

Effects of Lithium on Electrical Activity and Potassium Ion Distribution in the Vertebrate Central Nervous System

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(Accepted March 22nd, 1983)

Key words: lithium — motoneurons — glial cells — spinal cord — cortex — hippocampus — potassium

Three different regions of the vertebrate central nervous system maintained in vitro (frog spinal cord, guinea pig olfactory cortex and hippocampus) have been used to investigate how Li^+ influences membrane potential, membrane resistance, action potentials, synaptic potentials and the transmembrane K^+ -distribution of neurons and glial cells. In view of the therapeutic action of Li^+ in manic-depressive disease, a special effort was made to determine the threshold concentration for the actions of Li^+ on the parameters described above. It was observed that Li^+ induced a membrane depolarization of both neurons and glial cells, a decrease of action potential amplitudes, a facilitation of monosynaptic excitatory postsynaptic potentials and a depression of polysynaptic reflexes. The membrane resistance of neurons was not altered. Li^+ also induced an elevation of the free extracellular potassium concentration and a decrease of the free intracellular potassium concentration. Furthermore, in the presence of Li^+ a slowing of the recovery of the membrane potential of neurons and glial cells, and of the extracellular potassium concentration after repetitive synaptic stimulation was observed. The threshold concentrations for the effects of Li^+ were below 5 mmol/l in the frog spinal cord and below 2 mmol/l in the guinea pig olfactory cortex and hippocampus. The basic mechanism underlying the action of Li^+ may be an interaction with the transport-function of the Na^+/K^+ pump.

INTRODUCTION

Despite extensive investigations, the mechanism of the therapeutic and prophylactic action of lithium ions (Li^+) in manic-depressive disorder is still unclear. Besides a possible action on neurotransmitter metabolism and receptor sites, there are several indications that Li^+ might interact with the distribution and transport of potassium and sodium in the central nervous system. It has been reported in this context, that Li^+ induces a decrease in the K^+ concentration of brain slices^{19,42}. Ullrich et al.⁴⁰ described an elevation of the extracellular K^+ concentration in the cerebellum of rats which had been chronically treated with Li^+ . Furthermore, Li^+ inhibited the K^+ reuptake of brain cortical slices after a period of anaerobic incubation¹⁷ and also the K^+ uptake into astrocytes in primary cultures⁴¹. Complementary to these observations is the well-known interaction of Li^+ with the electrogenic Na^+/K^+ pump, as judged from the Li^+ -induced decrease or blockade of a membrane hyperpolarization following repetitive neuronal ac-

tivity in rat or rabbit vagus nerve^{6,30,33}, in amphibian optic nerve³⁶, in the crayfish stretch receptor²⁴ and in primary afferents of the frog spinal cord⁵. The latter effect has been investigated mainly using rather high concentrations of Li^+ , but it was also observed in the therapeutic concentration range⁶.

We have now extended the studies concerning Li^+ and the Na^+/K^+ pump by a comparative analysis of the effects of Li^+ on 3 different regions of the vertebrate CNS maintained in vitro. It has been investigated how Li^+ influences the membrane potential, membrane resistance, action potentials and synaptic potentials of neurons and glial cells in the isolated frog spinal cord, guinea pig olfactory cortex and hippocampus. Many of the experiments were performed using K^+ -sensitive microelectrodes parallel to conventional intra- or extracellularly located microelectrodes. Furthermore, dynamic changes of the extracellular K^+ concentration and of the membrane potential of neurons and glial cells have been observed under the influence of Li^+ . A special effort was made to determine the threshold for the actions of Li^+ on

the parameters mentioned above. A preliminary publication of part of the results has already been published¹².

MATERIALS AND METHODS

Frog spinal cord

Experiments were performed on *Rana esculenta* with the techniques described in detail elsewhere³⁴. After decapitation, a ventral laminectomy was performed in cooled Ringer solution. The spinal cord, including dorsal and ventral roots of the lumbar segments, was removed and placed in a recording chamber (vol. 1.5 ml), which was continuously superfused with Ringer solution by means of a roller pump (2.5 ml/min). The Ringer solution contained (mmol/l): NaCl 98, KCl 3.6, CaCl₂ 2.0, NaHCO₃ 12, glucose 10. The pH was continuously monitored and adjusted to 7.3–7.5 by varying the proportion of O₂ and CO₂ in the gassing mixture. The temperature of the perfusion fluid was adjusted to 18–21 °C. The dorsal roots of the lumbar segments of one side were placed on silver wire electrodes for stimulation and covered with vaseline; the ventral roots of the same side were drawn into glass suction electrodes for stimulation or recording from the ventral roots. The construction and calibration of the double-barrelled ion-sensitive microelectrodes are described elsewhere^{13,14}.

Guinea pig olfactory cortex

Male or female guinea pigs (about 300 g) were decapitated and the brain was rapidly removed onto wet filter paper. Using a plastic guide and a bowcutter, a surface slice (approximately 500 μm thick) of olfactory cortex was cut and placed in oxygenated Krebs solution at 30 °C. Slices were then transferred to a small temperature-controlled Perspex bath, sandwiched between two nylon meshes and completely submerged. Oxygenated Krebs solution at 30 °C was pumped through the bath at 8–10 ml/min. The Krebs solution contained (mmol/l): NaCl 118, KCl 3.0, CaCl₂ 1.5, NaHCO₃ 25, NaH₂PO₄ 1.2, MgCl₂ 1.0, glucose 10; this solution was continuously gassed with 95% O₂–5% CO₂, pH = 7.4. Stimulation of the lateral olfactory tract (LOT), intracellular recording with 4 mol/l potassium acetate-filled microelectrodes and extracellular recording with K⁺-sensi-

tive microelectrodes were performed using conventional methods previously described⁸.

Guinea pig hippocampus

Procedures used in the preparation, maintenance and fixation of slices from the guinea pig hippocampus were in general accordance to the techniques described in detail by Lee et al.²². The medium which was continuously pumped through the recording chamber (flow rate 1–2 ml/min; volume of the chamber 1.5 ml) was temperature-controlled at 35 °C and contained (mmol/l): NaCl 124, KCl 2.25, KH₂PO₄ 1.25, MgSO₄ 2.4, CaCl₂ 2.5, NaHCO₃ 25.7, glucose 10; the solution reached but did not cover the surface of the slices. Li⁺ was added via the solution and reached the chamber 3.5 min after the onset of superfusion. Stimulation of Schaffer collaterals was achieved by a bipolar tungsten wire electrode (resistance: 10 kΩ at 1700 Hz) positioned in the stratum radiatum of the CA3 region. Recordings were made from the CA1 region by two micropipettes (filled

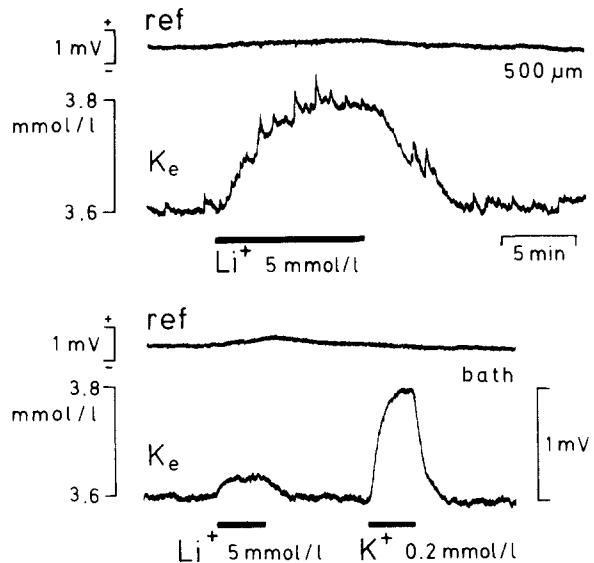


Fig. 1. Changes of extracellular potassium concentration ($[K^+]$ _e) in the isolated frog spinal cord during the application of Li⁺. The upper two traces show recordings of a K⁺-ion-sensitive microelectrode (ISME) at a depth of 500 μm below the lateral surface of the spinal cord. The small fluctuations in the K_e -trace are due to spontaneous synaptic activity (compare with Fig. 2A). The lower traces show measurements with the same K⁺-ISME after its withdrawal to the Ringer solution in the organ bath. LiCl (5 mmol/l) and KCl (0.2 mmol/l) have been added to the Ringer solution; the small change of the K_e -trace in the organ bath during the application of Li⁺ is therefore due to a change in the ionic strength.

with 2 M NaCl, tip diameter 1.5–2 μm) placed at a depth of about 150 μm below the surface of the slice in the soma and dendritic layer, respectively.

