Studies on the Collectability of ^{3}H-γ-Aminobutyric Acid from Rat Cerebral Cortex; Effects of Lithium[1]

Considerable information has accumulated on the physiological effects of Li^+ in animals, including man[2–8], and although the actions of Li^+ on the brain have been studied with relation to monoamine metabolism[9,10] and the action of cyclic-AMP[7,11], few studies have been made on the possible association between Li^+ and cerebral amino acids[12–18]. Using perfusion techniques which have been described previously[19,20], the effects of replacement of Na^+ by Li^+ and of electrical stimulation on the efflux of newly-acquired ^{3}H-GABA have been compared in vitro and in vivo in rat cerebral cortex.

Krebs bicarbonate-buffered media, containing 10 mM glucose, and various concentrations of LiCl (as substitutions for NaCl) were used. In experiments with cortical slices, electrical (unipolar) stimulation was carried out at 60 Hz, 20 mA (5 msec duration) for 1 min, and in rats anaesthetized with 'Dial Compound' the exposed cortex was stimulated with a bipolar electrode assembly at 30, 60 or 200 Hz, 1 mA (0.5 msec duration) for 1 min. The efflux of ^{3}H was monitored using a Packard Model 3375 liquid scintillation spectrometer and corrections for quenching were made by external standardization[21]. Aminooxyacetic acid-hemihydrochloride (AOAA, Mann Chemical Co.) was added to the incubation media during cortical slice experiments (5×10^{-5} M) and was injected s.c. in the in vivo experiments (40 mg/kg) to prevent the catabolism of newly-acquired GABA. The concentration of 2, 3-^{3}H-GABA (New England Nuclear Corp; specific radioactivity, 2.02 Ci/mmole) in the media used for labelling the slices (for 30 min at 37 °C) was 1×10^{-7} M, and in the in vivo experiments 35 µCi were applied directly to the exposed surface of the cortex for 20 min before beginning perfusion.

The table shows the effect of replacing about 80% of the total Na^+ content of the medium with Li^+ on the spontaneous efflux of ^{3}H from the slices and the effects of electrical stimulation at 60 Hz on this efflux. Substitution of Na^+ by Li^+ evoked a highly-significant increase in the efflux of ^{3}H from the slices which was comparable to that evoked by electrical stimulation[19], but the Li^+-evoked efflux was slower in onset and usually of longer duration. Further experiments were conducted using isotonic LiCl as the perfusion fluid during the entire course of the efflux studies (30 collections; 60 min), and under

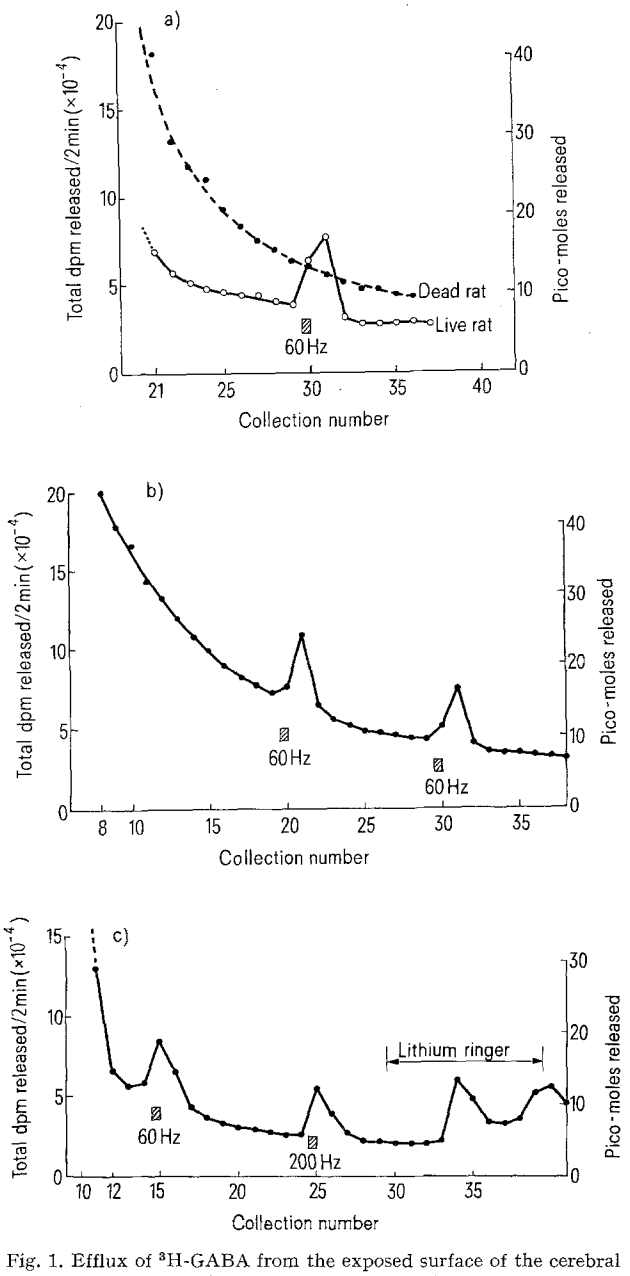

Fig. 1. Efflux of ^{3}H-GABA from the exposed surface of the cerebral cortices of Dial-anaesthetized rats. The cerebral cortex was exposed and 35 µCi of ^{3}H-GABA were placed into the perfusion cup for 20 min and then superfusion was begun. Times during which the cortex was stimulated electrically or superfused with medium containing Li^+ are indicated; each collection period was 2 min. a) Efflux from a living vs a dead rat; b) efflux produced twice in the same rat; c) electrically- and Li^+-evoked increases in ^{3}H-GABA efflux.

