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DEPRESSANT ACTION OF LITHIUM AT THE CRAYFISH NEUROMUSCULAR JUNCTION: PRE- AND POSTSYNAPTIC EFFECTS

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SUMMARY

1. The effects of replacement of external sodium ions with lithium have been studied at the excitatory neuromuscular junction of the crayfish.

2. Intracellularly recorded excitatory junctional potentials fall 45% in amplitude in the first 10 min after lithium substitution, and fail irreversibly in 48–120 min.

3. The quantal content of extracellularly recorded excitatory junctional potentials declines 25–70% within the first 10 min of Li^+ exposure. During the next 40–120 min the nerve terminal potentials and quantal release at individual synapses fail simultaneously and irreversibly.

4. The mean amplitude of the spontaneous miniature excitatory junctional potentials (m.e.j.p.s) is reduced 13% by Li^+ substitution, but recovers upon restoration of sodium. The mean frequency of m.e.j.p.s rises steadily during Li^+ exposure, and continues to increase after reintroduction of Na^+.

5. The postsynaptic response to iontophoretically applied L-glutamate falls 35–40% in 10 min, but never falls below 45% of the control level. The effect on the glutamate response is completely reversible with sodium restoration.

6. The effective resistance of the postsynaptic cells is unaffected or only increased slightly by lithium substitution.

7. Thus, the primary mechanism of transmission block by lithium appears to be decreased transmitter release and inexcitability of presynaptic terminals, probably as a result of intracellular accumulation of lithium.

INTRODUCTION

The use of lithium as a psychopharmacological agent in the treatment of mania has grown extensively in recent years (Johnson, 1975). However, the cellular basis of its observed tranquillizing action remains obscure. A substantial literature exists on the neurochemical effects of lithium (Schou, 1958; Schou, 1976) and its role in excitation of nerve and muscle (Bunney & Murphy, 1976); from which two important points emerge: (1) lithium can substitute for sodium as an inward current carrier during excitation (Overton, 1902; Hodgkin & Katz, 1949; Gallego & Lorente de Nó, 1952; Uehara, 1962; Condouris, 1963; Carmeliet, 1964; Gardner & Kerkut, 1968; Hille,

1972), but (2) it is ineffectively extruded from cells by the membrane sodium/potassium pump (Keynes & Swan, 1959; Connelly, 1959; Nakajima & Takahashi, 1966; Thomas, Simon & Oehme, 1975; de Weer, 1976).

In contrast, the number of studies dealing with lithium's influence on synaptic transmission processes is rather meagre and somewhat contradictory. A general result has been that transmission may continue after replacement of Na^+ by Li^+, but is finally blocked after a period of minutes to several hours. This has been shown in the amphibian neuromuscular junction (Onodera & Yamakawa, 1966; Kelly, 1968; Benoit, Audibert-Benoit & Peyrot, 1973; Balnave & Gage, 1974; Crawford, 1975), in mammalian sympathetic ganglia (Klingman, 1966; Pappano & Volle, 1967), and the crayfish neuromuscular junction (Ozeki & Grundfest, 1967). Another consistent finding is that, after a variable latency, Li^+ substitution always increases the rate of spontaneous transmitter release (Onodera & Yamakawa, 1966; Ghosh & Straub, 1967; Kelly, 1968; Okada, 1969; Carmody & Gage, 1973; Crawford, 1975). Paradoxically, evoked release is reported to increase in the presence of lithium by some authors (Balnave & Gage, 1974; Crawford, 1975; Branisteanu & Volle, 1975), to decrease by others (Onodera & Yamakawa, 1966; Katz, Chase & Kopin, 1968; Benoit *et al.* 1973), or to be little affected (Kelly, 1968).

Two non-mutually exclusive hypotheses have been put forth to explain the decline and eventual failure of synaptic transmission after replacement of Na^+ with Li^+: (1) Lithium acts presynaptically, transmission block occurring simultaneously with failure of the nerve terminal action potential; (2) the effects are postsynaptic, Li^+ being unable or having diminished ability to substitute for Na^+ during the action of transmitter agents.