RESULTS

Extracellular K⁺ concentration

The application of LiCl into the superfusion solution, either by exchanging it for an equimolar concentration of NaCl, or by adding it to the normal saline, resulted in an elevation of the free extracellular K⁺ concentration, [K⁺]_e, in the isolated frog spinal cord (Figs. 1 and 2A) and in the guinea pig olfactory cortex (Fig. 2B). The rise in [K⁺]_e started within the first 2 min after Li⁺ superfusion and a [K⁺]_e-peak was reached between 5 and 10 min later. During and after a long-lasting Li⁺-application, a slow decrease of [K⁺]_e to its baseline level, and an undershoot below the control values during the washing phase with normal saline, was observed. The threshold for this ef-

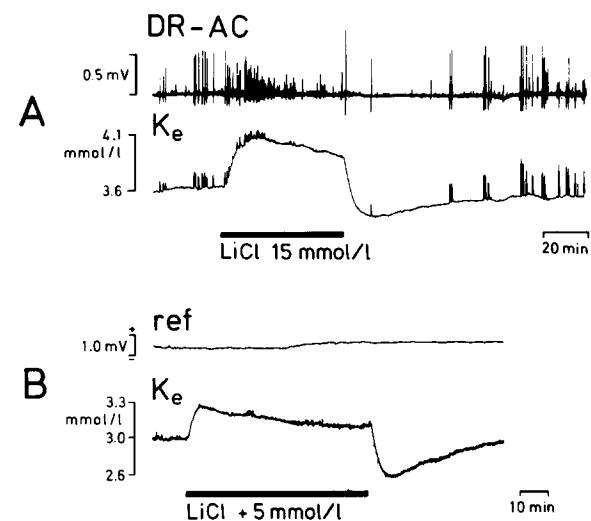


Fig. 2. A: effects of Li⁺ (15 mmol/l, exchanged for NaCl) on [K⁺]_e and spontaneous synaptic activity in the frog spinal cord during a long-lasting application. The upper trace of A shows the recording from a suction electrode containing one of the lumbar dorsal roots (DR; AC-coupled differential amplifier). The lower trace in A illustrates alterations of K_e during the same period and measured by a K⁺-ISME in the dorsal horn at a depth of 270 μm below the lateral surface of the spinal cord. Note the increase of spontaneous activity in the dorsal root during the beginning of the Li⁺-superfusion and the absence of spontaneous activity during the post Li⁺ K_e-undershoot. B: effects of Li⁺ (5 mmol/l added to the Krebs solution) on [K⁺]_e in the olfactory cortex slice during a long lasting application. The K⁺-ISME was placed at a depth of about 300 μm below the pia surface. Note also here the post Li⁺ K_e-undershoot.

fect was below 5 mmol/l LiCl in the spinal cord and below 2 mmol/l LiCl in the olfactory cortex. Quantitatively, in the isolated frog spinal cord at a depth of about 400–500 μm below the lateral surface, a maximal increase of [K⁺]_e from 3.6 to 3.8 \pm 0.05 mmol/l (mean \pm S.D.; n = 5) during the administration of 5 mmol/l Li⁺, and from 3.6 to 4.2 \pm 0.1 mmol/l (n = 10) during 15 mmol/l Li⁺, was measured. The given concentrations of Li⁺ refer to the values in the organ bath; the real concentration of Li⁺ 15 min after application in deeper regions of the spinal cord is about 65% of that in the bathing solution, as demonstrated with Li⁺-sensitive microelectrodes¹³. In brain slices from the guinea pig olfactory cortex at a depth of about 200–300 μm blow the pia, [K⁺]_e increased from 3.0 to 3.15 \pm 0.06 mmol/l (mean \pm S.D.; n = 3; 2 mmol/l Li⁺), from 3.0 to 3.3 \pm 0.09 mmol/l (n = 13; 5 mmol/l Li⁺) and from 3.0 to 3.9 \pm 0.3 mmol/l (n = 7; 15 mmol/l Li⁺).

Several experiments were performed to demonstrate that voltage changes measured by the K⁺-ion-sensitive microelectrodes (ISME; Corning Ion Exchanger 477317) in the presence of Li⁺ were indeed caused by an increase of [K⁺]_e. Firstly, increases of [K⁺]_e observed with low concentrations of Li⁺ added to the normal saline are not induced by an alteration of the ionic strength of the saline, since control measurements in the organ bath revealed much smaller voltage changes of the K⁺-ISME by application of Li⁺-containing solutions (Fig. 1). Moreover, in experiments with 15 mmol/l LiCl, the ionic strength of the solution was kept constant by exchanging Li⁺ for an equimolar concentration of NaCl. Secondly, identical changes of [K⁺]_e were observed when either Corning K⁺-exchanger or valinomycin-based K⁺-ISME were used. This fact indicates that the change of the ionic signal did not reflect an elevation of acetylcholine or other quaternary ammonium compounds in the spinal cord. Finally, 3 experiments were performed in the presence of TTX (1 $\mu\text{mol/l}$). This resulted in only a small reduction of Li⁺-induced increases of [K⁺]_e, indicating that an indirect action of Li⁺ on, for example, spontaneous synaptic activity is not the main reason for the elevation of [K⁺]_e.

[K⁺]_e-decay phase after repetitive stimulation

In addition to its effects on the [K⁺]_e-baseline, Li⁺ also exhibited an influence on the [K⁺]_e-decay phase

