Lithium Distribution Across the Membrane of Motoneurons in the Isolated Frog Spinal Cord

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Abstract. Lithium sensitive microelectrodes were used to investigate the transmembrane distribution of lithium ions (Li^+) in motoneurons of the isolated frog spinal cord. After addition of 5 mmol·l⁻¹ LiCl to the bathing solution the extracellular diffusion of Li⁺ was measured. At a depth of 500 µm, about 60 min elapsed before the extracellular Li⁺ concentration approached that of the bathing solution. Intracellular measurements revealed that Li⁺ started to enter the cells soon after reaching the motoneuron pool and after up to 120 min superfusion, an intra – to extracellular concentration ratio of about 0.7 was obtained. The resting membrane potential and height of antidromically evoked action potentials were not altered by 5 mmol·l⁻¹ Li⁺.

Key words: Lithium – Ion sensitive microelectrode – Intracellular lithium – Motoneurons – Spinal cord – Frog

Introduction

The mechanism of the therapeutic action of lithium (Li^+) is still unclear. One factor involved may be an influence upon other ions which are important for neuronal functions. In this context several investigations have shown that Li⁺ can interfer with the active and electrogenic transport of Na+and K⁺-ions observable after neuronal stimulation in rabbit or rat vagus nerve (Ritchie and Straub 1957; Ploeger 1974; Smith 1979), amphibian optic nerve (Tang et al. 1980), rat sympathetic ganglion (ten Bruggencate et al. 1981), frog spinal cord (Davidoff and Hackman 1980; Grafe et al. 1981) and rat cerebellum (Ullrich et al. 1980). The interpretation of these experiments, however, remained limited with respect to the concentration of Li⁺ in the nervous tissue as a function of the application time and to the unknown extra-/intracellular distribution ratio of Li⁺. The development of a lithium sensitive ion exchanger suitable for microelectrodes (Güggi et al. 1975) now offers the possibility for obtaining these data. Thomas et al. (1975) have applied lithium ion sensitive microelectrodes (Li⁺-ISMEs) to determine Li⁺ accumulation in snail neurons. We have extended this to vertebrate neurons and used Li⁺-ISMEs to investigate the distribution of Li⁺ across the membrane of motoneurons in the isolated frog spinal cord. The aims of this project were (a) to observe the kinetics of extracellular and intracellular Li⁺ in an isolated preparation after application of the ion into the superfusion fluid. (b) To determine the time required to reach a steady state concentration of Li⁺ inside the cell. (c) To measure the steady state intra-/extracellular concentration ratio.

Methods

Experiments were performed on the frog, Rana esculenta, using the approach described in detail by Sonnhof et al. (1975). Briefly, 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 (volume 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·1⁻¹): NaCl 98.0, KCl 3.6, CaCl₂ 2.0, NaHCO₃ 12.0, glucose 10.0. The pH was continuously monitored and adjusted to 7.3 - 7.5 by means of a variable mixture of O_2 and CO_2 . The temperature of the perfusion fluid was adjusted to 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 which permitted stimulation or recording from the ventral roots. In order to facilitate the insertion of microelectrodes, a large area of the meninges including the pia was carefully removed between ventral and dorsal roots. Electrode tracks aiming at motoneurones were guided by antidromic field potentials; motoneurones were identified by antidromic invasion from the stimulated ventral roots.

Ion sensitive microelectrodes (ISMEs) with outer tip diameters of approximately $0.5-1\,\mu m$ were drawn from borosilicate theta-capillaries. Whilst applying pressure (N_2) to one channel, a drop of hexamethyl-disilazane (Sigma, München, FRG) was backfilled into the other channel. The electrode was then inserted into a horizontal heating coil (approximately 400° C) and after baking for 20 min, a drop of lithium sensitive ion exchange resin (Güggi et al. 1975) was injected into the silanized tip under microscopic control. This channel was then back-filled with $150 \text{ mmol} \cdot 1^{-1}$ LiCl. The reference barrel was filled with $4 \mod \cdot l^{-1}$ potassium acetate for conventional recording of potentials. After insertion of Ag/AgCl wires, the electrodes were sealed with wax. The resistance of the ion sensitive barrel was about $10^{11} \Omega$, while that of the reference barrel ranged between 10^7 and $5 \cdot 10^7 \Omega$ (measured with dc-current). High-impedance buffer amplifiers and differential subtraction amplifiers were used to obtain the pure ion signal and the potential of the reference barrel. Both potentials were recorded on a chart recorder that filtered out high frequency components (above 10 Hz). Fast field potentials, synaptic and action potentials were recorded with the reference barrel and displayed on an oscilloscope. The amplitude of the antidromic spike was also monitored by the chart recorder using a peak height detector (Courtice 1977).