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Efflux of ^{3}H-GABA from slices of rat cerebral cortex in vitro

Condition	dpm in peak fraction dpm in fraction No. 9	Average collection No. for peak of radioactivity
Fresh slices		
No stimulation	0.9 ± 0.1 (5)	—
Stimulation during collection No. 10	3.2 ± 1.3 (4)	11
Li^+ during collections No. 10–20	2.0 ± 0.2 (4)	18
Frozen and thawed slices [a]		
Stimulation during collection No. 10	1.3 ± 0.1 (3)	12
Li^+ during collections No. 10–20	1.3 (2)	11

Means ± standard errors; numbers of experiments in parentheses. Fraction No. 9 was collected just prior to electrical stimulation or application of Li^+. In media containing Li^+, about 80% of the NaCl was replaced by LiCl. [a] Slices of cerebral cortex were frozen at −20 °C for 24 h prior to experiments.

these conditions, electrically-evoked release became negligible, presumably due to the marked increase in resting release (2 experiments; data not shown). When about 40% of the Na^+ of the medium was replaced by Li^+, both the electrically-evoked and spontaneous efflux were unaffected (1 experiment; data not shown). When medium containing Li^+ was applied at the same time as electrical stimulation, it had no obvious effect on the electrically-evoked increase in ^{3}H-GABA efflux but the additional efflux was prolonged (4 experiments; data not shown). The results in the table indicate further that both Li^+ and electrical stimulation caused negligible amounts of ^{3}H-GABA to be released from frozen and thawed slices which served as controls.

In the in vivo experiments the efflux of ^{3}H-GABA from the exposed cortex was increased by electrical stimulation in 39 of the 49 rats used; in some preparations it was possible to elicit increases in ^{3}H efflux as many as 5 times during the experiment. No clear-cut frequency dependence for ^{3}H-GABA release could be shown. Reasons for the ineffectiveness of electrical stimulation in some of the rats remain unknown but are perhaps related to cerebral anoxia caused by decreases in cerebral blood supply. The plots of Figure 1a indicate that it is necessary to have a living animal with intact cerebral blood supply to show electrically-induced increases in ^{3}H-GABA efflux. Figure 1b illustrates an experiment in which increases in ^{3}H-GABA efflux were caused twice by 60 Hz stimulation. Replacement of about 80% of the Na^+ of the superfusion fluid by Li^+ produced a dramatic increase in ^{3}H-GABA efflux which was at least as great as that evoked by electrical stimulation. (This effect was shown in 5 different rats.) It is noteworthy that the increase in collectability of ^{3}H-GABA caused by Li^+ occurred after a delay of about 8 min (Figure 1c). In further experiments with slices of cerebral cortex it was shown that substitution of about 80% of the Na^+ of the medium by Li^+ caused a marked reduction in ^{3}H-GABA uptake (Figure 2).

The results presented here indicate that the efflux of newly-acquired GABA from the cerebral cortex can be increased not only by electrical stimulation of the cortical surface but also by substituting large portions of the Na^+ of the superfusion fluid by Li^+. The increased collectability of GABA caused by Li^+ may be due to a combination of factors, the major ones being a blocking action of Li^+ on the processes of 'binding' and uptake of GABA, both of which have been shown to be Na^+-dependent[12, 16, 17, 22–29]. Further studies along these lines may provide useful information about the ionic bases of amino acid release mechanisms and on the interrelationships which exist between 'binding', uptake and release mechanisms. No definite conclusions can be drawn from the present study regarding the possible involvement of GABA in the therapeutic actions of Li^+ since such large amounts of Li^+ had to be used (as replacement for Na^+) to show effects on GABA movements.

Résumé. La libération de l'acide γ-aminobutyrique à la surface du cortex cerebral est augmentée par remplacement du Na^+ par le Li^+.

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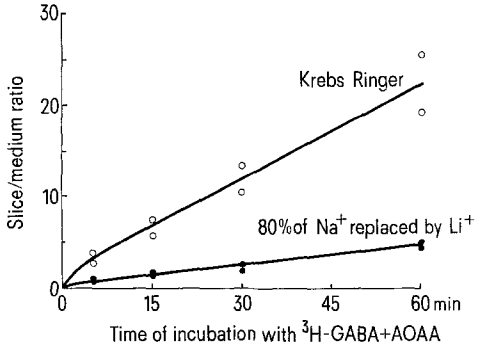

Fig. 2. Effect of substituting 80% of Na^+ by Li^+ on the uptake of ^{3}H-GABA by slices of rat cerebral cortex. Slices were incubated in the presence of 10^{-7} *M* ^{3}H-GABA and 5×10^{-5} *M* AOAA-HCl.

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