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SHORT COMMUNICATIONS

The effect of lithium treatment on the acetylcholine content of rat brain

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Lithium acts as a prophylactic agent in manic-depressive psychosis, and has therapeutic effects in different manic states [1, 2, 3]. The biochemical changes in mania and the possible mode of action of lithium have been documented in several reports. Some authors [4–6] described elevated intracellular sodium levels both in manic and depressive patients. It was suggested by several investigators [7, 8], that depression may be associated with a deficiency and mania with an excess of norepinephrine at the receptor sites of the brain. This hypothesis is, partly, in accord with the finding that lithium salts increase the rate of deamination as well as the turnover of norepinephrine and decrease norepinephrine available to receptors [9, 10]. According to Abdulla and Hamadan [11] mania is caused by intracellular cyclic AMP accumulation in the brain. Dousa and Hechter [12] pointed out that lithium inhibits the Tris fluoride-stimulated mitochondrial adenyl cyclase in rabbit brain. In previous studies lithium was found to produce transiently the release of acetylcholine during resting conditions from Auerbach's plexus of guinea-pig ileum [13, 14] and from slices of rat cerebral cortex [14, 15]. However, lithium reduced the output of acetylcholine from Auerbach's plexus of guinea-pig ileum [14], and from isolated superior cervical ganglion [16], when these tissues were stimulated electrically. It was also shown [14], that lithium inhibits acetylcholine synthesis in slices of rat cerebral cortex. However, the lithium concentration used in these experiments (117·9 mM) was much higher than that which occurs in patients or animals treated with lithium salts [17, 18]. We have therefore examined the effect of a lower concentration of lithium ions on acetylcholine synthesis. We studied also the effect of chronic treatment with LiCl on the acetylcholine content of rat brain.

Adult albino rats of both sexes weighing 100–150 g were injected intraperitoneally with LiCl in aqueous solution (4·7 mEq/kg, 200 mg/kg) in 0·1 ml/100 g twice daily for five doses. The animals were kept together, with water and food given *ad lib.* One hour after the last injection the rats were decapitated. The whole brain was removed immediately and divided into cerebral cortex, thalamus and striatum, brain stem, the rostral section being made immediately anterior to the colliculi superiors, and the whole cerebellum. The methods for *in vitro* experiments, and acetylcholine extraction and the calculation of the rate of acetylcholine synthesis were described previously [14]. Acetylcholine was assayed on guinea-pig ileum, as described by Paton and Vizi [19].

Lithium-treated animals exhibited diarrhoea, salivation and increased ingestion of water. Immediately after lithium injection the rats became agressive for several minutes due to the local irritative effect of the lithium solution. No other behavioural changes were observed during the experiment. Similar results were reported on mice [18]. The acetylcholine content of different parts of brain in the control and lithium-treated animals is shown in Table 1. There was a significant fall in the acetylcholine content of medulla oblongata–pons–mesencephalon in response to lithium treatment. The values for striatum + thalamus, cerebellum and cortex did not differ significantly from the controls. After isolated cortical slices had been exposed to lithium ions 27·6 mM for 1 hr, the acetylcholine content of slices was reduced significantly from 8·355 ± 0·26 to 6·341 ± 1·23 nmoles/g (mean ± S.D., n = 3, $P < 0.05$). During this period the release of acetylcholine was as high as 1·729 ± 0·71 nmoles/g per hr, indicating that the synthesis was completely blocked. However, under control conditions the rate of acetylcholine synthesis was as high as 1·539 ± 0·57 nmoles/g per hr (n = 3) without any significant change in before–after levels of acetylcholine showing that acetylcholine released was continually replaced by the synthesis.