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Usually, the calibrations for intracellular measurements were carried out using standard solutions of different LiCl concentrations (1, 3 and 5 mmol·1⁻¹) against a constant background of 100 mmol·1⁻¹ KCl plus 16 mmol·1⁻¹ NaCl or less (see below). The same standard Li⁺ concentrations in Ringer solution were used to obtain values for extracellular measurements (see Fig. 2B). This method avoided calculations using selectivity coefficients to be determined for each electrode (Thomas et al. 1975). The actual concentrations of Na⁺ in the intracellular calibration solutions were chosen by adjusting the voltage change between Ringer solution and various intracellular calibration solutions to the potential jump of the difference signal (Li_i) after withdrawal from the intracellular compartment (cf. Fig. 5).

 Li_i was calculated as a concentration, since the Ringer solution and the intracellular calibration solution had about the same ionic strength, Also the activity coefficient for Li⁺ is probably the same inside the cell as outside (for discussion see Thomas 1978). Some electrodes were tested for their selectivity coefficients against Na⁺ and Ca²⁺. Using a computer program based on the Nikolsky equation and calibration solutions containing a constant background concentration of Na⁺ and different concentrations of Li⁺, a selectivity coefficient against Na⁺ of 0.046 \pm 0.014 (mean \pm SD; n = 20) was obtained. A similar program, using different concentrations of Ca²⁺ in solutions with constant amounts of Na⁺ and Li⁺ was used to determine the selectivity coefficient against Ca²⁺ (0.18 \pm 0.08; n = 11). The Mg²⁺ interference of the Li⁺ ion exchanger resin was not tested; according to the literature it is unlikely to be important (Li⁺:Mg²⁺ = 1:0.003; Güggi et al. 1975).

The pure ion potential of extracellularly placed Li-ISMEs in the absence of Li⁺ had a certain value because of the presence of interferring ions in the extracellular space. Upon application of Li⁺, this potential (Li_e) changed according to the changing extracellular Li⁺ concentration. Intracellularly, a similar ion potential was observed in the absence of Li⁺; ("apparent" Li_i = $E_{Li} - E_m$; E_{Li} = voltage of ion barrel, E_m = voltage of reference barrel). This "apparent" Li_i is set by the intracellular activities of interferring ions, and may change even in absence of Li⁺ (see Fig. 2). In particular, an alteration of the Na⁺ activity is the most important factor.

Results

Extracellular Li⁺ Distribution

Experiments were performed with extracellularly placed Li-ISMEs in order to quantify the entry of Li⁺ into the spinal cord as a function of time. Figure 1 illustrates changes of Li_e at a depth of 500 µm below the lateral surface of the cord (location of the motoneuron pool); the time course of Li⁺ changes in the bathing chamber is shown to the left. Although a constant Li⁺ level in the bath was reached within 2 min, it took about 60 min until Lie within the motoneuron pool approached the applied concentration of $5 \text{ mmol} \cdot l^{-1}$. Even after such a long Li⁺ application, Li_e was still about $0.5 \text{ mmol} \cdot 1^{-1}$ less than the bath concentration. Observations from 5 different spinal cord preparations have shown that a latency of 1-2 min passed between the time of Li⁺ entrance at the surface and a measurable Li⁺ signal at a depth of 400-500 µm; Li_e then increased to $3.58 \pm 0.34 \text{ mmol} \cdot 1^{-1}$ (mean \pm SD; n = 5) after 30 min and to 4.33 \pm 0.47 mmol·1⁻¹ (n = 3) after 60 min of application.