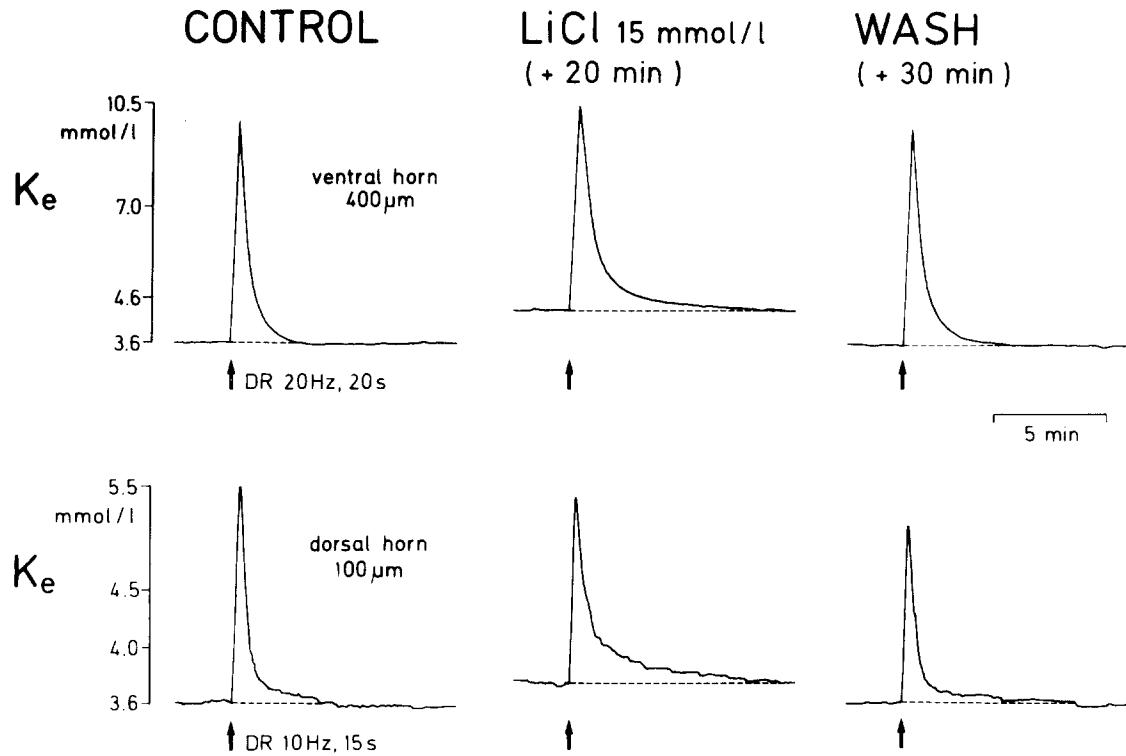


Fig. 3. Effects of Li^+ on the $[\text{K}^+]_e$ -decay phase after repetitive stimulation of a dorsal root (DR) in the isolated frog spinal cord. The figure originates from two different experiments, in which K^+ -ISME have been used to measure the kinetics of K_e -changes during and after repetitive stimulation of a dorsal root. The same stimulation parameters have been used before, during and after Li^+ -application (15 mmol/l, exchanged for NaCl). The upper trace illustrates a recording in the ventral horn (400 μm below the lateral surface) and the lower trace one in the dorsal horn (100 μm below the lateral surface). Note the Li^+ -induced slowing of the $[\text{K}^+]_e$ -decay phase.

after repetitive synaptic stimulation. In particular, this effect was observable in the isolated frog spinal cord. In normal Ringer's solution, repetitive stimulation of a dorsal root (stimulation parameters: 10–20 Hz, 10–20 s) resulted in a $[\text{K}^+]_e$ -elevation in the dorsal and ventral horn of the spinal cord. After the end of the stimulation, several minutes elapsed until $[\text{K}^+]_e$ had reached its resting baseline level (Fig. 3). A post-tetanic $[\text{K}^+]_e$ -undershoot was not detected, in accordance with observations from other authors^{25,35}. In the presence of Li^+ , a slowing of the $[\text{K}^+]_e$ -recovery phase was observed in both the ventral and the dorsal horns. The effects of Li^+ at a concentration of 5 mmol/l were weak, whereas clear changes were measured with 15 mmol/l LiCl (12 experiments).

In the olfactory cortex slice, similar though less prominent effects of Li^+ on the $[\text{K}^+]_e$ -decay phase after repetitive afferent stimulation (20 Hz, 5–10 s) were measured. Also in this preparation, a post-tet-

anic $[\text{K}^+]_e$ -undershoot did not occur. Li^+ , at a concentration of 5 mmol/l, induced a slowing of the $[\text{K}^+]_e$ -recovery phase in 4 out of 6 experiments (see Fig. 8).

Membrane potential, membrane resistance and action potentials

Li^+ induced a membrane depolarization in frog spinal motoneurons and in neurons of the guinea pig olfactory cortex. The depolarizations, however, were more pronounced in the latter preparation. Spinal motoneurons of the frog did not show a clear change of the resting membrane potential at 5 mmol/l, whereas 15 mmol/l LiCl produced a depolarization of 3.2 ± 1.2 mV (mean \pm S.D.; $n = 15$; see Figs. 4 and 5). Li^+ had a fast onset of action and recovery from the Li^+ -induced membrane depolarization was observed within a few minutes after washing with normal Ringer solution. A change in the input resistance of frog motoneurons was not observed

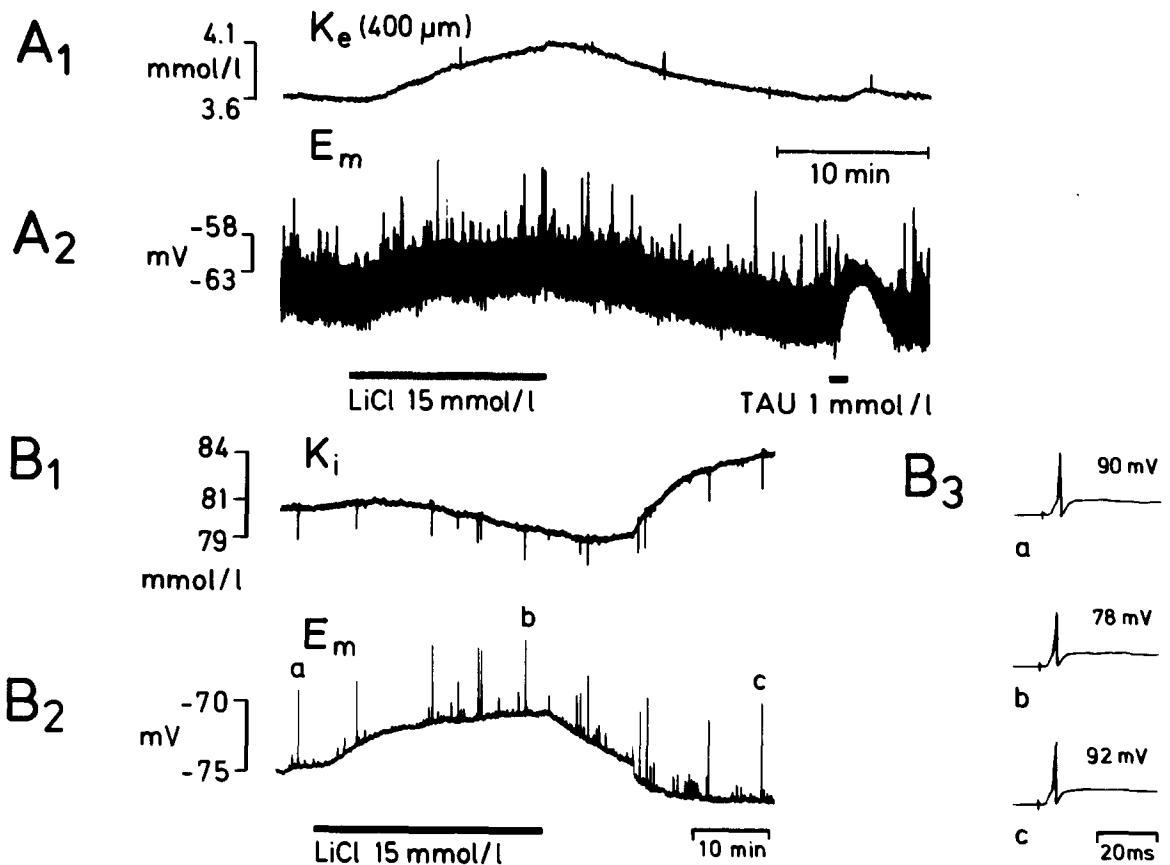


Fig. 4. Effects of Li^+ on membrane potential, membrane resistance, action potential amplitude and extra- and intracellular K^+ -concentrations of frog spinal motoneurons. A₁ shows the recording of a K^+ -ISME at a depth of 400 μm below the lateral surface of the spinal cord. A conventional single-barrelled microelectrode was positioned intracellularly in a motoneuron at the same time (A₂). The deflections in the membrane potential trace (E_m) consist of hyperpolarizing constant current pulses (duration 100 ms; current 10 nA; frequency 0.2 Hz) and depolarizing voltage deflections, which represent spontaneous synaptic activity. Application of LiCl (15 mmol/l, exchanged for NaCl) induced an increase of $[\text{K}^+]_e$ and a depolarization of the motoneuron membrane without changing the input resistance. As a control, later on taurine (TAU) was applied into the superfusion solution which induced a typical decrease of the input resistance. B: impalement of a motoneuron with a double-barrelled K^+ -ISME. Determination of changes of the free intracellular K^+ -concentration (B₁) simultaneous to changes of the membrane potential (B₂) during the action of Li^+ . Amplitudes of synaptically induced action potentials compared before, during and after Li^+ in B₃.

