LITHIUM AND SYNAPTIC TRANSMISSION IN THE MAMMALIAN BRAIN

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Despite extensive investigations the mechanism of the therapeutic and prophylactic actions of lithium (Li) in manic-depressive disorder is unclear (10, 25, 35). Clinically relevant effects are obtained at plasma levels of 0.8 - 1.5 mmol/l and only after repeated administration for at least several days. One possibility of studying the effect of relatively low (but unknown) concentrations of Li on single cells in the central nervous system is the technique of microionophoresis which may admittedly only examine acute effects unrelated to the long term effects. Segal (37) and Siggins et al. (39) using this approach found an antagonistic effect of Li against amine transmitter actions. Haas and Ryall (19) observed an excitatory action of Li on many neurones in several areas of the cat and rat central nervous system. In the cat spinal cord only Renshaw cells but not other interneurones or motoneurones were excited with a typically slow onset of action. As the excitation was blocked by dihydro-beta-erythroidine a likely explanation was that Li increased the release of acetylcholine from the terminals of recurrent motoneurone collaterals as it has been described for the peripheral endings of motor nerves (9, 11, 12). Supraspinal neurones were also excited by locally applied Li ions with a certain predilection for cholinoceptive cells. Fig. 1 illustrates the effect of ionophoretically applied Li on an identified neurosecretory neurone from the supraoptic nucleus. These cells have been shown to be readily excited by acetylcholine (8, 15, 21).

If the effect of Li were mainly on cholinergic nerve terminals a destruction of these endings should abolish or reduce the Li effect. This was found not to be the case in hippocampus 3 to 7 days after lesioning the cholinergic septohippocampal fibers in 5 rats (Haas, unpublished).

In the majority of in vitro studies on peripheral synapses or invertebrate neurones high concentrations of Li have been employed and these may be expected to affect excitable membranes by virtue of the fact that Li substitutes for sodium in diffusion across Na-pores but is not handled effectively by the sodium pump (6, 27). Accumulation of Li in nerve endings has been suggested to lead to depolarization (34) which increases miniature endplate potential (mepp) frequency (14). At the neuromuscular junction Li causes an increase of end plate potentials (epp) and mepp frequency prior to failure of transmission (9, 11, 12, 26). In sympathetic ganglia 20 - 154 mM Li blocks synaptic transmission (28, 30) after a transient increase (30).

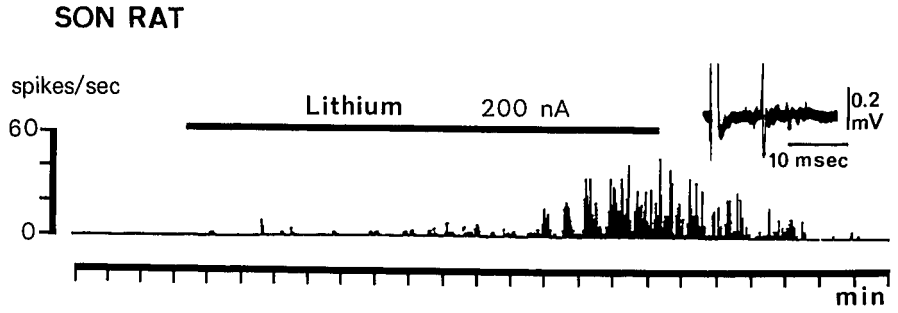

Figure 1. Ratemeter recording from a silent supraoptic neurosecretory neurone (identified by its antidromic invasion after electrical stimulation of the hypophyseal stalk under visual control in a urethane anaesthetized rat, see insert). Lithium was ejected from a multibarrel micropipette with 200 nA for the time indicated by a bar.

Studies using low concentrations of Li suggest a block of the electrogenic sodium pump in non myelinated nerve fibers (32, 33) and in rat cerebellar cortex (41). Interesting and partially unexpected information has recently come from invertebrate systems. Partridge and Thomas (31) found that Li injected into snail neurones causes a rise in potassium permeability possibly via an increase of intracellular ionized calcium. Subsequently Thomas et al. (40) reported that Li is in fact actively transported out of the cells and that intracellular levels are much below extracellular levels. Aldenhoff and Lux (1) have measured the intracellular calcium concentration in such neurones directly and shown a significant increase when Li was applied extracellularly in millimolar concentrations. In the light of these results the hippocampal slice preparation offered itself favorably for an investigation of Li effects on central synaptic transmission and membrane properties of cortical neurones in mammalian brain.