The present study examines the effects of Na^+ substitution by Li^+ on transmission at the crayfish excitatory neuromuscular junction emphasizing independent assessment of pre- and postsynaptic actions of the ion. Our results indicate that, following treatment with lithium, transmission efficacy is initially reduced by a decrease in both quantal output and postsynaptic sensitivity. Eventually, blockade occurs due to loss of nerve terminal excitability, presumably as a result of lithium accumulation.

METHODS

The experiments were performed on the abductor of the dactylopodite (opener of the claw) of the first walking leg of the crayfish *Orconectes virilis* and *Procambarus clarkii*. The dissection and experimental chamber were similar to those previously described by Dudel & Kuffler (1961). Intracellular potentials were recorded with conventional 10–20 MΩ, 3 M-KCl microelectrodes. Extracellular potentials were monitored with 0·5–3·0 MΩ, 3 M-NaCl, LiCl or KCl microelectrodes. For determination of muscle fibre input impedance, current was delivered via a 2 M K-citrate electrode with a 300 MΩ series resistor and variable ±90 V voltage source. Iontophoretic application of L-glutamate was similar to that described by Takeuchi & Takeuchi (1964). L-Glutamate solutions (1 M) were adjusted to pH 8·0 with Tris-base, KOH, or LiOH. Results obtained were independent of the type of recording or iontophoretic electrode used. Electronic signal averaging was accomplished with a Northern Scientific NS-550 Digital Memory Oscilloscope with 1024 bit memory and

25 μsec/bit temporal resolution. Averaged electronic signals and individual oscilloscope traces were photographed directly from the CRT face.

The normal bathing solution consisted of (mM): NaCl, 195; $CaCl_2$, 15; KCl, 5; $MgCl_2$, 3; Tris-maleate buffer, 10 (pH 7·0–7·2). Lithium medium was prepared by stoichiometric substitution of NaCl with LiCl. Heavy metal contamination of LiCl and LiOH was < 0·002 % by weight (Baker 'Analyzed Reagents', J. T. Baker Chemical Co., Phillipsburg, N.J.).

The bathing medium was exchanged by voiding and refilling the experimental chamber (volume < 1·5 ml) 3 or more times with the test solution, followed by continuous superfusion at ~ 3 ml min^{-1} at constant volume. In most instances, the preparation was allowed to equilibrate at least 10 min before measurements were taken. Samples of Li^+ bathing medium taken from the chamber after the standard exchange procedure showed no detectable Na^+ when vaporized in a Bunsen flame and compared to a 'standard' solution containing 194 mM-Li^+ and 1 mM-Na^+. Experiments were conducted at room temperature, 20–23 °C.

RESULTS

Each fibre of the crayfish opener muscle receives multiterminal innervation from the same excitatory axon (Wiersma, 1961). In addition, electrotonic decrement over the entire length of a single muscle fibre rarely exceeds 40 % (Dudel & Kuffler, 1961; Ortiz, unpublished observation). Thus, the intracellularly recorded excitatory junctional potential (e.j.p.) represents the integrated activity of many individual synaptic junctions widely distributed over the muscle fibre. Consequently, amplitude fluctuations of the e.j.p. reflect not only the statistical nature of transmitter release at individual junctions, but also some spatial attenuation of signals arising at junctions located at different distances from the recording site. Typical records of the intracellular e.j.p. are shown in Fig. 1 A. Three responses are shown in each column, and some variability in amplitude is evident. The e.j.p.s in this experiment failed after 48 min in Li^+ medium and did not recover after 44 min in Na^+. In order to quantify the change in e.j.p. amplitude with time, the mean amplitude was determined by signal averaging the responses to 100 stimuli applied at 1–3 s^{-1}. These values are plotted for three different nerve-muscle preparations (Fig. 1 B). Lithium was introduced at $t = 0$ and averages taken at 10–20 min intervals until transmission failure (i.e. no response to 100 stimuli). After failure, Na^+ medium was re-introduced and averages taken for up to 45 min. The average amplitude of the e.j.p. declined ~ 45 % within 10 min after exposure to Li^+ and thereafter declined at a slower rate; transmission failure occurring 40–120 min after introduction to Li^+. Under these conditions, the average time to failure was 81·3 ± 23·3 min (± S.D., $N = 6$). Transmission was not restored during the Na^+ 'recovery' period. In preparations similarly dissected and stimulated in Na^+ media, the e.j.p.s could be reliably evoked for up to 6 h.

