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Phosphorylation of brain synaptosomal proteins in lithium-treated rats*

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It has been suggested that the state of phosphorylation of proteins in the synaptic membrane could influence the ease of passage of the nerve impulse [1, 2] and that phosphorylation may play a key role in nervous tissue function [1, 3–5]. It has also been proposed that synaptic phosphorproteins may influence neurotransmitter release [6, 7] and/or ion conductance and membrane polarization [3]. Lithium is known to alter certain behaviours such as aggression [8], and to cause impairment of learning and memory. Acute lithium treatment has been reported to be directly depressant to the central neurons [9, 10], altering the neuronal discharge frequency [10]. In the present study, the state of phosphorylation of the synaptic membranes in lithium-treated rats has been examined.

Materials and methods

Chemicals. The various chemicals used were obtained as follows: acrylamide and methylene-bis acrylamide from BDH Chemicals Ltd., Poole, England; glycine, sodium dodecyl sulfate and mercaptoethanol from the Sigma Chemical Co., St. Louis, MO, U.S.A.; and TEMED from Koch Light Ltd., Buckinghamshire, England. Tissue solubiliser was obtained from the Eastman Kodak Co., Rochester, NY, U.S.A. The rest of the chemicals were of "Analar" grade, obtained from the Chemicals Division, Glaxo Laboratories, Bombay, India. ^{32}Pi and [γ-^{32}P]ATP were obtained from the Bhabha Atomic Research Centre, Bombay, India.

Animals and diet regimen. Male Wistar rats, weighing between 150 and 200 g, were fed nutritionally adequate standard laboratory diet in which Li_2CO_3 (40 m-equiv/kg diet) was incorporated for 20 days [11].

In vivo *phosphorylation.* For the *in vivo* phosphorylation studies, ^{32}P was injected intracranially into the frontal cerebral cortex of rats maintained under mild ether anaesthesia [12]. Each animal was injected with 40 μl (2 × 20 μl bilaterally) of carrier-free ^{32}P orthophosphate (total 40 μCi) in an aqueous solution.

The rats were killed 1 hr after ^{32}P injection, and the whole brain was removed. The synaptosomes were separated [13], and the membranes were isolated therefrom by published procedures [14]. Phosphorylated protein from the membrane was isolated after fractionation with $HClO_4$ [15] and dissolved in tissue solubilizer, and an aliquot was taken for counting of radioactivity by liquid scintillation spectrometry. The protein concentration was determined by the method of Lowry *et al.* [16].

In vitro *phosphorylation.* Phosphorylation of the synaptic membranes was carried out with 100 μl of synaptosomal membrane fraction (4 mg/ml), and the incubation medium was made up to contain 5 μM [γ-^{32}P]ATP [15]. The reaction was terminated after 2 min by addition of an equal volume of 0.5 N perchloric acid and was purified, as described earlier, and counted [15].

Polyacrylamide gel electrophoresis and autoradiography. Acrylamide gel electrophoresis of the synaptosomal fraction obtained from rats injected with 1000 μCi ^{32}P was carried out at room temperature, using a constant current of 25–30 mA/slab. The protein concentration that loaded in each track was 100 μg. After electrophoresis, the slab was fixed with propanol–acetic acid (25%:10%) and stained with Coomassie Blue. The stained gels were dried on a filter paper under vacuum and placed in contact with an X-ray film for 8 days. The autoradiogram was scanned in a densitometer.

Results

The *in vivo* incorporation of ^{32}P into the total rat brain synaptosomal fraction was found to increase significantly after 20 days of feeding Li_2CO_3 at the level of 40 m-equiv/kg diet. This increase in the labelling of the total synaptosomal fraction was also reflected in an increase in the radioactivity associated with the purified protein (Table 1). However, when the phosphorylation of the synaptosomal proteins was examined *in vitro*, a small but insignificant decrease was observed in the lithium-fed rats.

Polyacrylamide slab gel electrophoresis of the synaptosomal membrane protein from control and lithium-treated rats did not show an alteration in either the number of bands or the protein content per band (Fig. 1A). The *in vivo* incorporation of ^{32}P was, in general, higher in the synaptosomal membranes from the lithium-treated animals as compared to controls. However, there were large differences in the extent of lithium-induced changes in the various bands (Fig. 1C; Table 2). Whereas the four bands with the highest mobilities (Nos 11–14) showed lower radioactivity in the lithium-treated rats compared to the control rats, all the others showed an increase in radioactivity as a result of lithium treatment. The maximum increase was shown by bands 6 and 7, while 1 and 2 showed a marginal increase.

* Address for correspondence: Dr. G. B. Nadkarni, Head, Biochemistry and Food Technology Division, Modular Laboratories, Bhabha Atomic Research Centre, Bombay 400 085, India.