Fig. 1. Extracellular Li⁺ transients in the isolated frog spinal cord. Changes in bath and tissue Li⁺ concentration induced by steplike addition of $5 \text{ mmol} \cdot 1^{-1}$ LiCl to the superfusate (time indicated by bars; corrected for the delay caused by the tube system). The electrode position in the tissue was 500 µm below the lateral surface of the spinal cord within the motoneuron pool

Li⁺ Distribution across the Motoneuron Membrane

Before describing intracellular Li⁺ measurements, it is necessary to report some of our general observations with the Li⁺-ISME in the intracellular fluid. Due to the sensitivity of the ion exchanger to Na⁺ and Ca²⁺, and the existing concentration gradients for those ions, a voltage jump of the difference signal ("apparent" Li_i) of up to 50 mV could be observed upon impalement of a cell. Additionally, changes in the membrane potential (E_m) and the height of the antidromically evoked action potentials (a. AP) were accompanied by voltage shifts of "apparent" Li, Such effects were frequently seen in the period following the impalement; one typical example is illustrated in Fig. 2A. At the beginning of the recording (1 min after impalement) E_m was -63 mV and the a. AP was 75 mV. E_m then slowly rose to -73 mV and simultaneously the height of the a. AP increased first to 86 mV and was then blocked. In this situation, the antidromic stimulation induced a ventral root EPSP (Sonnhof et al. 1977). An a AP of 92 mV could only be elicited when superimposed on a spontaneous synaptic depolarization. Parallel to the increase in resting membrane potential and a. AP amplitude, a voltage shift of the Li⁺-ISME of 15 mV occurred, probably due to the outward pumping of Na⁺ during the "sealing in" of the electrode. Opposite, but similar changes in the Li,-voltage were observed when the membrane depolarized as a result of cell deterioration. Therefore, we restricted our analysis of intracellular Li⁺ concentrations to neurons, which had stable E_m and a. AP. Maximally, a change of 5 mV in the E_m and 10 mV in the a. AP was tolerated (16 motoneurons from 15 spinal cord preparations).

Figure 3 illustrates a typical experiment showing the transmembrane Li⁺ movement into a motoneuron. After the impalement of the cell, we measured the intracellular Li⁺ accumulation after application of 5 mmol $\cdot 1^{-1}$ Li⁺ into the bathing solution (Fig. 3C). After about 2 min (range 2– 5 min) Li⁺ entered the motoneuron, whilst E_m (Fig. 3D) and the height of the a. AP (Fig. 3E) remained unchanged. Thirty minutes later the impalement became unstable and the electrode was withdrawn from the cell (not illustrated). This same electrode was then positioned extracellularly close to the recorded neuron and, after a washing period of about 1 h, Li⁺ was reapplied. The kinetics of the extracellular Li⁺ diffusion was then recorded (Fig. 3B).



Fig. 2A and B. Intracellular performance and calibration of Li⁺-ISME. (A) Changes in the ion signal (Li_i), resting membrane potential (E_m), and antidromic spike (a. AP) during the period following impalement of a motoneuron. Simultaneous to the increase in E_m and the a. AP amplitude, a voltage shift of "apparent" Li_i occurred. (The *asterisk* after Li_i points to the fact that no Li⁺ is present.) This is probably caused by a decrease in the intracellular Na⁺ concentration since recordings with Na⁺-ISMEs showed the same behaviour (unpublished). The ventral roots were stimulated every 10s; towards the end of the trace the a. AP did not invade the soma of the motoneuron. Figures above the a. AP record give the actual size of the a. AP (mV) as read from the oscilloscope. The upward deflections on the E_m -trace are spontaneously occurring synaptic potentials; a. APs are not visible due to the slow frequency response of the pen recorder. (B) Voltage response of a representative Li⁺-ISME with different concentrations of Li⁺ (0, 1, 3 and 5 mmol·l⁻¹) added to the normal Ringer solution (a) and to an "intracellular calibration solution" containing 100 mmol·l⁻¹ KCl and 16 mmol·l⁻¹ NaCl (b). Due to the sensitivity of the ion exchanger to Na⁺ and Ca²⁺, a voltage jump of 35 mV occurred upon changing from solution a to b. Also, the electrode was more sensitive to Li⁺-ions in the low Na⁺- and low Ca²⁺-background solution b



Fig. 3A—E. Li⁺ entry into a motoneuron. Traces (A) and (B) illustrate the reference signal (*ref*) and the difference signal (Li_e) of a Li⁺-ISME positioned extracellularly at a depth of 450 µm below the lateral surface of the spinal cord. The following 2 traces (C, D) are the corresponding signals obtained with the same electrode inside a motoneuron (depth 440 µm). The upward deflections on the E_m -trace are spontaneously occurring synaptic potentials. A record of the amplitude of antidromic action potentials (a. AP), as elicited by stimulation of the ventral roots (0.1 Hz) is shown in E. The cell was lost 28 min after the start of Li⁺ superfusion (5 mmol·1⁻¹, exchanged for NaCl and indicated by arrow and vertical line)