It was thought that failure of transmission might be due to accumulation of Li^+ which entered during presynaptic action potentials, as opposed to passive entry, although both may occur. To distinguish between these alternative routes of entry of Li^+, we tried stimulating the presynaptic nerve fibres continuously, instead of only during occasional tests as above. When continuous 50 ms, 100 Hz trains were applied

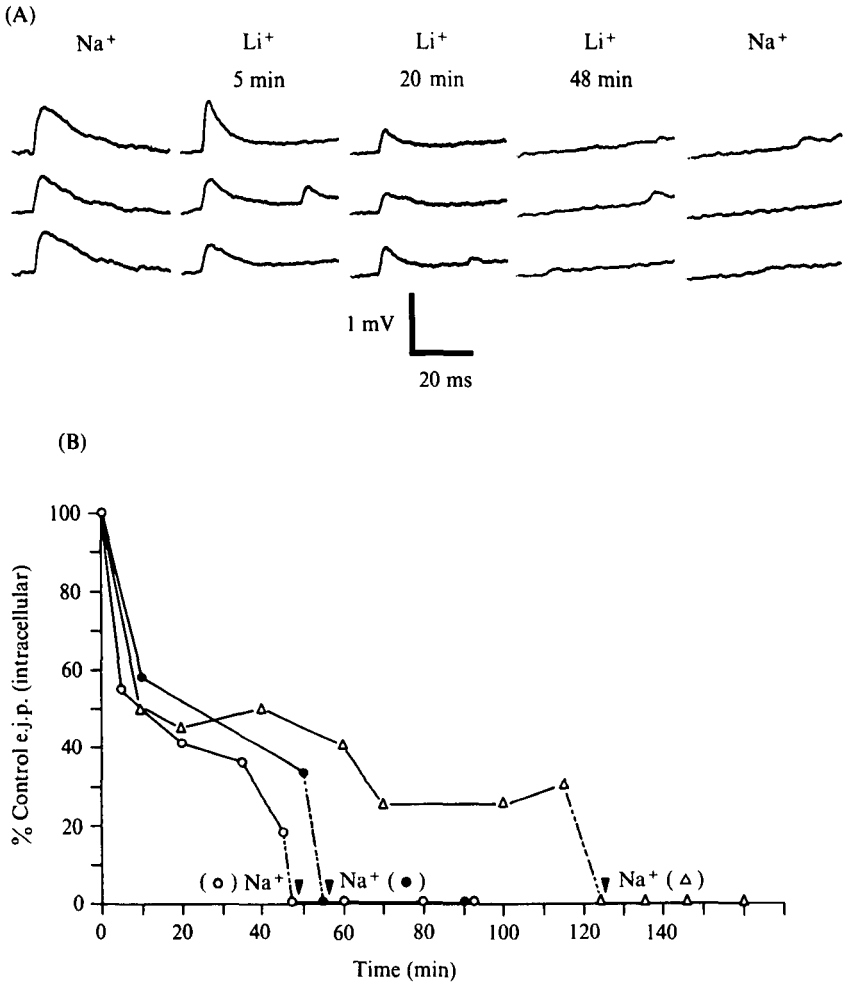

Fig. 1. Effect of Li^+ replacement on e.j.p. amplitude (A) Traces of individual intracellular e.j.p.s recorded from a single muscle fibre during decline and failure following Li^+ exposure. Transmission failure occurred after 48 min in Li^+ (no response to 100 stimuli on memory oscilloscope; not shown). Re-exposure to Na^+ for 45 min did not restore transmission. Stimulus frequency 1 Hz. (B) Amplitude of average e.j.p. (as percentage of pre-Li^+, Na^+ control) in three experiments. Zero-time represents Na^+ control amplitude and exposure to Li^+ medium. Subsequent to response failure, Na^+ was reintroduced (arrows) in each experiment. Interrupted line indicates transmission failure between measurements. Stimulus frequency 1 Hz.

at 1 s^{-1} in Li^+ medium, the average time to failure decreased to $32{\cdot}5 \pm 11{\cdot}1$ min ($N = 6$). This stimulus produced many more action potentials in the presynaptic terminals than our previous method, and the result suggested that lithium entry during action potentials contributed significantly to transmission block. Parenthetically, during continuous stimulation with pulse trains in Li^+ and Na^+ medium we often observed the type of long-term facilitation described by Sherman & Atwood (1971).