(Fig. 4). In olfactory cortex neurons, a clear membrane depolarization was detected. This depolarization was concentration-dependent and ranged between 2.5 and 5 mV at 5 mmol/l Li^+ , ($n = 5$), and between 6 and 8 mV at 15 mmol/l Li^+ ($n = 3$). Again, no change in the input resistance of the neurons was observed (measured with constant hyperpolarizing current steps). It should be mentioned that the Li^+ -induced membrane depolarization was most prominent in neurons with high resting membrane potentials (> -70 mV; see Figs. 6 and 7).

We have also investigated how Li^+ influences the membrane potential kinetics (of neurons in the frog

spinal cord and guinea pig olfactory cortex) after repetitive synaptic stimulation. In the spinal cord, a post-tetanic membrane hyperpolarization of motoneurons was typically observed after repetitive stimulation of a dorsal root (10–20 Hz; 10–20 s). It is very likely, that this hyperpolarization results from the activation of an electrogenic Na^+/K^+ pump^{4,5,14,25}. As illustrated in Fig. 5, 15 mmol/l LiCl reduced or even abolished this post-tetanic membrane hyperpolarization. In addition, there was a membrane depolarization and an increase of spontaneous synaptic potentials. Also in the olfactory cortex, a Li^+ -induced change in the post-tetanic behaviour of neurons was found.

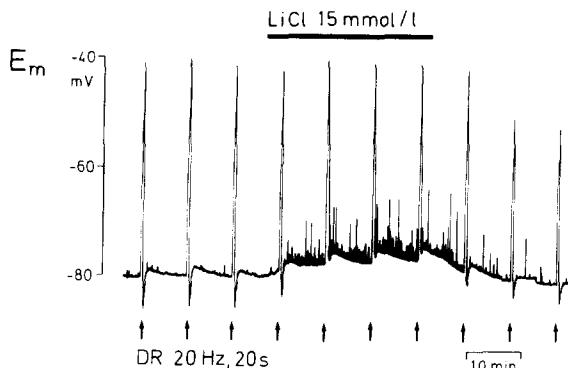


Fig. 5. Effects of Li^+ on the post-tetanic membrane hyperpolarization in a frog spinal motoneuron. A dorsal root was stimulated repetitively (frequency 20 Hz; duration 20 s) in regular intervals of about 8 min (indicated by arrows). The post-tetanic membrane hyperpolarization observable in normal Ringer solution was completely abolished in the presence of Li^+ (15 mmol/l, exchanged for NaCl). Simultaneously, a membrane depolarization and an increase of spontaneous synaptic activity was observed.

However, in contrast to the uniform appearance of a post-tetanic hyperpolarization in frog motoneurons, more variable post-tetanic recovery phases were observed in olfactory cortex neurons. Sometimes, the membrane potential exhibited a long-lasting post-tetanic membrane depolarization whereas in other cases a membrane hyperpolarization was observed (stimulation of the lateral olfactory tract (LOT) with 10–30 Hz; 5–10 s). Additionally, and in contrast to the frog spinal motoneurons¹⁴, a clear change of the input resistance was seen during the post-tetanic recovery phase. Therefore, it is not possible, at the moment, to decide which mechanism is mainly responsible for the post-tetanic membrane behaviour of cortical neurons. Neurons with high membrane potentials (-75 to -85 mV), however, showed a typical long-lasting post-tetanic membrane depolarization (see Figs. 6 and 7). In these cells, Li^+ induced a slowing of

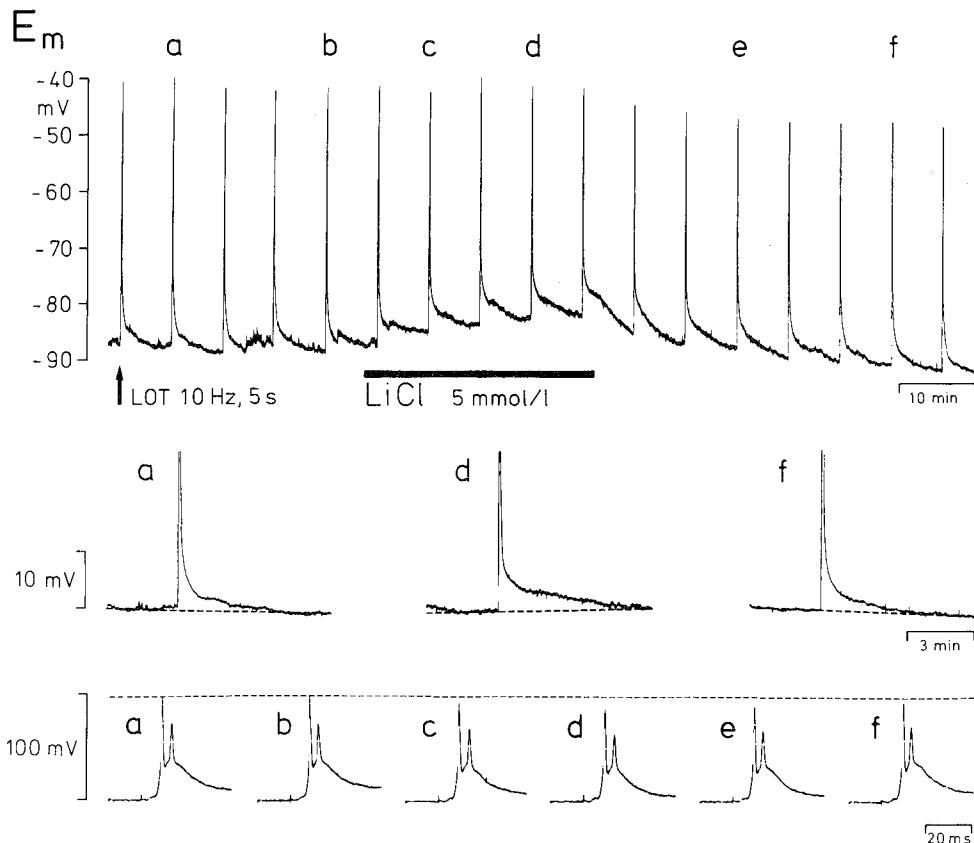


Fig. 6. Effects of Li^+ on a neuron of the guinea pig olfactory cortex slice. The uppermost trace shows the membrane potential of a cortical neuron with a slow time base. The lateral olfactory tract (LOT) has been stimulated repetitively (frequency 10 Hz; duration 5 s) in regular intervals of about 7 min. During the period marked by the bar, Li^+ (5 mmol/l) was exchanged for an equimolar concentration of NaCl in the Krebs solution. The trace in the middle of the figure illustrates with a faster time base the post-tetanic membrane behaviour before (a), during (d), and after (f) application of Li^+ . The lowermost traces show action potentials evoked by the first shock of the trains and taken at the times indicated by the letters above the uppermost trace. Note, that Li^+ induced a decrease of the action potential amplitude.

the post-tetanic membrane recovery (5 mmol/l). This effect of Li⁺ was observed in addition to a membrane depolarization.

Also, it was investigated how Li⁺ influences the amplitude of action potentials in spinal and cortical neurons. In the spinal cord, no change of the action potential amplitude was observed with 5 mmol/l Li⁺ (see also ref. 13). However, 15 mmol/l, a concentration that produced a membrane depolarization of the motoneurons, decreased the amplitude of synaptically induced action potentials. This effect is illustrated in Fig. 4B. The observation stems from one of the 3 experiments, in which the Li⁺-induced membrane depolarization was measured in motoneurons impaled with a double-barrelled K⁺-ISME. This enabled us to follow changes of the free intracellular K⁺-concentration ([K⁺]_i) during the action of Li⁺. A small decrease in [K⁺]_i was detected with a slow onset of action. The maximum decrease of [K⁺]_i in the 3 motoneurons, in which it was measured, ranged between 1 and 2.5 mmol/l. A Li⁺-induced decrease in the amplitude of synaptically induced action potentials was also observed in neurons from the guinea pig olfactory cortex (5 mmol/l; see Fig. 6).