In several other experiments we have observed Li_i after changing from Li^+ Ringer to normal Ringer solution. After a *short* application (15-20 min), Li_i still increased for several



Fig. 4. Extra- and intracellular Li⁺ transients upon brief periods of Li⁺ application. LiCl (15 mmol $\cdot 1^{-1}$, exchanged for NaCl) was applied via the bathing solution at the time indicated by the bar. In this figure, the Li_i calibration is given in both mmol $\cdot 1^{-1}$ and mV, thereby revealing that the sensitivity of the Li⁺-ion exchanger is much greater in a solution with an ionic background similar to the intracellular fluid. The registration of the membrane potential is superimposed by synaptic potentials induced by dorsal root stimulation (0.05 Hz). The extracellular Li⁺ measurement was obtained during a second Li⁺ application after withdrawal of the electrode from the motoneuron (depth 480 µm) to a place close to the neuron (depth 430 µm)

minutes despite removal of the ion from the superfusion fluid; about twice the application time was required until Li⁺ was completely removed from the cell (see Fig. 4). In 2 other preparations we impaled motoneurons after Li⁺ had been applied for up to 5h. In these cases, after changing back to normal Ringer 3-5 min elapsed before Li_i started to fall and reached the zero level about 120 min later.



Fig. 6. Intracellular Li⁺ levels after long lasting Li⁺ applications. Summary of 5 motoneurons from experiments in which Li, was observed for application times of 60-90 min. On the left side the depth of the motoneuron, the resting membrane potential (E_m) and height of the antidromically evoked action potential (a. AP: bl. = antidromic invasion blocked) at the beginning and end of each recording are given. The traces illustrate the kinetics of the intracellular Li⁺ levels redrawn from the original data. The values at the end of the traces give the intracellular Li⁺ levels at the end of the measurement. The continuous application of 5 mmol·1⁻¹ LiCl, either added to the Ringer solution (3 cells) or exchanged for NaCl (2 cells), was started at the vertical line

Steady State Measurements

Our longest intracellular recording is illustrated in Fig. 5 and shows that even after an application time of 120 min Li, did not reach a steady state. The ratio between Li, and Li, at this time was 0.7. Observations on 5 different motoneurons, recorded for 60-90 min, are summarized in Fig. 6. In 2 of these examples a stable intracellular Li⁺ level could be

Fig. 5

Steady state distribution of Li_i. After impalement of the motoneuron and a control period, LiCl $(5 \text{ mmol} \cdot l^{-1}, \text{ exchanged for NaCl})$ was continuously perfused through the bathing chamber, resulting in an increase of Li_i. At the same time, the membrane potential (E_m) remained stable (the antidromic action potential was blocked, not illustrated). After about 120 min, the recording became unstable and the electrode was withdrawn from the cell and placed into the bathing solution, which still was a Ringer solution with $5 \text{ mmol} \cdot l^{-1}$ LiCl. At the arrow (w) the LiCl solution was exchanged for normal Ringer. Then the ISME was calibrated using concentrations of 0, 1, 3 and 5 mmol $\cdot l^{-1}$ LiCl in solutions containing 100 mmol $\cdot l^{-1}$ KCl and 16 mmol $\cdot l^{-1}$ NaCl (a) and 100 mmol $\cdot l^{-1}$ KCl and 8 mmol $\cdot l^{-1}$ NaCl (b). The intracellular Li⁺ concentration after 120 min was calculated as $3.6 \text{ mmol} \cdot 1^{-1}$. For this calculation the calibration b was used because the baseline was identical to the intracellular level. Potentials in the E_m -trace during the calibration procedure are junction potentials at the bath ground (Ag/AgCl)

observed, whereas in the remaining neurons Li, was still increasing after 90 min superfusion. However, in all cells studied so far, Li, did not exceed the extracellular Li⁺ concentration. Our measurements also showed that Li+ (5 mmol·1⁻¹) did not alter E_m and a. AP. Summarizing our longest intracellular recordings, E_m was $-67.0 \pm 3.8 \text{ mV}$ (mean \pm SD, n = 7) before and $-68.3 \pm 5.8 \text{ mV}$ (n = 7) after superfusion of 5 mmol·1⁻¹ Li⁺ for 30–120 min. The corresponding data for the a. AP are $84.0 \pm 15.2 \text{ mV}$ (n = 4) before and $85.0 \pm 20.0 \text{ mV}$ (n = 4) after Li⁺ superfusion. In the remaining 3 motoneurons the a. AP was blocked.