The observed effects of Li^+ substitution on the intracellular e.j.p., however, provide little information as to the site(s) of Li^+'s action since the magnitude of the intracellular e.j.p. depends on several factors: (1) quantity of transmitter released pe

impulse by presynaptic terminals; (2) sensitivity of postsynaptic receptors; (3) magnitude of net inward postsynaptic ionic current resulting from transmitter action; and (4) the passive electrical properties of the muscle fibre. Several other experimental techniques were employed in an attempt to differentiate pre- and postsynaptic effects of Li^+ substitution.

Presynaptic effects

Transmitter release. When an extracellular microelectrode is positioned within a few microns of a junctional region of a muscle fibre it is possible to record the change in the extracellular electrical field produced by current flow through both the presynaptic nerve terminal membrane during the action potential and the postsynaptic membrane during transmitter action (del Castillo & Katz, 1956; Dudel & Kuffler, 1961). Junctional areas were located by probing a fibre surface with a low resistance (0·5–3·0 MΩ) microelectrode while stimulating the excitatory axon at 1–5 s^{-1}. When a synaptic region was well localized, 100 responses were simultaneously averaged and photographed. To check recording stability, the bath was exchanged using normal Na^+ medium and the recording repeated. If the average amplitude of the extracellular e.j.p. from three such trials varied by $\leqslant$ 10%, the Na^+ bathing solution was replaced by Li^+ medium and the recording procedure repeated at approximately 10 min intervals. No stimulation was applied between recording runs. The preparation was re-exposed to Na^+ when no response to 100 stimuli was detected on the memory oscilloscope. The recording procedure was continued throughout the recovery period. The series of individual oscilloscope traces in Fig. 2A are taken from a typical experiment and demonstrate two important effects observed during Li^+ exposure: (1) an increase in the number of instances in which the nerve impulse failed to release transmitter and (2) an abrupt failure in transmission (in this instance after about 80 min of Li^+ exposure). Quantitatively, the former is expressed as the change in the mean number of transmitter quanta released per impulse, i.e. mean quantal content, $m_0 = \ln (N/n_0)$, where N is the number of nerve impulses and n_0 the number of times these impulses failed to release transmitter (del Castillo & Katz, 1954). Two criteria were used to ensure that the increased number of transmission failures observed immediately after Li^+ exposure was not due to conduction block at the nerve terminal: (1) the extracellularly recorded excitatory nerve terminal potential (e.n.t.p.) was discernible in individual traces and/or (2) the averaged amplitude of the e.n.t.p. remained unchanged from its pre Li^+ control value. Fig. 2B shows the change in m_0 (expressed as a percentage of its pre Li^+ control value) during the course of four experiments. The mean quantal content, m_0, decreased 25–70% within 10 min after Li^+ exposure, but then remained relatively stable until transmission failed abruptly due to failure of impulse conduction at the nerve terminal (i.e. no detectable e.n.t.p. or e.j.p. in response to 100 stimuli on the memory oscilloscope). In several experiments in which the e.n.t.p. was large no change in its amplitude was seen during the first 10–20 min of Li^+ exposure although quantal content had substantially decreased during this period. With continued exposure, however, e.n.t.p. amplitude usually declined by 20–50% prior to failure. That this decline did not result from small movements of the microelectrode or muscle was evidenced by the fact that average amplitudes of spontaneous m.e.j.p.s declined only slightly during Li^+ exposure.