Glial cells

In the following section, the effects of Li⁺ on glial cell will be described. These investigations were performed on glial cells of olfactory cortex slices, because in this preparation glial cells were impaled rather frequently. The application of Li⁺ resulted in a membrane depolarization and a slowing of the membrane potential recovery phase after repetitive stimulation of the LOT. The membrane depolarization started within the first 3 min of the onset of the Li⁺-superfusion, and a maximum was reached 5–10 min later. The effect was reversed within a few minutes after the end of Li⁺-superfusion (see Fig. 8). Quantitatively, a membrane depolarization of 2.2 ± 1 mV (mean \pm S.D.; n = 9) during 5 mmol/l Li⁺ (either added to the normal Krebs solution or exchanged for an equimolar concentration of NaCl) was observed. However, these data only refer to measurements from glial cells at a depth of about 100–460 μ m below the pia surface of the brain slice. Very often glial cells just below the pia were impaled. In these cells, a Li⁺-induced membrane depolarization (5 mmol/l Li⁺) was not detected. This fact is probably due to a lack

of [K⁺]_e elevation in the superficial tissue layers which are in close contact to the fast-flowing bath solution. In the 3 glial cells on which 15 mmol/l LiCl was tested, a membrane depolarization of about 5–7 mV was observed. Furthermore, a dependence of the Li⁺-induced membrane depolarization on the resting membrane potentials of the glial cells was also observed. The amplitude of the depolarization was maximal at high resting potentials (> -80 mV).

At the time of the membrane depolarization, a Li⁺-induced slowing of the membrane potential recovery phase of glial cells after repetitive stimulation

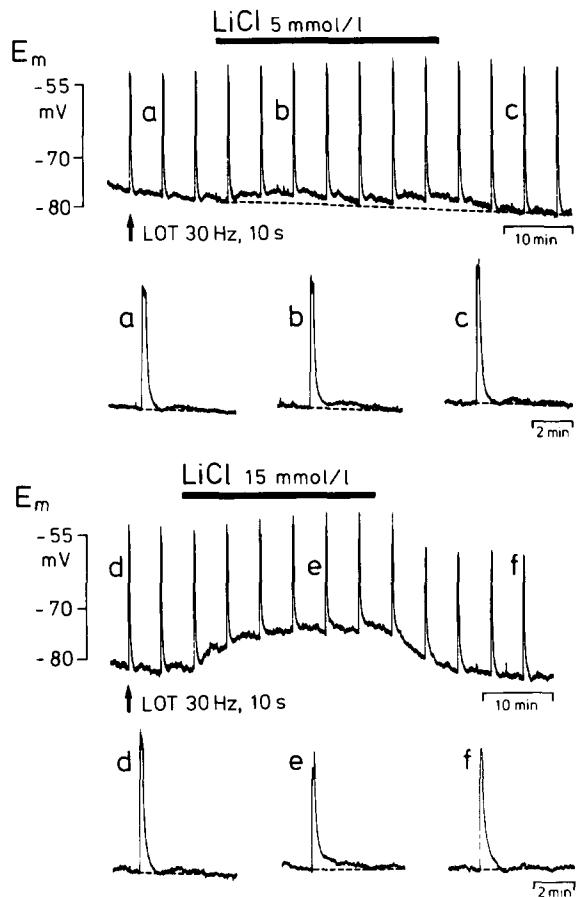


Fig. 7. Concentration-dependency of Li⁺-effects on a cortical neuron. The upper-most trace shows the membrane potential of a cortical neuron with a slow time base. The LOT has been stimulated repetitively (frequency 30 Hz; duration 10 s) in regular intervals of about 6 min. During the period marked by the bar, Li⁺ (5 mmol/l) was exchanged for an equimolar concentration of NaCl in the Krebs solution. The second trace from above illustrates the posttetanic membrane behaviour with a faster time base before (a), during (b), and after (c) application of Li⁺. The two lower traces show the same parameters during the application of 15 mmol/l Li⁺ (same neuron).

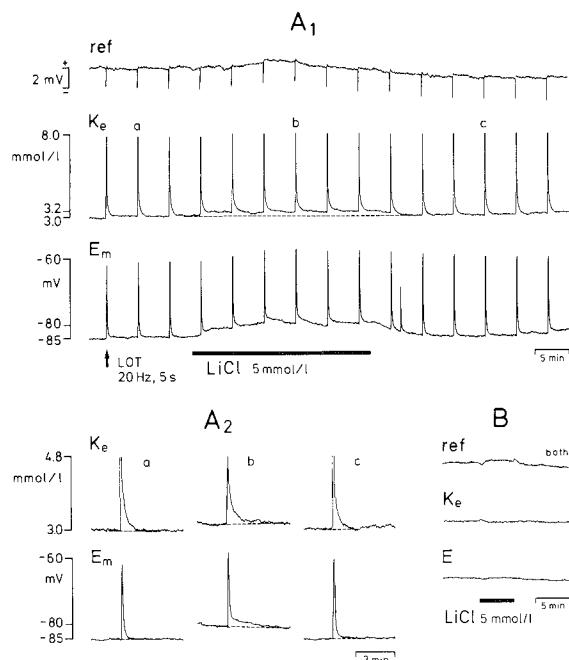


Fig. 8. Effects of Li^+ on a cortical glial cell. In this experiment, a simultaneous recording inside a glial cell ($270 \mu\text{m}$ below surface, conventional electrode) and outside with a K^+ -ISME has been performed. The LOT has been stimulated repetitively (frequency 20 Hz; duration 5 s) in regular intervals. A₁ illustrates changes of K_e and the glial cell membrane potential (E_m) during the influence of Li^+ (5 mmol/l, exchanged for NaCl) with a slow time base. A₂ shows the post-tetanic behaviour of $\text{K}_{e,c}$ and E_m with faster time base and B illustrates recordings of the K^+ -ISME (ref., K_e) and the single capillary (E) after withdrawing these electrodes into the Krebs solution of the organ bath.

of the LOT was also observed. A typical example is illustrated in Fig. 8. In this experiment a conventional microelectrode was used to record intracellularly from a glial cell ($247 \mu\text{m}$ below the pia) and an additional K^+ -ISME was used to measure the free extracellular K^+ concentration. Short stimulation trains were applied to the LOT at regular intervals. A display with a faster time base (Fig. 8A₂) reveals a Li^+ -induced slowing of the post-tetanic behaviour of the glial cell membrane potential and the $[\text{K}^+]_e$ -signal. Similar observations were made in 5 of the 7 experiments in which the action of Li^+ (5 mmol/l) on glial cells was investigated.

Li^+ and synaptic potentials

It has been shown that Li^+ (15 mmol/l) reduces the amplitudes of polysynaptic reflexes and increases the frequency of spontaneous synaptic activity in the iso-

lated spinal cord of the frog¹². Both effects could be imitated by an elevation of the extracellular potassium concentration from 3.6 to 4.6 mmol/l. It is also known that Li^+ (2–5 mmol/l) is able to increase the amplitude of extracellularly recorded EPSP and synaptically evoked population spikes in brain slices from the rat hippocampus¹⁵. In order to support our hypothesis, that effects of Li^+ on the CNS are mainly due to an alteration of the potassium homeostasis leading to a membrane depolarization, we have now performed some experiments in which effects of Li^+ and K^+ on field potentials of the hippocampus have