Discussion

We have used Li⁺-ISMEs (Güggi et al. 1975; Thomas et al. 1975)in order to determine the kinetics and the steady state intracellular Li⁺ concentration in frog motoneurons. Results based on this method are reliable, with respect to the Li⁺ kinetics, since the response time of the electrodes is much faster than the Li⁺ kinetics. On the other hand, the validity of the quantitative analysis is limited due to the partial sensitivity of the Li⁺ exchanger to other interferring ions. In particular, Na⁺ and Ca²⁺ (Güggi et al. 1975) have to be taken into account for (a) the composition of the intracellular calibration solution and (b) possible changes of the intracellular activities of these ions during the action of Li⁺. Our intracellular calibration solutions contained $100 \text{ mmol} \cdot l^{-1}$ KCl and 16 or $8 \text{ mmol} \cdot l^{-1}$ NaCl. The concentration of K⁺-ions is based on data obtained with K⁺-ISMEs in frog motoneurons (Sonnhof and Bührle 1981) for the membrane potential range of -60 to -75 mV, to which our analysis was restricted. The concentration of Na⁺-ions has been chosen by comparing the voltage jump of the difference signal (Li_i) during withdrawal after an impalement and the change from the Ringer to the intracellular calibration solutions (see Fig. 5). However, the unknown composition of the intracellular fluid remains a source of error.

A second problem is a possible change in the activity of intracellular Ca²⁺- and /or Na⁺-ions during the action of lithium. Aldenhoff and Lux (1980) reported that application of $1-40 \text{ mmol} \cdot 1^{-1}$ LiCl resulted in a concentration -

independent elevation of intracellular Ca^{2+} by 100 nmol $\cdot l^{-1}$ in snail neurons. Such an increase would not result in a measurable potential change with the Li⁺-ISMEs we used. On the other hand, as described in the results, we frequently observed voltage shifts of the Li⁺-ISME during changes in the membrane potential and/or the amplitude of the antidromic action potential. This may result from changes in intracellular Na⁺-concentration. Therefore, our analysis was restricted to motoneurons which had stable membrane potentials and constant action potential amplitudes. Li⁺ itself $(5 \text{ mmol} \cdot 1^{-1})$ seemed not to alter these parameters. In spite of possible small errors due to the factors discussed above, our data obtained from motoneurons of the isolated frog spinal cord are comparable with the transmembrane distribution of Li⁺ in snail neurons (Thomas et al. 1975) and cultured neurons and glial cells (Janka et al. 1980a, b) showing a nonpassive distribution of Li⁺ with an intra- to extracellular ratio of <1. If Li⁺ is passively distributed, the ratio should be much higher, closer to the K⁺ distribution ratio. An analysis of Li⁺ levels in monkey and human brain after chronic, therapeutic, oral dosage also revealed a ratio close to 1 (Spirtes 1976). In contrast, in cultured glioma cells an intra-/ extracellular Li⁺ ratio of up to 5.6 was observed (Gorkin and Richelson 1979). The non-passive distribution of Li⁺ does not necessarily imply an active outward transport of Li⁺ by the Na^+/K^+ -pump (Ritchie and Straub 1980), since a Na⁺/Li⁺ exchange system has been shown to maintain a low intracellular Li⁺ concentration in red blood cells (Duhm et al. 1976; Ehrlich and Diamond 1980; Tosteson 1981) and possibly also in cultured neurons (Janka et al. 1980c).

The time course of the Li⁺ movement into the frog spinal motoneurons is similar to the observations in snail neurons (Thomas et al. 1975) and cultured neurons (Richelson 1977; Janka et al. 1980a, b) showing that considerable amounts of Li⁺ are measurable intracellularly within a few minutes. This may indicate that the Li⁺ induced impairment of stimulus induced electrogenic Na⁺/K⁺-transport observed in experiments involving application of Li⁺ via the superfusion fluid might involve an interaction of Li⁺ at the intracellular binding site of a mechanism, which is normally engaged in the Na⁺/K⁺-transport.

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