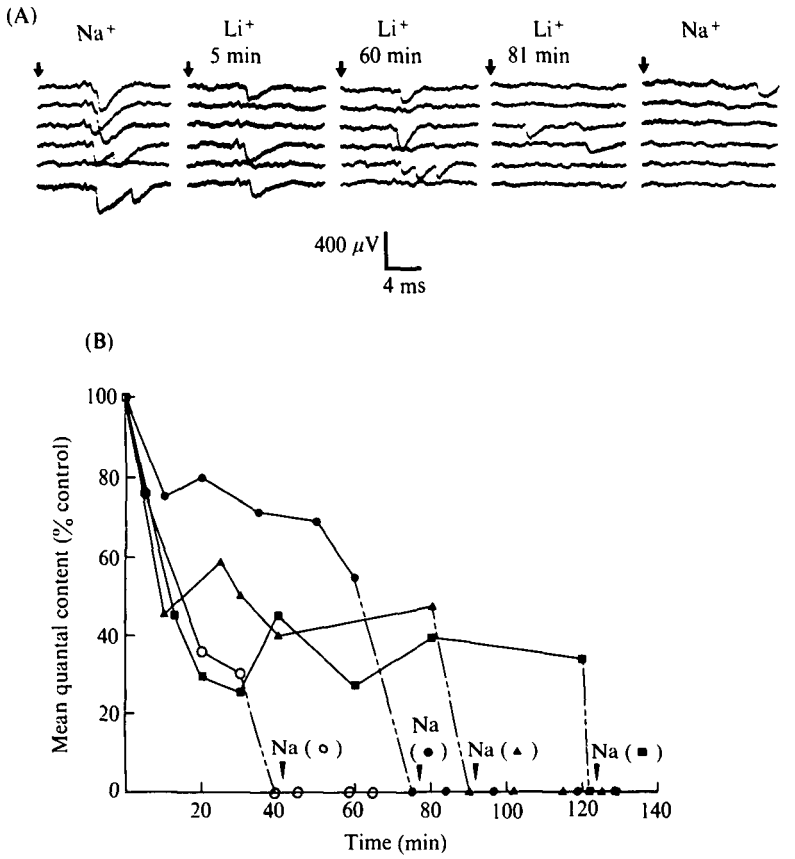

Fig. 2. Effect of Li^+ replacement on extracellular e.j.p. (A) Individual responses recorded from a single junctional area before, during and after 85 min exposure to Li^+. The proportion of transmission failures increased within 10 min with abrupt, irreversible failure occurring after 81 min. Arrows indicate nerve stimulus. Stimulus frequency 5 Hz. (B) Mean quantal content, $m_0 = \ln N/n_0$, as % Na^+ control (ordinate) during course of four experiments. Li^+ media was introduced immediately after Na^+ control measurement at $t = 0$. Transmission failed abruptly (interrupted line between points) and irreversibly in all experiments but time to failure varied widely among junctions. Arrows indicate reintroduction of Na^+ medium immediately after failure. Stimulus frequency 1–5 Hz.

Time to conduction block varied greatly among individual nerve terminals, but usually occurred 30–120 min after replacement of Na^+ with Li^+. After failure, re-introduction of Na^+ (arrows) for up to 48 min did not restore excitability (Fig. 2). Not surprisingly, activity was occasionally seen at individual terminals after the intracellular e.j.p. in the same muscle fibre had become unmeasurably small, and conversely, individual terminals were seen to fail with no obvious concomitant reduction in the intracellular e.j.p. These observations support the conclusion that the decline and eventual failure of the intracellular e.j.p. results from depressed quantal output and differential failure rates at individual junctions.

Average unit response. The average unit response V_1, $V_1 = \bar{V}/m_0$, where $\bar{V}$ is the average extracellular e.j.p. amplitude (obtained by signal averaging), in three experiments declined 17·7 (± 15·9 S.D.) % from control values during Li^+ exposure. The