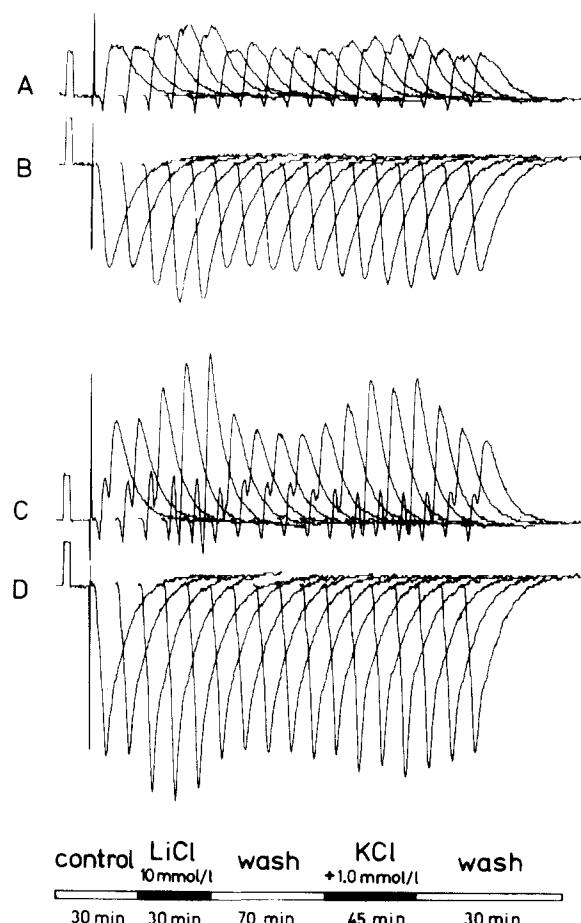


Fig. 9. Effects of Li^+ and K^+ upon evoked field potentials in the CA₁ region of a hippocampal slice. Traces A and C were recorded from the pyramidal cell layer, traces B and D from stratum radiatum. Potentials were evoked at a frequency of 0.2 Hz throughout the whole experiment. Measurements (4 sweeps averaged) were taken every 10 or 15 min during each experimental step using 5 different input intensities, two of which are plotted here: traces A and B were taken at 2.8 V and traces C and D at 4.55 V (90 μs stimulation pulse).

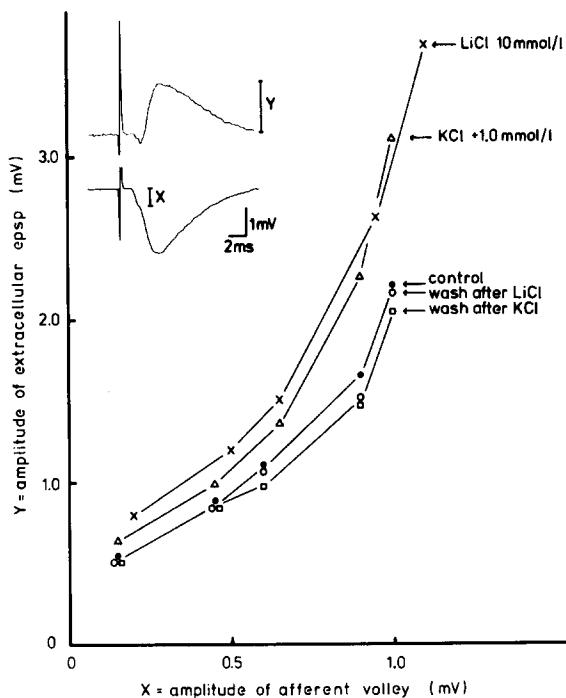


Fig. 10. Input-output graph of the effect of Li^+ and K^+ upon the extracellular EPSP. At 5 different stimulation intensities afferent volley amplitudes (abscissa) measured in the dendritic layer were correlated to the extracellular EPSP amplitudes (ordinate) measured in the somatic layer (see inset). The curves illustrate control values, values in the presence of Li^+ , during elevated K^+ (application time 20 min) and values in the recovery periods (70 min after Li^+ and 30 min after K^+). Data were taken from the experiment illustrated in Fig. 9.

been compared (8 brain slices from 8 guinea pigs). In these experiments Li^+ produced an increase in the amplitudes of evoked field potentials (Fig. 9). The action of Li^+ started 7–8 min after the onset of the Li^+ superfusion. Maximal values were reached after 20–30 min. Quantitatively, 5 mmol/l Li^+ added to the normal saline increased the extracellular EPSP by $19 \pm 8\%$ (mean \pm S.D.) and the population spike by $61 \pm 4\%$. 10 mmol/l Li^+ exchanged for an equimolar concentration of NaCl in the saline caused an increase of $46 \pm 8\%$ of the extracellular EPSP and of $149 \pm 29\%$ of the population spike. Furthermore, population spikes were already seen at lower stimulation intensities than in control solution (Fig. 9A). The amplitude of the afferent volleys was unchanged by Li^+ in 7 experiments. In the one experiment in which afferent volleys increased by $8 \pm 2\%$, the increase of the EPSP amplitude was independent of the increase of the afferent volley as judged from an input-output correlation (Fig. 10).

The excitatory effects of Li^+ could be imitated by increasing the K^+ concentration in the superfusion fluid. An elevation from 3.5 to 4.5 mmol/l caused a maximal increase of the extracellularly recorded EPSP by $34 \pm 3\%$, and of the population spikes by $148 \pm 33\%$. Similar to the action of Li^+ , population spikes already appeared at lower stimulation intensities. Afferent volley amplitudes always remained unchanged. With respect to the time course, an increase of EPSP amplitude was first seen about 10 min after the start of the K^+ superfusion; a maximal change was reached at around 30 min. Both Li^+ and K^+ effects upon field potentials in hippocampal slices were reversible. K^+ effects could be washed out within 15–20 min, whereas Li^+ effects took much longer to be completely reversed. During the washout phase after Li^+ , a transient decrease of the EPSP amplitude by $12 \pm 3\%$ was seen and the control values were then reached after about 50 min of washing (Fig. 9C).

DISCUSSION

The effects of Li^+ on the electrical activity and potassium ion distribution of frog spinal cord, guinea pig olfactory cortex and hippocampus observed in our experiments seem to be mainly a consequence of an interaction of Li^+ with the transport function of the Na^+/K^+ pump. We will therefore start the discussion with the possible mechanisms underlying this interference. Li^+ may have 3 sites of action on the Na^+/K^+ pump. (a) Li^+ might compete with K^+ for a common extracellular binding site. This possibility is supported by the demonstration that the uptake of Li^+ into neurons of primary brain cell cultures¹⁸, neuroblastoma cells¹¹, neuroblastoma/glioma hybrid cells²⁹ and in brain slices⁴² depends on the extracellular K^+ concentration. Low $[\text{K}^+]_{\text{e}}$ facilitates, whereas high $[\text{K}^+]_{\text{e}}$ inhibits, the Li^+ -uptake. Ouabain reduced the uptake of Li^+ into human neuroblastoma cells by up to 50%³². Furthermore, Li^+ inhibited the K^+ reuptake in brain cortical slices after a period of anaerobic incubation¹⁷ and also the K^+ uptake into astrocytes in primary cultures⁴¹. Finally, it is also known that Li^+ is able to substitute for K^+ in activating the transmembranal Na^+ transport³³. (b) Li^+ -influx into the intracellular space of neurons may decrease the free intracellular Na^+ concentration and thereby result in a reduced stimulation of the Na^+/K^+

pump from the interior of the cell. In fact, a diminution of $[Na^+]$ _i in the presence of high extracellular Li⁺ concentrations has been described in the crayfish stretch receptor⁹ and in neurons of the snail after the application of 40 mmol/l Li⁺ (ref. 27) and even with 1 mmol/l Li⁺ (ref. 1). The decrease of $[Na^+]$ _i may show up quite rapidly, since Li⁺ is able to enter neurons within 1 min after reaching the extracellular membrane surface, as has been measured with Li⁺-ISME in snail neurons³⁸ and frog motoneurons¹³. The time course of the Li⁺-induced membrane depolarization is therefore consistent with an intracellular site of action of Li⁺. Evidence that Li⁺ does not activate the Na⁺-site of the Na⁺/K⁺ pump has been obtained by Thomas³⁷ using direct injections of Li⁺-ions into snail neurons. (c) A third possibility is that Li⁺ may change the electrogenic Na⁺/K⁺ pump mode to an electroneutral K⁺/K⁺ pump mode, as suggested by Duham⁷. K⁺/K⁺ exchange is activated by a lowering of $[Na^+]$ _i and by inorganic phosphate, the concentration of which can rise substantially in nerve tissue upon repetitive stimulation³¹. Under these circumstances, Li⁺ would be able to replace external K⁺ in the K⁺/K⁺ exchange mode. All the 3 sites of action just mentioned might contribute to the interaction of Li⁺ with the transport function of the Na⁺/K⁺ pump. Keeping in view such possible mechanisms, the following discussion will be devoted to the actions of Li⁺ on electrical activity and on potassium ion distribution in the CNS.