MATERIALS AND METHODS

Male Wistar rats of 100 - 150 gramms were decapitated during halothane anaesthesia and the hippocampus dissected free. Transverse slices of 400 - 500 µm thickness were cut with a tissue chopper and transferred to a perfusion chamber (20). The perfusion fluid had a temperature of 32°C (sometimes 31° or 36°) and contained: (mM) Na 150, K 6.25, Cl 134, Ca 2.5, Mg 2.0, HPO_4 1.25, SO_4 2.0, HCO_3 26, glucose 10, and was equilibrated with 95% O_2/5% CO_2. Warm, moist gas mixture was deflected over the slices which were covered by a thin film of fluid.

Extracellular recordings of field potentials from CA 1 dendritic and somatic areas were made with glass micropipettes filled with 2 M NaCl and having resistances of 1 to 10 MΩ. Stimulation of stratum radiatum or the alveus was achieved with pipolar electrodes made from 75 µm

insulated tungsten wire. Stimuli were usually delivered at 0.2 Hz, had a duration of 0.2 msec and an intensity of 30 - 150 μA. For intracellular recording pipettes were filled with 4 M K-acetate or 3 M K-chloride and had resistances of 20 - 100 MΩ. For recording and current injection a high input impedance amplifier (WPI 707) was used. Signals were amplified, viewed on a storage oscilloscope, averaged, photographed and stored on magnetic tape for later analysis. Membrane potentials were continuously plotted on a chart recorder.

Lithiumchloride (1 - 10 mM, usually 2 mM) was added to the perfusion fluid or applied locally to the slices by pressure ejection from a micropipette. When sodium chloride (upto 10 mM) was added to the medium no changes in resting or synaptic potentials occured. Concentrations in the slices were probably considerably lower particularly when perfusion times shorter than 20 - 30 min were used.

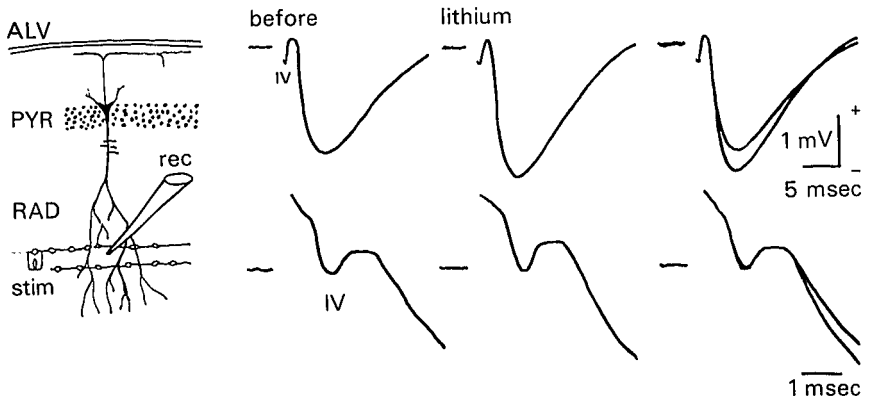

Figure 2. Left: Schematic representation of stimulating and recording arrangement. Right: Averaged (8 sweeps) extracellularly recorded epsps. Upper traces show e-epsps (25 msec sweeps) before and after 30 min of perfusion with 2 mM lithium. Lower traces show, with 5 msec sweeps, no change in the input volley (IV) but an increase in the e-epsp slope. The rightmost traces are superimposed: before and during lithium. Recovery was obtained 40 min after washout. ALV: alveus, PYR: str. pyramidale, RAD: str. radiatum.

RESULTS

Field potentials

Field potentials were recorded from 35 slices (27 rats). Stimulation of stratum radiatum caused typical extracellular epsps (e-epsps) and population spikes in the apical dendritic and pyramidal layer of area CA 1 (5). E-epsp peak voltages were measured in 7 preparations

with stimulus intensities well below threshold for population spikes. In 5 slices e-epsps increased by 16.4 ∓ 5.3 (SD) %, 2 slices were unaffected by 2 mM Li perfused for 20 to 30 min. Such an experiment is illustrated in fig. 2. Peak voltage and slope of the e-epsp rose during perfusion with 2 mM Li in the absence of changes in the input volley. Orthodromically elicited (synaptic) population spikes were also enhanced during perfusion with 2 mM Li by 36.5 ∓ 13.4 (SD) %, (n=4) while antidromic population spikes were unchanged. These effects were also seen with local application of Li (fig. 3).