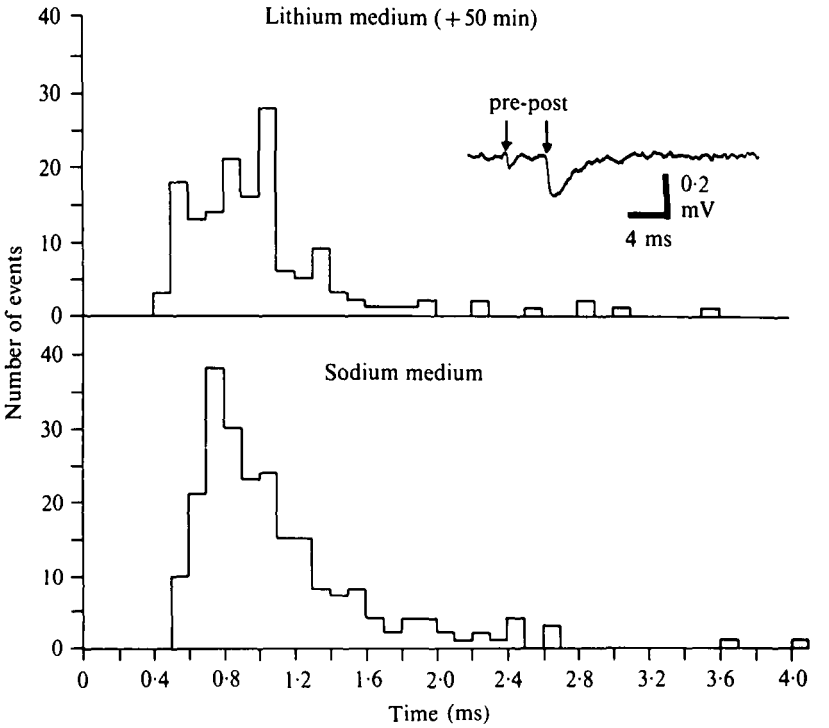

Fig. 3. Effects of Li^+ replacement on synaptic delay. Synaptic delay histograms of extracellular e.j.p.s in Na^+ and after 50 min in Li^+. Mean latencies are $1{\cdot}07 \pm 0{\cdot}48$ (S.D.) and $0{\cdot}97 \pm 0{\cdot}45$ (S.D.) ms for Na^+ and Li^+, respectively. The difference is not statistically significant. The inset indicates delay measurement criteria in a particularly long latency event.

reduction in V_1 suggests that during Li^+ exposure the postsynaptic response to transmitter is somewhat diminished. Nevertheless, the signal-to-noise ratio in all experiments remained sufficiently large that distinguishing response from failure was unequivocal and thus introduced no error into the calculation of m_0.

Synaptic delay

The amplitude of the intracellular e.j.p. is dependent on both the time course of transmitter release at individual nerve terminals and the near synchronous discharge of endings distributed over the muscle fibre. No significant change in synaptic delay or mean conduction delay (measured from stimulus artifact to e.n.t.p.) was observed upon replacement of Na^+ by Li^+ or during Li^+ exposure. The mean synaptic delays in four experiments were $0{\cdot}97 \pm 0{\cdot}45$ S.D. ms and $1{\cdot}07 \pm 0{\cdot}48$ S.D. ms in Na^+ and Li^+, respectively Fig. 3 typifies the results obtained. In both Na^+ and Li^+ the minimum synaptic delay was about 0·45 ms. These results indicate that Li^+ replacement does not significantly alter the time course of the transmitter release process.

Effect of ouabain Since a well-known action of lithium ions on excitable membranes is to inhibit the membrane Na-K pump, we wished to see if the lithium effect at the crayfish synapse was mimicked by another pump-blocking agent. In one preparation, we applied 10^{-4} M ouabain in Na^+ medium while stimulating the exciter axon at 1 s with 50 ms, 100 Hz trains. Initially, the e.j.p. amplitude was not greatly affected but