One of the effects, which was consistently observed during Li⁺-application, was a depolarization of neurons in the frog spinal cord and guinea pig olfactory cortex. A membrane depolarization after a complete exchange of Li⁺ against Na⁺ has been also described on crayfish stretch receptors^{9,26} and on bullfrog ganglion cells²⁰. In brain slices of rat hippocampus even low levels of Li⁺ (2–5 mmol/l) caused a remarkable depolarization of CA1 pyramidal cells¹⁵. It has been suggested that the mechanisms underlying the Li⁺-induced membrane depolarization might be the reduced activity of an electrogenic sodium pump^{15,26}, a decrease of the intracellular potassium concentration^{9,20} and also part of it an increase of excitatory transmitter release¹⁵. Our data showing an increase of $[K^+]$ _e and a decrease of $[K^+]$ _i clearly demonstrate a Li⁺-induced shift of the K⁺ equilibrium potential towards more depolarized levels. This ac-

tion of Li⁺ seems to be the main reason for the membrane depolarization of the neurons. The lack of a change of the membrane resistance and the depolarization of glial cells is in support of this view. A reduced activity of the electrogenic sodium pump, however, cannot be excluded because our measurements with K⁺-ISME are not accurate enough to determine the potassium distribution exactly at the membrane surface of the neurons. This information is necessary to allow us to calculate whether the shift of the K⁺ equilibrium potential alone is sufficient to account for the membrane depolarization. The basic mechanism leading to the shift of the K⁺ equilibrium potential, however, might be an interaction of Li⁺ with the transport function of the Na⁺/K⁺ pump (see first paragraph of Discussion).

A depolarization of the postsynaptic membrane is also the most likely mechanism underlying the increase of population spikes seen in the presence of Li⁺. The population spike is a function of the number of discharging cells. Thus, during a Li⁺-induced membrane depolarization the number of cells from which a given input intensity elicits action potentials will increase. This view is strongly supported by the finding that increases in population spikes very similar to those seen in the presence of Li⁺ were elicited by an elevation of $[K^+]$ _e. The facilitation of the EPSP amplitude, on the other hand, probably results from an action on the presynaptic terminal. Li⁺ might induce a depolarization of the presynaptic membrane or a change in the free intracellular Ca²⁺ concentration in analogy to its effect on snail neurons¹. However, the similarity in the action of K⁺ and Li⁺ might indicate that a depolarization of the nerve terminal is the main mechanism underlying the facilitation of the EPSP amplitude.

Li⁺ induced a slowing of the post-tetanic $[K^+]$ _e recovery phase to the resting value. This observation in the isolated spinal cord of the frog and guinea pig olfactory cortex resembles the reduction in the post-stimulus $[K^+]$ _e-undershoot measured in isolated rat sympathetic ganglia³ and in the rat cerebellum⁴⁰ during acute or chronic application of Li⁺. It is well-known that the Na⁺/K⁺ pump is an important factor determining the decay of K⁺ potentials in the central nervous system²¹. Consequently, any impairment of the transport function of the Na⁺/K⁺ pump by Li⁺ should cause a slowing of the post-tetanic $[K^+]$ _e re-

covery phase, as has been observed also in the present experiments. Li^+ additionally induced a reduction in the amplitude of membrane hyperpolarization occurring in frog spinal motoneurons after repetitive stimulation of a dorsal root. It has been demonstrated that this hyperpolarization results from the activation of an electrogenic Na^+ pump^{4,5,14,25}. The reduction of the hyperpolarization in the presence of Li^+ is therefore also consistent with an impairment of the Na^+/K^+ pump.

Without appropriate measurements it remains unclear whether the effects of Li^+ observed in our in vitro experiments are involved in the therapeutic action of Li^+ in man. Usually, no significant changes of the serum^{16,23} and CSF²⁸ K^+ -concentration during Li^+ -therapy have been found. However, changes in serum $[\text{K}^+]$ are not likely to appear, since the adrenal-renal regulatory mechanisms would correct any deviation from homeostatic norm. Even a patient suffering from Li^+ -intoxication (serum Li^+ concentration 3.8 mmol/l) did not show a serum K^+ concentration outside the normal range², although a Li^+ -induced hyperkalemia (6.2 mmol/l) has been reported as well¹⁰. On the other hand, measurements of the extracellular K^+ -concentration alone do not describe the transmembranal K^+ -gradient, i.e. the K^+ -equi-

librium potential. However, the cardiotoxicity of Li^+ in humans (depression, sometimes inversion, of the T-wave in the electrocardiogram³⁹) has been postulated to reflect an intracellular myocardial K^+ -depletion. If a similar depletion of intracellular $[\text{K}^+]$ occurs in the brain of patients, it may play a part either in the therapeutic action of Li^+ , or in its toxic effects, or both. In addition, effects of Li^+ on the kinetics of dynamic changes of $[\text{K}^+]$ during phasic neuronal activity have also been observed in our investigation. It may be, therefore, that effects of Li^+ on the kinetics of the membrane potential of neurons and glial cells after repetitive synaptic stimulation are also important for its therapeutic action. At present, however, there is no possibility to determine whether such an effect of Li^+ occurs in the brain of the patients.

ACKNOWLEDGEMENTS

We would like to thank Dr. J. Rimpel and Ms. C. Koestler for participation in several of the frog spinal cord experiments and Dr. A. Barber for correcting the English. Expert technical assistance was given by Mrs. C. Müller, Ms. G. Schneider and Mrs. E. Hennoch. The work was supported by a grant given by the Wilhelm Sander Stiftung.

REFERENCES

- 1 Aldenhoff, J. B. and Lux, H. D., Effect of lithium on calcium-dependent membrane properties and on intracellular calcium-concentration in helix neurons. In H. M. Emrich, J. B. Aldenhoff and H. D. Lux (Eds.), *Basic Mechanisms in the Action of Lithium*, Excerpta Medica, 1982, pp. 50–63.
- 2 Askenasi, R., Linkowski, P. and Leeman, M., Seizure due to lithium intoxication, *Nouv. Presse Med.*, 10 (1981) 3481–3483.
- 3 Bruggencate, G. ten, Ullrich, A., Galvan, M., Förstl, H. and Baierl, P., Effects of lithium application upon extracellular potassium in structures of the peripheral and central nervous system of rats. In D. W. Lübbbers et al. (Eds.), *Progress in Enzyme and Ion-Selective Electrodes*, Springer-Verlag, Berlin, 1981, pp. 135–140.
- 4 Czech, G., Kříž, N. and Syková, E., Extracellular potassium accumulation in the frog spinal cord induced by stimulation of the skin and ventrolateral columns, *J. Physiol. (Lond.)*, 320 (1981) 57–72.
- 5 Davidoff, R. A. and Hackman, J. C., Hyperpolarization of frog primary afferent fibers caused by activation of a sodium pump, *J. Physiol. (Lond.)*, 302 (1980) 297–309.
- 6 Den Hertog, A. and Ploeger, E. J., Mechanism of action of lithium salts, *Psychiat. Neurol. Neurochir.*, 76 (1973) 529–535.
- 7 Duhm, J., Note on the interaction of lithium ions with the transport function of the Na^+/K^+ -pump. In H. M. Emrich, J. B. Aldenhoff and H. D. Lux (Eds.), *Basic Mechanisms in the Action of Lithium*, Excerpta Medica, 1982, pp. 21–27.
- 8 Galvan, M., Grafe, P. and ten Bruggencate, G., Convulsant action of 4-aminopyridine on the guinea-pig olfactory cortex slice, *Brain Research*, 241 (1982) 75–86.
- 9 Giacobini, E., Hovmark, S. and Stepita-Klauco, M., Studies on the mechanism of action of lithium ions. II. Potassium sensitive influx of lithium ions into the crayfish stretch receptor neuron determined by microphlame photometry, *Acta physiol. scand.*, 80 (1970) 528–532.
- 10 Goggans, F. C., Acute hyperkalemia during lithium treatment of manic illness, *Amer. J. Psychiat.*, 137 (1980) 860–861.
- 11 Gorkin, R. A. and Richelson, E., Lithium transport by mouse neuroblastoma cells, *Neuropharmacology*, 20 (1981) 791–801.
- 12 Grafe, P., Rimpel, J., Reddy, M. M., Koestler, C. and ten Bruggencate G., Effects of lithium on motoneurons, synaptic transmission and extracellular potassium in the isolated frog spinal cord. In H. M. Emrich, J. B. Aldenhoff, and H. D. Lux (Eds.), *Basic Mechanisms in the Action of Lithium*, Excerpta Medica, 1982, pp. 92–101.
- 13 Grafe, P., Rimpel, J., Reddy, M. M. and ten Bruggencate G., Lithium distribution across the membrane of motoneurons in the isolated frog spinal cord, *Pflügers Arch. ges. Physiol.*, 393 (1982) 297–301.