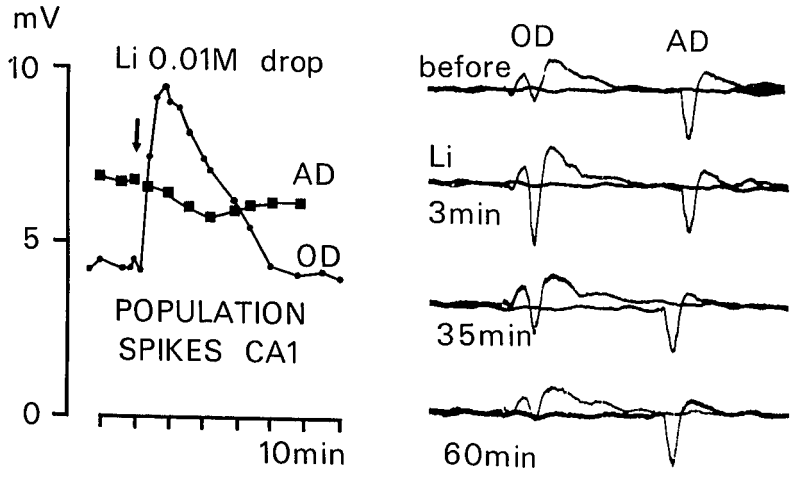

Figure 3. Action of locally applied Lithium on population spikes elicited by stimulation of the alveus (antidromic, AD) and the stratum radiatum (orthodromic, OD), see fig. 2. Each point or square represents an average of 8 spikes. Li was applied as a microdrop (ca 10 nl). Right: superimposed oscilloscope traces of synaptic (OD) and antidromic (AD) population spikes evoked alternatively with 5 sec intervals, sweep duration is 50 msec. Time after Li drop application is indicated in min.

On three slices Li (5 mM) was perfused for 30 min at room temperature (21°C). In all cases no effect was observed although the usual enhancement of e-epsps was obtained with a second perfusion done 1 hr after warming up the slices to 35°C. Ouabain (10^{-5} - 10^{-4} M) increased e-epsps in a similar manner and depolarized pyramidal cells.

Intracellular observations

Intracellular recordings were obtained from 22 cells in 22 slices from 16 rats. Membrane resting potentials were higher than 60 mV, action potentials larger than 80 mV and input resistances above 25 MΩ. In 13 of 15 cells tested perfusion with 1.5 - 10 mM Li caused a depolarization of 7.6 ∓ 3.1 (SD) mV, and 6.0 ∓ 2.8 (SD) mV in the six cells

exposed to 2 mM Li. One cell was unaffected and one cell was hyperpolarized by 4 mV. Similar but stronger depolarizations were also obtained with rubidiumchloride and caesiumchloride (2 mM) but not with sodiumchloride (upto 10 mM). Membrane conductance, measured by intracellular current injection through a bridge circuit was usually unchanged but was increased by upto 20 % in 3 cells during the intense firing at the peak of the Li action. Action potentials were reduced in size during depolarization but the half falling time was unchanged except for 2 cells which were exposed to 10 mM Li for more than 30 min where a widening and an increase of the latency of antidromically elicited action potentials occurred. Excitability by depolarizing current injection and spontaneous firing were enhanced during the Li evoked depolarizations but no difference to control was observed when the cells were artificially repolarized.