It has been shown that lithium ions can inhibit acetylcholine synthesis even at a low concentration. During chronic exposure to lithium the level of acetylcholine in the brain stem of rats was significantly reduced. Since lithium ions can accumulate inside the nerve cell [20, 21], exceeding the extracellular concentration, they can reach the level sufficient to inhibit acetyl-CoA-synthetase [22], an enzyme partly responsible for acetylcholine synthesis. It has also been shown in this study, that lithium at a

Table 1. Acetylcholine content of different areas of rat brain after subchronic LiCl treatment

	Acetylcholine content (mean ± S.E.M.) Control (nmoles/g)	Li^+-treated (nmoles/g)	P
Cortex	4·82 ± 0·55 (n = 4)	4·08 ± 0·60 (n = 4)	>0·3
Striatum + thalamus	9·39 ± 0·87 (n = 4)	7·17 ± 0·50 (n = 4)	>0·05 < 0·1
Medulla oblongata	8·00 ± 0·48 (n = 4)	6·10 ± 0·40 (n = 4)	<0·05
Cerebellum	4·29 ± 0·30 (n = 4)	3·16 ± 0·51 (n = 4)	>0·1

Rats were injected intraperitoneally twice daily for 5 doses with 200 mg/kg (4·7 mEq/kg) LiCl. One hr after the last injection the brain was carefully removed, blotted and chilled and separated into cortex, striatum + thalamus, medulla oblongata and cerebellum. The medulla oblongata corresponds to the medulla oblongata, pons and mesencephalon.

concentration which may occur in patients treated with this agent, can alter the acetycholine level in the CNS of the rat.

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The effect of lithium on liver glycogen concentration in the rat

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Lithium salts are currently used in the treatment and prophylaxis of manic depressive psychosis [1]. The precise mechanism of action of this cation is unknown. Several diverse side effects, for example, its action on the kidney, thyroid and body water metabolism have been reviewed by Davis and Fann [2]. Patients receiving lithium tend to increase in weight [3], a feature which has been ascribed to an effect of the cation on carbohydrate metabolism [4]. Lithium increases glucose uptake in rat hemidiaphragm [5] this effect being accompanied by alterations in tissue glycogen distribution. Data on liver glycogen concentrations in response to lithium administration are contradictory. For example, Krulic and Zvolsky [6] reported an increase in rat liver glycogen following lithium administration whereas the converse had been found by Plenge *et al.* [7]. Glucagon is known to stimulate hepatic glycogenolysis and has been considered to be of importance in the mechanism of lithium induced changes in hepatic glycogen concentration [8].

In this paper the effect of varying doses of lithium on liver and skeletal muscle glycogen has been studied in rats of differing weights and measurements of plasma glucagon have been made.

METHODS

Sixty-nine female Wistar rats were studied. They were divided into three groups according to weight. Animals in group *A* weighed 90–110 g, those in group *B* 130–150 g and those in group *C* 175–195 g. The animals were fed a normal laboratory diet with water *ad lib.* All experiments were performed in the morning and food was withdrawn during this time. The appropriate dose of Lithium chloride (Analar) was made up to an injection vol of 1·0 ml with deionised water and physiological saline was used as the control injection. All injections were administered intraperitoneally. Animals were killed 3 hr after injection by a blow on the head. Venous blood was obtained from the jugular veins; the liver and the skeletal muscle from the hind leg was removed and placed in ice-cold physiological saline.

Serum lithium was estimated by flame photometry. Liver and muscle glycogen was estimated as follows: 1 g of tissue was homogenised in 10 ml physiological saline. Sodium acetate buffer (pH 4·8) was prepared containing the glycogen debranching enzymes. 50 μg/ml glucosidase and 100 μg/ml amylo-α-1,4-α-1,6-glucosidase (EC 3.2.1).

Incubation of 100 μl homogenate and 50 μl buffer was carried out at 30° for 60 min. Glucose was then estimated by the hexokinase method [9] and tissue glycogen expressed as mg/g wet tissue wt. *N*-terminal plasma glucagon (total plasma glucagon) was measured by radio-immunoassay [10] in pooled plasma samples from each dose range.

The statistical test used was Student's *t*-test.

RESULTS

Serum lithium varied between 0·2 and 0·4 m-mole/l. following the 200 μmole dose, 0·6–1·8 m-mole/l. following the 500 μmole injection and between 1·9 and 2·3 m-mole/l. at higher dose levels of injected lithium chloride. The effect of injected LiCl on liver glycogen levels in the different groups is shown in Table 1.

In all groups there was a significant fall of liver glycogen concentration 3 hr after injection of 500 μmoles of LiCl