- 14 Grafe, P., Rimpel, J., Reddy, M. M. and ten Bruggencate G., Changes of intracellular sodium and potassium concentrations in frog spinal motoneurons induced by repetitive synaptic stimulation. *Neuroscience*, 7 (1982) 3213–3220.
- 15 Haas, H. L., Lithium and synaptic transmission in the mammalian brain. In H. M. Emrich, J. B. Aldenhoff and H. D. Lux (Eds.), *Basic Mechanisms in the Action of Lithium*, Excerpta Medica, 1982, pp. 71–79.
- 16 Hullin, R. P., The effects of lithium on electrolyte balance and body fluids. In F. N. Johnson (Ed.), *Lithium Research and Therapy*, Academic Press, 1975, pp. 359–380.
- 17 Israel, Y., Kalant, H. and Le Blanc, A. E., Effects of lower alcohols on potassium transport and microsomal adenosine-triphosphatase activity of rat cerebral cortex. *Biochem. J.*, 100 (1966) 27–33.
- 18 Janka, Z., Szentivanyi, I. and Rimannoczy, R., The influence of external sodium and potassium on lithium uptake by primary brain cell cultures at 'therapeutic' lithium concentration. *Psychopharmacology*, 71 (1980) 159–165.
- 19 Kjeldsen, C. S., Lund-Andersen, H. and Hertz, L., Effects of lithium ions in a pharmacological concentration on potassium and sodium ions in rat brain cortex slices. *Biochem. Soc. Trans.*, 1 (1973) 111–114.
- 20 Koketsu, K. and Yamamoto, K., Effects of lithium ions on electrical activity in sympathetic ganglia of the bullfrog. *Brit. J. Pharmacol.*, 50 (1974) 69–77.
- 21 Krnjevic, K. and Morris, M. E., Factors determining the decay of K^+ -potentials and focal potentials in the central nervous system. *Canad. J. Physiol. Pharmacol.*, 53 (1975) 923–934.
- 22 Lee, K., Oliver, M., Schottler, F. and Lynch, G., Electron microscopic studies of brain slices: the effects of high-frequency stimulation on dendritic ultrastructure. In G. A. Kerkut and H. V. Wheal (Eds.), *Electrophysiology of Isolated Mammalian CNS Preparations*, Academic Press, 1981, pp. 189–211.
- 23 Myers, J. B., Morgan, T. O., Carney, S. L. and Ray, C., Effects of lithium on the kidney. *Kidney Int.*, 18 (1980) 601–608.
- 24 Nakajima, S. and Takahashi, K., Post-tetanic hyperpolarization and electrogenic Na^+ -pump in stretch receptor neurone of crayfish. *J. Physiol. (Lond.)*, 187 (1966) 105–127.
- 25 Nicoll, R. A., Dorsal root potentials and changes in extracellular potassium in the spinal cord of the frog. *J. Physiol. (Lond.)*, 290 (1979) 113–127.
- 26 Obara, S. and Grundfest, H., Effect of lithium on different membrane components of crayfish stretch receptor neurons. *J. gen. Physiol.*, 51 (1968) 635–654.
- 27 Partridge, L. D. and Thomas, R. C., The effects of lithium and sodium on the potassium conductance of snail neurones. *J. Physiol. (Lond.)*, 254 (1976) 551–563.
- 28 Paul, H. A., Pilz, H. and Munz, E., Der Lithiumgehalt im Liquor cerebrospinalis bei Therapie mit Lithiumsalzen. *Nervenarzt*, 44 (1973) 210–211.
- 29 Reiser, G. and Duhm, J., Transport pathway for lithium ions in neuroblastoma \times glioma hybrid cells at 'therapeutic' concentrations of Li^+ . *Brain Research*, 252 (1982) 247–258.
- 30 Ritchie, J. M. and Straub, R. W., The hyperpolarization which follows activity in mammalian non-medullated fibres. *J. Physiol. (Lond.)*, 136 (1957) 80–97.
- 31 Ritchie, J. M. and Straub, R. W., Phosphate efflux and oxygen consumption in small non-myelinated nerve fibers at rest and during activity. *J. Physiol. (Lond.)*, 287 (1979) 315–327.
- 32 Saneto, R. P., Srivastava, S. K., Werrbach-Perez, K. and Perez-Polo, J. R., Lithium uptake at physiological ion concentration in a human clonal neuroblastoma cell line. *J. Neurochem.*, 34 (1980) 1523–1524.
- 33 Smith, I. C. H., The electrogenic potential in rat C nerve fibers: some effects of lithium and thallium. *J. Physiol. (Lond.)*, 294 (1979) 135–144.
- 34 Sonnhof, U., Grafe, P., Krumnikl, J., Linder, M. and Schindler, L., Inhibitory postsynaptic actions of taurine, GABA and other amino acids on motoneurons of the isolated frog spinal cord. *Brain Research*, 100 (1975) 327–341.
- 35 Sykova, E., Shirayev, B., Kříž, N. and Vyklícký, L., Accumulation of extracellular potassium in the spinal cord of frog. *Brain Research*, 106 (1976) 413–417.
- 36 Tang, C. M., Cohen, M. W. and Orkand, R. K., Electrogenic pumps in axons and neuroglia and extracellular potassium homeostasis. *Brain Research*, 194 (1980) 283–286.
- 37 Thomas, R. C., Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. *J. Physiol. (Lond.)*, 201 (1969) 495–514.
- 38 Thomas, R. C., Simon, W. and Oehme, M., Lithium accumulation by snail neurones measured by a new Li^+ -sensitive microelectrode. *Nature (Lond.)*, 258 (1975) 754–756.
- 39 Tilkian, A. G., Schroeder, J. S. and Hultgren, H., Cardiotoxicity of lithium salts. In M. R. Bristow (Ed.), *Drug-Induced Heart Disease*, Elsevier/North-Holland Biomedical Press, 1980, pp. 293–303.
- 40 Ullrich, A., Baierl, P. and Bruggencate, G. ten, Extracellular potassium in rat cerebellar cortex during acute and chronic lithium application. *Brain Research*, 192 (1980) 287–290.
- 41 Walz, W. and Hertz, L., Acute and chronic effects of lithium in therapeutically relevant concentrations on potassium uptake into astrocytes. *Psychopharmacology*, 78 (1982) 309–313.
- 42 Wraae, O., Hillman, H. and Round, E., The uptake of low concentrations of lithium ions into rat cerebral cortex slices and its dependence on cations. *J. Neurochem.*, 26 (1976) 835–843.