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SHORT COMMUNICATIONS

Stimulation by lithium ions of the incorporation of [U-^{14}C]glucose into glycogen in rat brain slices

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Although lithium salts have been used for many years in the treatment of manic-depressive disorders [1], little is known about the mechanisms by which lithium affects brain function.

One of the cellular effects of lithium, first described by Bhattacharya [2], is a stimulation of glucose uptake by skeletal muscle *in vitro*. This action of lithium was confirmed by others [3–5], and it was also demonstrated that lithium ions increase the synthesis of glycogen by skeletal muscle [3–5]. The experiments of Diamond and Fishman [6] indicate that lithium influences carbohydrate metabolism of nervous tissue as well. These authors showed that sodium and lithium ions stimulated the formation of radioactive CO_2 from [U-^{14}C]glucose in K-depolarized rat brain synaptosomes. Studies with intact animals have also provided evidence that lithium affects carbohydrate metabolism in brain. DeFeudis [7, 8] injected [U-^{14}C]glucose into mice and found that the radioactivity in brain 30 min later was higher in animals pretreated with LiCl than in controls. The author did not attempt to determine the nature of the radioactive metabolites found in the brain after glucose administration. Plenge *et al.* [9] gave intraperitoneal or intracisternal injections of LiCl to rats and demonstrated an increase in total glycogen content of brain tissue. In a subsequent paper, Plenge [10] reported that the specific activity of brain glycogen was the same in controls and lithium-treated rats 12 min after the injection of [U-^{14}C]glucose although the total glycogen increased with lithium, as had been reported previously [9]. The specific activity of blood glucose did not differ between the two groups of animals. It appears to us that a greater amount of [U-^{14}C]glucose must have been incorporated into glycogen in the lithium-treated rats to result in an equal specific activity of glycogen despite a markedly higher total pool of the polysaccharide.

In view of the inconclusive evidence of an effect of lithium on glycogen synthesis in brain, we carried out experiments to determine whether lithium affected the incorporation of glucose into glycogen in rat brain slices.

Male Wistar strain rats (100–125 g) were fed *ad lib.* and killed by decapitation. The calvarium was opened quickly and the forebrain separated from the cerebellum and lower brain stem. The forebrain was removed and two superior and two lateral cortical slices approximately 0.4 mm thick were prepared with a Stadie–Riggs microtome wetted with iced 0.15 M NaCl. Two slices (one lateral and the contralateral superior slice) were added to Erlenmeyer flasks containing 1.5 ml of prewarmed medium and incubated in a shaker bath at 37° with 100% oxygen in the gas phase. The medium had the following composition: 0.040 M HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, pH 7.2), 0.005 M $MgCl_2$, 0.005 M KCl, 0.006 M [U-^{14}C]glucose (sp. act., 1.88×10^5 cpm/μmole) and 0.108 M NaCl. When LiCl was added, the concentration of NaCl was adjusted to maintain isosmolarity. At the end of incubation, the tissues were added to 10% KOH at 100°. After 5 min in KOH, an aliquot was diluted and neutralized with HCl for protein determination by the method of Lowry *et al.* [11]. After 10 min, 10 μl of 1% glycogen was added to the remaining KOH and the glycogen precipitated by addition of ethanol to give a concentration of 66%. The precipitate was resuspended in water and the incorporation of glucose into glycogen determined by the filter paper method of Thomas *et al.* [12]. This involves four washings of the filter paper with 66% alcohol and one with acetone. Counting was done in LSC-complete (Yorktown Research) with a Packard liquid scintillation counter.

Figure 1 shows the incorporation of glucose into glycogen during incubation of various periods of time.

After a short lag period, there is a close to linear increase in radioactive glycogen in controls and in the presence of lithium ions. The stimulation of incorporation of glucose into glycogen by 25 mM LiCl is statistically significant ($P < 0.01$) at all times greater than 10 min.

The results of experiments with different concentrations of LiCl are presented in Fig. 2. Brain slices were incubated for 1 hr with [U-^{14}C]glucose. As the concentration of LiCl in the medium was raised, there was a progressive increase in the incorporation of isotope into glycogen. A significant effect of lithium was obtained at a concentration as low as 0.5 mM and the maximum effect of the lithium ion was observed at about 5 mM. There is a suggestion that the effect of 10 mM LiCl was less than maximal but the difference between the actions of LiCl at 5 mM and 10 mM was not significant and this point needs further study. We have no explanation for the fact that the control value for incorporation of glucose into glycogen for 60 min was higher in these experiments than in the experiments presented in Fig. 1. It is possible that the time of year or

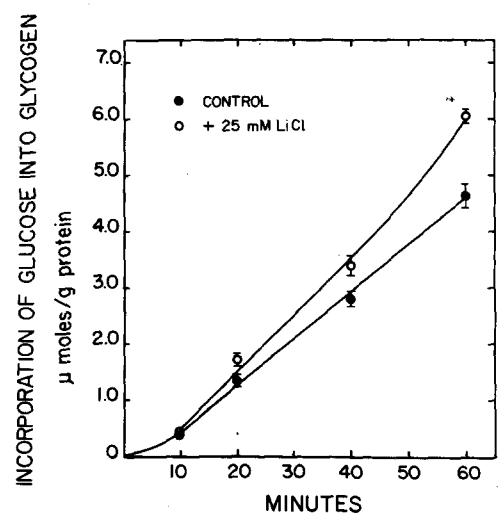

Fig. 1. Incorporation of [U-^{14}C]glucose into glycogen by rat brain slices. Slices were incubated at 37° with and without 25 mM LiCl. Values are expressed as means ± S. E. M. N = 6–13. Significance of Li effect: $P < 0.01$ for all times greater than 10 min.