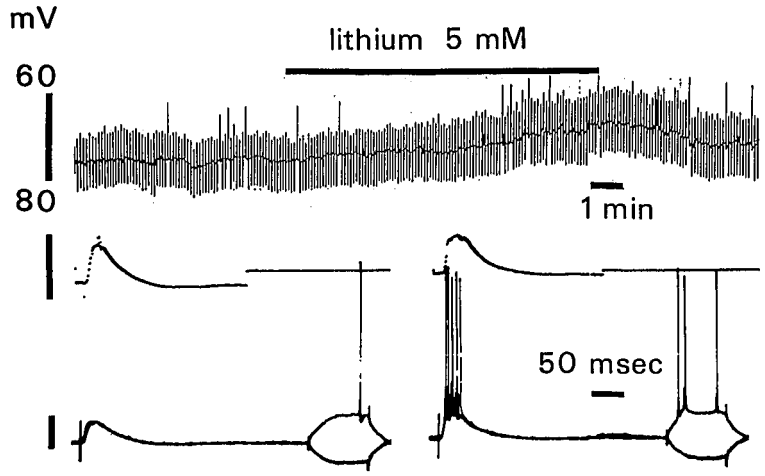

Figure 4. Intracellular recording from a CA 1 pyramidal neurone. Upper trace shows chart record with epsps and hyperpolarizing pulses of - 0.5 nA (upward and downward deflections respectively, reduced by the slow frequency response of the writer). Lower traces show averaged (8 sweeps) and original traces with an epsp (-ipsp) before and during Li perfusion and responses to $\mp$ 0.5 nA current injection. Calibration bars on the left are between 60 and 80 mV.

Epsps and ipsps evoked by stratum radiatum stimulation were clearly enhanced in 4 cells, in the other recordings the change of the epsp-ipsp sequence during depolarization and the advent of action potential firing did not allow a conclusion wether the synaptic potentials were enhanced (fig. 4). Three cells were recorded with K-Cl filled electrodes in order to investigate spontaneous depolarizing ipsps (2) which were readily observed during continuous chloride injection (- 0.1 - 0.5 nA). Li perfusion always lead to an increase in size and number of these

potentials. This is illustrated in fig. 5 on a cell which was depolarized by 7 mV during a perfusion with 5 mM Li. Artificial adjustment of the membrane potential was not used here because of the resulting change in Cl injection into the cell. When periods of 10 sec were analyzed 88 ipsps with an average height of 3.5 ∓ 2.0 (SD) mV were counted before and 128 events with 4.8 ∓ 2.7 (SD) mV during Li perfusion.

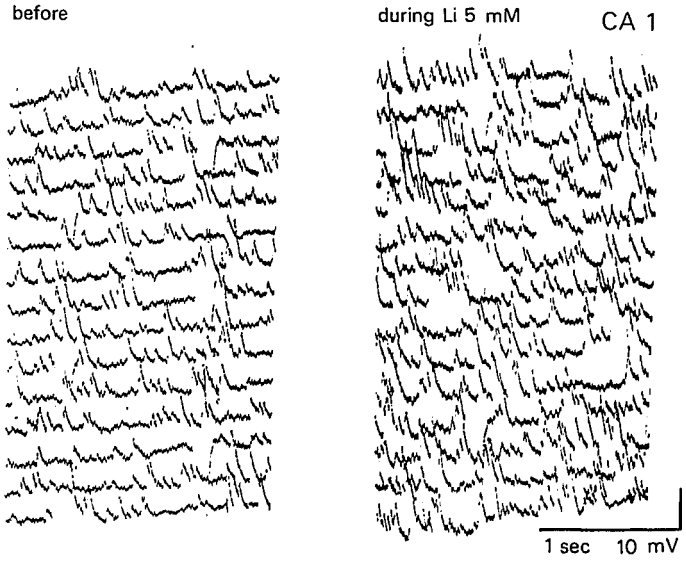

Figure 5. Intracellular recording from a CA 1 pyramidal neurone registered on a fiber optic oscillograph. Chloride ions have been continuously injected for 1 hr prior to the control record (before): Spontaneous depolarizing ipsps (2) are stable in size and number. On the right the cell is depolarized by 7 mV after 10 min perfusion with 5 mM Li.

Afterhyperpolarizations after bursts of action potentials elicited by intracellular depolarizing current injection which are probably produced by a calcium activated potassium conductance (24) were studied in 5 cells. The long lasting potentials are voltage dependent and were therefore recorded on the same voltage levels enforced by current injection. Under these conditions, there was no change in 4 cells but an increase was seen appearing with a delay of 10 min after the Li induced depolarization in one cell. Five further cells were investigated during tetrodotoxin poisoning (1 µg/ml). This treatment promptly blocked fast action potentials but did not prevent the depolarizing actions of Li. The threshold for eliciting presumable calcium spikes (18,36) determined with current pulses at equal voltage levels was unchanged by Li. In two preliminary experiments conducted in a medium in which synaptic potentials had been blocked by adding magnesium (10 mM final concentration) 2 mM Li for 20 min was ineffective in depolarizing the cells.

