with a fatal outcome was reported by Amdisen *et al.* (4). At death 21 days after the intoxication, Li levels in the cerebral white matter, cerebral gray matter, brain stem and cerebellum gray matter were 0.85, 0.72, 0.51 and 0.35 meq/kg wet wt., respectively and the level of Li in serum was 0.6 to 0.4 meq/l. Francis and Traill (9) also reported Li levels in two patients who died of asthma or cardiac disease after 3 or 4 days of Li treatment for mania. Li levels in the pons were higher than in the cerebrum or cerebellum.

It is unlikely that the uneven distribution is due to different histomorphological states of the nerve cells or nerve fiber densities. The difference may be either the result of a different rate of influx and/or of active (10) efflux. Ebadi *et al.* (5) found no correlation between regional distribution of Na^+-K^+ ATPase and Li levels in rat brain.

As for the relationship between the side effects of Li and its regional distribution in the brain, Ebadi *et al.* (5) suggested that signs and symptoms such as tremor, ataxia, muscular hyperirritability, choreoathetotic movements and hyperactive tendon reflexes might in part be related to the accumulation of Li in the basal ganglia. Francis and Traill (9) mentioned that the tremors and ataxia might be associated with higher Li levels in the pons. We have reported a case of Li intoxication in which the patient had acute symptoms such as delirious states, hyperactive tendon reflexes, tremor and dyskinesia (11). Cerebellar symptoms such as intention tremor, scanning speech, bradylalia, truncal ataxia and clumsiness in finger-nose test, however, persisted for about a year. In the present work using dogs, cerebellar symptoms were also apparent, however, the Li level in the cerebellum was not high. The extent of the influence on the functions of nerve cells seems to be related to differences in the vulnerability of respective nerve cells to the ion.

The effects of Li ion on 5-HT metabolism have also been reported and here again the results were inconsistent. In subacute or chronic studies, Sheard and Aghajanian (12), Perez-Cruet *et al.* (13), Grahame-Smith and Green (14), Judd *et al.* (15) and Shaw and Ratcliffe (16) reported increased turnover rates of 5-HT metabolism. Bliss and Ailion (17) observed no change. Corrodi *et al.* (18), Ho *et al.* (19) and Segawa and Nakano (20) found a reduction in the metabolism. These studies differed widely in experimental designs, doses of Li, procedure and duration of its administration, condition of animals, methods used to examine the metabolic turnover rate, parts of the brain examined and so on. Knapp and Mandell (21) reported that interactions between the dose and time were implicit in the effects of Li on the parameters

of biosynthetic capacity for 5-HT in the rat brain and that receptor mediated neuronal feedback regulation was involved.

In the present study, large doses were administered and the animals were maintained under severely intoxicated conditions for prolonged periods of time (25 to 110 days). 5-HT and 5-HIAA were generally decreased in various parts of the brain ; decreases in 5-HT in the medulla, cerebral gray matter and cerebral white matter and of 5-HIAA in the pons, lenticular nucleus, cerebellum and inner capsule were statistically significant.

In relatively similar conditions, Amdisen *et al.* (4) reported that levels of 5-HIAA in the caudate nucleus and in the pons, and 5-HT in the pons of a patient who died 21 days after the onset of Li intoxication were 0.34, 0.47 and 0.04 μg/g, respectively. These values were lower than respective control values : 0.70, 1.04 and 0.06 μg/g, although they did not comment on this point as these were relatively small reductions compared to a more marked reduction of homovanillic acid in this brain. Ho *et al.* (19) found a significant reduction of 5-HT levels in the rat hypothalamus and brain stem and no changes in the cerebral cortex, cerebellum and diencephalon, 28 days after daily injections of 2 meq/kg of LiCl, a time when some of the animals showed signs of intoxication. They found a significant reduction of 5-HT turnover rate in the hypothalamus and a significant increase in the cerebellum. In whole brain studies, the turnover rate was slightly but not significantly reduced.

Correlations between the Li levels and the extent of the regional 5-HT or 5-HIAA changes were not observed in our experiment. The extent of the changes seems to be dependent not on the levels of Li but rather on the vulnerability of the brain regions.

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