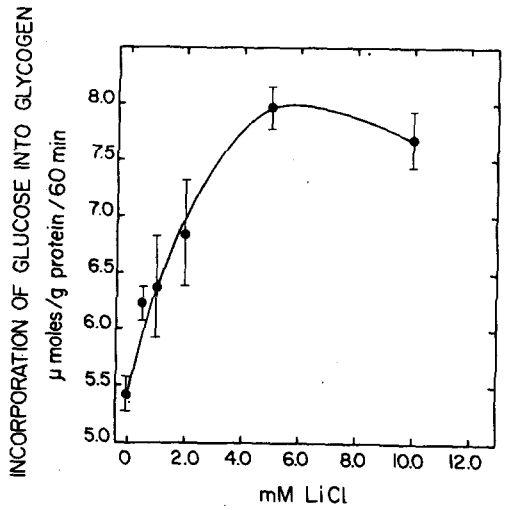

Fig. 2. Effect of LiCl on the incorporation of [U-^{14}C]glucose into glycogen by rat brain slices. Slices were incubated at 37° for 1 hr. Values are expressed as means ± S. E. M. N = 6. Significance of effects of Li: 0.5 mM, $P < 0.005$; 1 mM, not significant; 2 mM, $P < 0.025$; 5 mM, $P < 0.001$; and 10 mM, $P < 0.001$.

variation in the animal population could be involved, since the experiments were carried out several months apart.

The experiments reported here demonstrate that lithium ions have a direct stimulatory effect on the rate of glycogen synthesis from glucose in brain, as has been shown with skeletal muscle [3–5]. In the isolated rat diaphragm, this increase in glycogen synthesis is associated with activation of glycogen synthase (4,13). It would be of interest to determine whether lithium also increases glycogen synthase activity in brain.

The effect of lithium on glycogen metabolism occurs at concentrations similar to those estimated to exist in brain tissue of patients undergoing therapy with lithium salts. From studies of the distribution of lithium in rats injected with non-toxic doses of lithium carbonate it was found that the concentration of lithium in the brain was about one half of that found in erythrocytes [14]. In a group of men responding to lithium therapy, a mean erythrocyte level of 0.64 mM was observed in samples of blood drawn in the morning before the patient received lithium carbonate [15]. At this time the tissue lithium content would be expected to be at the lowest point of the daily fluctuation. An estimated minimum lithium concentration of 0.32 mM would indicate that the brain levels in man during lithium therapy are not far from those shown here to be effective in influencing glycogen metabolism *in vitro*.

Whether the effects of lithium on carbohydrate metabolism in the central nervous system bear any relation to the therapeutic effects of the ion is unknown. One may speculate that the metabolic alterations in the cell produced by lithium could affect membrane properties or the synthesis, release or action of neurotransmitters. The studies of Gibson and Blass [16] are of considerable interest in this connection. These authors showed with intact rats and mice that conditions that partially inhibited brain carbohydrate metabolism, but did not alter the cellular content of ATP, caused large changes in the ability of the brain to synthesize acetylcholine.

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REFERENCES

1. J. F. J. Cade, *Med. J. Aust.* **36**, 349 (1949).
2. G. Bhattacharya, *Nature, Lond.* **183**, 324 (1959).
3. T. Clausen, *Biochim. biophys. Acta* **150**, 66 (1968).
4. E. S. Haugaard, R. A. Mickel and N. Haugaard, *Biochem. Pharmac.* **23**, 1675 (1974).
5. E. S. Haugaard, A. Frazer, J. Mendels and N. Haugaard, *Biochem. Pharmac.* **24**, 1187 (1975).
6. I. Diamond and R. A. Fishman, *J. Neurochem.* **21**, 1043 (1973).
7. F. V. DeFeudis, *Archs int. Pharmacodyn. Thér.* **193**, 322 (1971).
8. F. V. DeFeudis, *Archs int. Pharmacodyn. Thér.* **197**, 141 (1972).
9. P. Plenge, E. T. Mellerup and O. J. Rafaelsen, *J. psychiat. Res.* **8**, 29 (1970).
10. P. Plenge, *Int. Pharmacopsychiat.* **11**, 84 (1976).
11. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
12. J. A. Thomas, K. K. Schlender and J. Larner, *Analyt. Biochem.* **25**, 486 (1968).
13. R. S. Horn, O. Walaas and E. Walaas, *Biochim. biophys. Acta* **313**, 296 (1973).
14. A. Frazer, J. Mendels, S. K. Secunda, C. M. Cochrane and C. P. Bianchi, *J. psychiat. Res.* **10**, 1 (1973).
15. J. Mendels and A. Frazer, *J. psychiat. Res.* **10**, 9 (1973).
16. G. E. Gibson and J. P. Blass, *J. Neurochem.* **27**, 37 (1976).