DISCUSSION

Adding lithiumchloride to the perfusion fluid of hippocampal slices consistently leads to a depolarization of CA 1 pyramidal cells and an increase in synaptic transmission. It has been shown in several preparations and with different methods that Li increases resting release (9, 11, 12, 26, 42) but also a moderate increase in evoked release was found in vegetative ganglia (30) and the neuromuscular junction (12). Release could not be measured directly in the hippocampus but the increase in epsps which was best quantified with extracellular field potential recording is consistent with an increase in evoked release. An increase in evoked and resting release of excitatory transmitters from the abundant inputs to the pyramidal cell dendrites could be at least partly responsible for the depolarization during Li perfusion. Such a combined effect would be expected to be reduced but not blocked by high magnesium and during tetrodotoxin poisoning. Release of inhibitory, hyperpolarizing transmitters is likely to be equally affected by Li (an increased release of GABA from superfused rat cerebral cortex by Li has in fact been reported (13)). Although only ipsps but no epsps (both representing evoked release) seem to occur spontaneously in CA 1 pyramidal cells the resting release of excitatory transmitter may overcome the hyperpolarizing influences during the presence of Li. GABA, the inhibitory transmitter, has also a partially depolarizing action (4) and causes a conductance increase (which was in fact observed in some cells during Li perfusion). The spontaneous ipsps, best seen as depolarizing potentials after a shift of the chloride equilibrium potential, are shown to become larger and more frequent during Li perfusion. In this situation Li seemed to cause stronger depolarizations. Although this effect suggests an increased inhibitory transmission it could also be explained by an increased firing of interneurones.

If Li entered cells like sodium but is less effectively extruded (27) a depolarization and an increased resting release from depolarized terminals would result (14). Such a Li accumulation is unlikely to occur with the low concentrations employed and the relatively short time course. Intracellularly injected Li to a level of 1 - 2 mM in snail neurones increases potassium conductance and hyperpolarizes (31) but this situation may not occur with "Li-treatment" levels (40). Millimolar concentrations of Li in the extracellular fluid nevertheless lead to an increase of intracellular calcium (1) which, if occurring also in nerve endings, would facilitate release. As such a facilitation really occurs at the neuromuscular junction even in the absence of extracellular calcium (11, 12), the most probable explanation is that Li leads to mobilization of intracellular calcium (presumably from mitochondria (3)) rather than to an enhanced calcium influx. This is again in keeping with our present results which suggest an increased release but no change in calcium currents and calcium activated potassium current. The evidence is however indirect as the failure to show changes in threshold and configuration of the calcium spikes in CA 1 pyramidal cell somata may not necessarily reflect the events in nerve endings.

The question remains how Li exerts this effect and how it relates to the clinical efficacy. One attractive possibility, an impairment of the Na-K transport has been suggested by several investigators (11, 28, 32, 33, 42) also in chronically treated animals (41). Low "clinical" concentrations of Li block the Na-K pump like cardiac glycosides and this has been shown to result in an increased release (16) independent of extracellular calcium (7) and could account for cell depolarization through sodium accumulation too. Such a mechanism is however likely to affect primarily nerve endings and thin dendrites with a large surface relative to the enclosed volume.

Segal (37) has described a Li-noradrenaline antagonism in the hippocampus which may now be explained by the suggestion of the same author that noradrenaline acts by activating the sodium pump (38).

Our present experiments support this mode of action as 1. ouabain and Li had similar actions (depolarization, epsp increase) and 2. at low temperature, when the pump activity is low, Li was ineffective.

Ryall and I (19) have previously suggested that the action of Li in the central nervous system may be chiefly due to a facilitating effect on transmitter release with a certain predilection for cholinoceptive neurones. Such neurones may have a high resting sodium conductance (29) and a high sodium pump activity and may therefore be particularly sensitive to the actions of Li. Li is likely to produce a depolarization by sodium accumulation in these cells in addition to its action on release.

If an increase in synaptic transmission occurs in the brain of patients receiving Li it should be reflected by an increase in evoked potentials. This has indeed been found in animals (22) and man (23).

In conclusion, low levels of lithium (1 - 5 mM) cause a depolarization of and an increased synaptic transmission on CA 1 pyramidal cells. The effects could be explained by a reduction of the sodium pump activity.

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