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THE DISTRIBUTION OF LITHIUM, SODIUM AND MAGNESIUM IN RAT BRAIN AND PLASMA AFTER VARIOUS PERIODS OF ADMINISTRATION OF LITHIUM IN THE DIET

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1 The tissue solubilizer Soluene-100 provides an efficient and easy means of preparing small amounts of rat tissue for cation analysis.

2 Administration of lithium ions to rats for two days to 42 days by the addition of lithium chloride to the diet at a concentration of 30 mmol/kg dry weight results in (a) the uniform distribution of lithium throughout the brain at a concentration comparable to that found in plasma; (b) decrease in the brain sodium concentration; (c) a decrease in brain magnesium concentration and an increase in plasma magnesium concentration; (d) no change in brain water content.

3 The inclusion of LiCl in the diet at a concentration of 30 mmol/kg dry food gives consistent and predictable plasma and brain levels of lithium in the rat without the occurrence of serious side effects over periods of up to 42 days.

Introduction

Interest in lithium as an agent for the treatment of the affective disorders has grown steadily over the past few years. However, despite its intensive use, little is known of the biochemical basis for its behavioural effects. For the interpretation of biochemical and behavioural studies of lithium action, a knowledge of the distribution of lithium ions and other cations in the brain is desirable.

In previous studies various methods of administration have been used, including intravenous (Schou, 1958), intraperitoneal and oral by stomach tube (Morrison, Pritchard, Braude & D'Aguanno, 1971), and by adding lithium to the drinking water (Birch & Jenner, 1973). The consistent finding in whole brain studies was that lithium entered the brain more slowly than it did most other tissues. However, the concentration ratio between plasma and brain depended on the route and duration of administration, as well as the nature and amount of the lithium salt given.

Some workers have examined the distribution of lithium in the brain and its effect on the concentrations of other electrolytes (Ho, Gershon & Pinckney, 1970; Edelfors & Gøthgen, 1971). A higher concentration of lithium was found in the hypothalamus.

This paper describes the development of a simple assay for cations in small amounts of rat tissue. The method to be described uses Soluene-100 (Packard Scientific), a quaternary ammonium base, to solubilize tissues. The use of this agent has previously only been described for the estimation of zinc, copper, iron and manganese (Jackson, Michael & Schumacher, 1972). This new method of assay has been used to study the distribution of lithium, magnesium and sodium in rat brain after administration of lithium in the diet for periods ranging from 2 to 42 days.

Methods

Animals

The animals used were male CFY strain Wistar rats (Carworth Europe) which were 28 days old at the beginning of each experiment. They were allowed to acclimatize to laboratory conditions for one week. During this time they were kept three to a cage with a 12 h light-dark cycle (LD 12 : 12) (lights on 08 h 00 min-20 h 00 min) and given food (Carworth Dixon C-DD-R) and distilled water *ad libitum*.

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Management

After acclimatization the conditions were kept constant but lithium or sodium was added to the food. A slurry of 50% ground food pellets and 50% distilled water containing either sodium chloride or lithium chloride at concentrations of 30 mmol/l or 45 mmol/l was then given *ad libitum*, and replenished every other day. Daily intake of food and water was measured for each group of rats on alternate days, and the individual body weights were recorded. Treatment groups were as follows:

(1) Lithium chloride 30 mmol/kg dry food for 2 days (Li 30 2 days)
(2) Lithium chloride 30 mmol/kg dry food for 14 days (Li 30 14 days).
(3) Lithium chloride 30 mmol/kg dry food for 42 days (Li 30 42 days).
(4) Lithium chloride 45 mmol/kg dry food for 14 days (Li 45 14 days).

The controls for each of the groups consisted of rats given sodium chloride in the diet at the same molar dose and for the same length of time as the lithium chloride. A second control group given a normal diet (no additional sodium) was also included for use in the brain sodium concentration measurements.

Tissue preparation

At the end of the treatment period all animals were killed by cervical dislocation between 10 h 00 min and 11 h 00 min. The neck vessels were severed and blood collected in heparinized tubes. The brains were immediately dissected out (except for the olfactory lobes), rinsed with distilled-deionized water to remove extraneous matter and as many surface blood vessels as possible, and blotted dry. They were then dissected into seven regions following the scheme of Glowinski & Iversen (1966), except that a circular cut was made around the hypothalamus to reduce contamination with cortical tissue.

The blood collected from each rat was separated by centrifugation and the plasma stored at -20° C until assayed.

Each brain region from each rat was placed in a tared vial with an air-tight closure and weighed. Soluene-100 was added to the vial to give an approximate 10-fold tissue dilution. The tissue was left to solubilize for 24 to 48 h at room temperature. Immediately before assay all the solubilized samples were diluted approximately three-fold with 2-ethoxyethanol and weighed. The exact dilution of the tissue sample was calculated on a weight for weight basis. The same method was used to prepare whole-brain samples for assay. Cortical sections from an additional group of rats similar in all respects to Li 30 14-day rats but killed at 19 h 00 min were also prepared in the same manner.