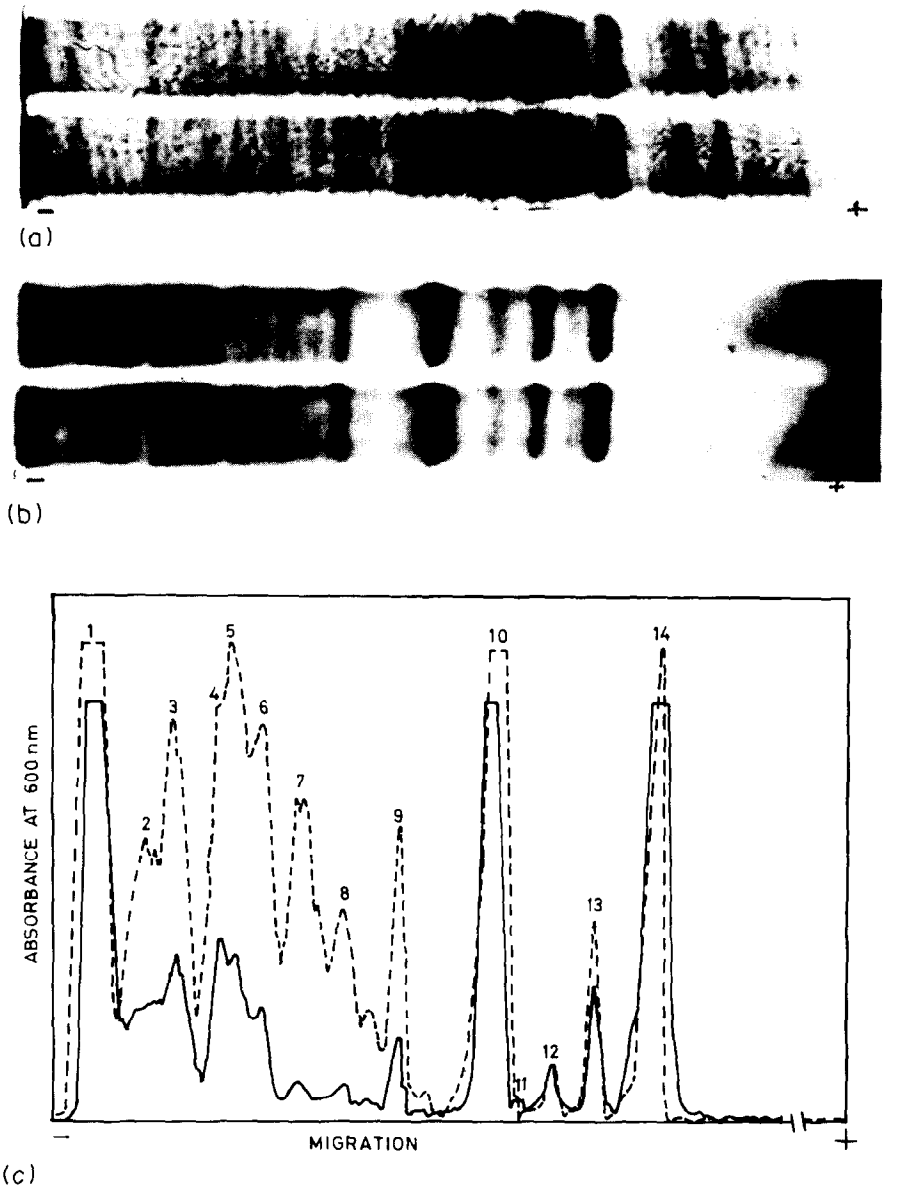

Fig. 1. Gel electrophoretic separation of synaptosomal proteins. (A) Stained protein bands. Top, control; bottom, lithium-treated. (B) Autoradiogram of the dried gel. Top, lithium-treated; bottom, control. (C) Densitometric scan of the autoradiogram. Solid line, control; broken line, lithium-treated.

Discussion

In the present study, it was observed that lithium treatment resulted in a large increase in the *in vivo* ^{32}P-incorporation into proteins of synaptosomal membranes. However, *in vitro* phosphorylation was unaffected; the possible reasons that could underlie such differences in phosphorylation between synaptosomal membranes *in vivo* and *in vitro* have been discussed [17].

The possibility that an alteration in phosphorylation may be responsible for lithium-induced effects is suggested by the following observations in literature. The extent of phosphorylation of synaptosomal protein has been reported to be correlated with both neuronal electrical activity [1–3] and behavioural performance [15, 18].

Although the precise mechanism by which lithium influences phosphorylation of synaptosomal proteins is not known, two likely explanations may be offered. Lithium has been known to alter the turnover of norepinephrine in brain [19] and to inhibit it release from neurons [20]. Norepinephrine is postulated to be involved in the alteration of the state of phosphorylation of synaptosomal membranes [21]. The other possibility involves the mediation of ACTH, since lithium administration has been shown to enhance corticosterone synthesis through its effect on the

Table 1. Phosphorylation of synaptosomal proteins in rats treated with lithium carbonate*

Fraction	Incorporation of ^{32}P (cpm/mg protein) Control	Lithium-treated
A. *In Vivo*		
Synaptosomes	1840 ± 140	3500 ± 540†
Purified protein	489 ± 60	1221 ± 150‡
B. *In vitro*		
Purified protein	301 ± 17	237 ± 28§

* Results are averages ± S.E.M. of seven (*in vivo*) and six (*in vitro*) different experiments.
† $P < 0.05$ (Student's *t*-test).
‡ $P < 0.01$ (Student's *t*-test).
§ Not significant.

Table 2. Relative distribution of radioactivity in the protein band*

Peak No.	Radioactivity as indicated by area under the peak Control	Lithium-treated	% of Control
1	129	150	116
2	64	72	112
3	44	97	220
4, 5	78	193	247
6	22	64	291
7	20	86	430
8	17	28	165
9	17	34	200
10	93	130	140
11, 12	27	5	18
13	33	19	58
14	141	70	50

* The autoradiogram was scanned in a densitometer, and the area under each peak was integrated to give a measure of the radioactivity.

pituitary adrenal axis [22], and the intraventricular administration of ACTH is reported to enhance the phosphorylation of synaptosomal membrane fraction *in vitro* [23].

The phosphorylation of brain synaptosomal membrane fraction was increased in rats fed a lithium-carbonate-containing diet for 20 days. The possible significance of this effect to the mechanism of action of lithium in therapy is discussed.

Biochemistry and Food Technology Division
Bhabha Atomic Research Centre
Bombay 400 085, India

MALINI VATAL
ANANT S. AIYAR*

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* Present address: Protein Foods and Nutrition Development Association of India, Mahalaxmi Chambers, 22 Bhulabhai Desai Road, Bombay 400 026, India.