The wet ashing method described by Chang, Gover & Harrison (1966) was used to prepare one set of cortex samples.

Assay

All samples treated with Soluene were assayed using a Hilger-Watt Atomspek AA2 atomic absorption spectrophotometer. Lithium assays were carried out with instrument settings recommended by the manufacturer for lithium, except that the burner height was increased to 5 mm and the air flow was increased by 10%.

A tissue blank prepared from the brain of a control animal treated as described above, and a reagent blank consisting of one part Soluene-100 to two parts 2-ethoxyethanol, were analysed with each batch of samples. Lithium standards were prepared by adding aqueous lithium chloride solutions to the reagent blank.

Samples of plasma, and aqueous samples obtained by the wet ashing method, were assayed for lithium using the instrument settings actually recommended by the manufacturer for lithium. Before assay all aqueous samples were diluted one part to three parts with a solution containing 6.5 g $La(NO_3)_3$, 0.67 g CsCl and 10 ml conc. HCl per litre of distilled water.

Sodium and magnesium determinations were carried out on aliquots of the diluted solubilized samples further diluted one part to ten parts and one part to four parts with 2-ethoxyethanol respectively. To the samples for magnesium determinations HCl and $La(NO_3)_3$ were added to a final concentration of 0.1N and 0.1%, respectively. Internal standards were used for both ions and instrument settings were those recommended by the manufacturer for sodium and magnesium.

Brain water content

Animals of the same type as those used for the cation analysis were given lithium chloride or sodium chloride in the food at a dose level of 30 mmol/kg of dry food for 14 days. At the end of this period they were killed and the brains were dissected to give the seven regions. Each region was weighed and then dried by heating at 110°C to constant weight.

Results

The analytical method used here has been compared with the standard technique of wet ashing as described by Birch & Jenner (1973) based on the procedure of Chang *et al.* (1966). The results of estimations of lithium, sodium and magnesium obtained by the two methods on similarly treated animals were not significantly different ($P > 0.05$, t test). When lithium is administered in the diet at a concentration of 30 mmol/kg of dry diet, an even distribution of lithium throughout the brain is reached within two days and remains essentially unaltered for up to 42 days. The values obtained after 14 days are representative of the entire experimental period (Table 1). Some of the brain regions show statistically significant differences in lithium concentrations after the various durations of administration; all values fall within a narrow range, however, and it is not possible to decide whether these differences have any real biological significance.

When the lithium dose was increased to 45 mmol/kg dry food, the brain region distribution of lithium remained similar but the concentrations increased (Table 1). However, the brain/plasma lithium ratio increased so that the concentrations of lithium in the hippocampus, mid-brain, corpus striatum and cortex were all significantly higher than the plasma lithium concentrations. The only exception to this was the hypothalamus where the ratio remained constant.

The whole brain concentration of lithium after the 30 mmol dosage level for 14 days is not significantly different from the plasma concentra-

Table 1 Rat brain and plasma concentrations of sodium, magnesium and lithium after the administration of a normal diet or diets with added NaCl (30 mmol/kg dry food) or LiCl (30 mmol or 45 mmol/kg dry food) for 14 days

	Normal diet	*Sodium diet NaCl 30 mmol/kg dry food*		*Lithium diet LiCl 30 mmol/kg dry food*			*Lithium diet LiCl 45 mmol/kg dry food*
	Na	Na	Mg	Na	Mg	Li	Li
Pons and medulla	51.7 ±0.91 (6)	52.8 ±0.89 (10)	5.69 ±0.38 (10)	47.3‡ ±0.95 (10)	5.03 ±0.24 (10)	0.49* ±0.021 (12)	0.74 ±0.029 (6)
Cerebellum	52.7 ±0.96 (6)	51.7 ±0.79 (10)	5.61 ±0.24 (10)	48.9‡ ±1.46 (10)	4.42‡ ±0.14 (10)	0.46* ±0.025 (12)	0.79 ±0.031 (6)
Hypothalamus	52.8 ±0.77 (6)	53.1 ±0.92 (10)	6.11 ±0.37 (6)	48.7‡ ±1.68 (10)	4.35 ±0.40 (6)	0.48* ±0.017 (12)	0.63* ±0.037 (5)
Hippocampus	52.5 ±1.10 (6)	52.8 ±1.46 (10)	5.86 ±0.37 (6)	52.1 ±0.92 (10)	4.62‡ ±0.38 (6)	0.61 ±0.023 (12)	1.0* ±0.047 (6)
Mid-brain	54.7 ±0.58 (6)	55.1 ±0.89 (10)	5.28 ±0.33 (10)	52.6‡ ±0.85 (10)	4.58 ±0.20 (10)	0.55 ±0.021 (12)	0.90* ±0.023 (6)
Corpus striatum	51.3 ±0.96 (6)	51.5 ±1.61 (10)	7.41 ±0.36 (6)	49.6 ±0.85 (10)	4.49‡ ±0.39 (6)	0.64 ±0.021 (11)	1.16* ±0.051 (5)
Cortex	51.8 ±0.73 (6)	51.2 ±0.47 (10)	5.67 ±0.21 (10)	47.5‡ ±1.71 (10)	5.03‡ ±0.14 (10)	0.63 ±0.022 (12)	1.22* ±0.040 (5)
Cortex (19h 00min)	–	–	–	–	–	0.52† ±0.040 (5)	–
Whole brain	–	–	–	–	–	0.55 ±0.037 (6)	0.92 ±0.063 (6)
Plasma	–	–	0.69 ±0.03 (13)	–	0.90‡ ±0.03 (12)	0.59 ±0.020 (12)	0.79 ±0.031 (6)

Values are expressed as mmol/kg fresh tissue or as mmol/l of plasma and represent the mean ± s.e. mean. The number of observations is in parentheses. All values are from rats killed at 10h 00min unless otherwise stated.

* Significantly different from plasma concentration
† Significantly different from cortex (10h 00min) concentration
‡ Significantly different from Na diet concentration

} $P < 0.05$, t test

tion and falls midway in the brain regions' lithium concentration range (Table 1). After the 45 mmol lithium dose the whole brain/plasma ratio of lithium concentration is increased and the whole brain lithium concentration again falls mid-way in the brain regions' range of lithium concentration.

The results show a small but significant drop in the lithium concentration in the cortex over the 9 h period from 10 h 00 min to 19 h 00 min. This period coincides with the normal light period for these animals (08 h 00 min to 20 h 00 min)

Unlike Edelfors & Gøthgen (1971), the present study revealed a significant decrease in the sodium content of all brain regions except the hippocampus and corpus striatum in rats given lithium for 14 days (Table 1).

The content of magnesium in animals treated with lithium for 14 days was decreased in all regions, reaching significance in all but the pons and medulla and mid-brain (Table 1). In accord with other studies (Birch & Jenner, 1973; Neilson, 1964a, b) in both man and animals, plasma magnesium was elevated in all the rats given lithium for 14 days.

Food intake for the first two days of lithium feeding was approximately 15% below control levels, but it increased to within 5% of control levels within the next four days. Weight increase of the lithium-treated rats was decreased for the first four days but returned to normal values over the rest of the experimental period.

Discussion

Pilot studies have shown that the Soluene method of tissue preparation is also suitable for K^+ determinations using a Technicon Auto Analyser and flame photometer. Calcium estimation on the other hand proved troublesome. This was due to precipitation problems which unlike those encountered during magnesium estimation could not be overcome by the addition of an acid. The limits of sensitivity of this method are set by the degree of dilution required to allow the sample to pass through the atomizer of the atomic absorption spectrophotometer.

These results differ in most respects from the only previous study which is at all comparable (Edelfors & Gøthgen, 1971) although few experimental details were given in that report which only appeared as an abstract. In those studies, following oral lithium administration for two or five weeks, comparatively high lithium levels were found in the hypothalamus compared with other brain regions. At the same time, the water to dry matter ratio decreased in all parts of the brain except the hypothalamus by about 50%. However, we found no significant change in water content of fresh brain tissue following lithium administration. Birch & Jenner (1973) also found no significant alteration of water content of whole brain after lithium administration.

Slight regional differences in water content were noted by us in both lithium- and sodium-treated animals. These were similar to those reported by Donaldson, St. Pierre, Minnich & Barbeau (1973), thus showing that any variation of the magnitude reported by Edelfors & Gøthgen (1971) should have been extremely obvious.

The absolute values of sodium and magnesium concentration in the brain regions of the sodium-treated control animals were very similar to those reported by Donaldson *et al.* (1973) using the same dissection technique but a dry ashing method for tissue preparation. Magnesium plasma levels in the sodium-treated controls were very similar to those reported by Mellerup, Plenge & Rafaelson (1973).

Comparing the results of the present study with those of Birch & Jenner (1973), both show a decrease in brain magnesium proportionally depending on the dose of lithium, although in the latter case lithium was administered by intraperitoneal injection or in the drinking water. Also, Mellerup *et al.* (1973) showed an increase in plasma magnesium after a single intraperitoneal injection of 900 μmol of lithium chloride which is comparable to the increased magnesium level we obtained with a very different dosage and route of administration.

It has been suggested (Baer, Kassir & Fieve, 1970) that the decrease in tissue sodium concentration in lithium-treated animals may only be apparent due to an elevated tissue sodium concentration in the controls if they are given extra sodium in the diet. However, the very close similarity in the sodium levels of the two non-lithium groups used here do not support this theory.

Many reports concerning tissue distribution of various ions after lithium administration have already been published (Schou, 1958; Ho *et al.*, 1970; Frazer, Mendels, Secunda, Cochrane & Bianchi, 1973). However, none have dealt with the regional distribution of sodium, magnesium and lithium in the rat brain after the administration of LiCl in the food at a dose producing a steady plasma/brain lithium ratio approaching unity, plasma levels within the human therapeutic range and no serious side effects when given for up to 42 days.

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