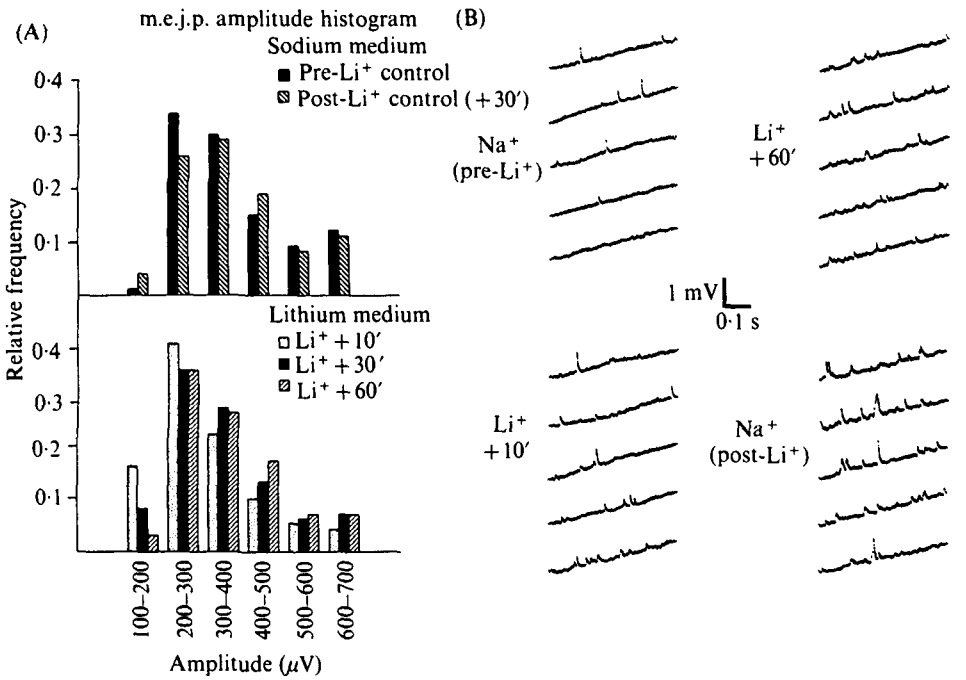

Fig. 4. Effect of Li^+ replacement on m.e.j.p. amplitude. (A) Amplitude histograms of m.e.j.p.s recorded in Na^+ (upper) and during 60 min exposure to Li^+ (lower). Spontaneous m.e.j.p.s < 100 μV are not included. Mean amplitude decreased about 13% during Li^+ exposure. (B) Individual oscilloscope traces from the same experiment. Note progressive increase in frequency throughout experiment.

with continued ouabain exposure the amplitude gradually increased due to long-term facilitation (Sherman & Atwood, 1971), with transmission failure occurring after 100 min exposure. These results were similar to those obtained in Li^+ media under these stimulation conditions and suggest the Li^+ may act by interfering with an active ion pump (see Discussion).

Postsynaptic effects

Three types of experiments were employed to assess the effects of Na^+ substitution by Li^+ on the postsynaptic membrane: (1) recording of spontaneous miniature junctional potentials (m.e.j.p.s); (2) iontophoretic application of L-glutamate; and (3) determination of current–voltage relations in single muscle fibres.

Miniature excitatory junctional potentials. Intracellular m.e.j.p.s result from spontaneous release of transmitter quanta at numerous junctions widely distributed over the surface of the muscle fibre (Dudel & Kuffler, 1961). When a microelectrode is inserted in a small diameter muscle fibre relatively large amplitude m.e.j.p.s (i.e. > 100 μV) may be recorded. Their amplitude fluctuation, like that of the intracellular e.j.p., reflects both size variation of individual quantal events and some spatial attenuation due to distance. For any given recording geometry, however, a characteristic amplitude distribution results. Thus, comparison of the amplitude histograms obtained from a single muscle fibre exposed to Na^+ and Li^+ during continuous

Table 1. *Effects of replacement of Na^+ with Li^+ on spontaneous miniature junctional potentials. Amplitude values* ± S.D.

Experiment	Evoked e.j.p. amplitude (% of control)	m.e.j.p. amplitude (μV)	%Δ	Frequency (s^{-1})
1. Control (pre-Li^+)	100	121 ± 43	0	2·21
Li^+ + 10′	54	105 ± 32	−12	2·87
Li^+ + 30′	34	102 ± 43	−15	3·09
Li^+ + 60′	(Failure)	108 ± 31	−10	3·31
Control (post-Li^+)	(Failure)	118 ± 42	−2	5·08
2. Control (pre-Li^+)	—	429 ± 133	0	0·81
Li^+ + 10′	—	361 ± 128	−16	1·83
Li^+ + 30′	—	396 ± 134	−8	2·70
Li^+ + 70′	—	411 ± 130	−3	3·60
Control (post-Li^+)	—	434 ± 138	0	3·98
3. Control (pre-Li^+)	—	122 ± 77	0	—
Li^+ + 10′	—	104 ± 56	−20	—
Li^+ + 30′	—	102 ± 56	−22	—
Control (post-Li^+)	—	154 ± 73	+25	—

recording should reveal any significant shift in the responsiveness of the postsynaptic membrane to spontaneously released transmitter. Typical results are presented in Fig. 4. The upper histogram (Fig. 4A) shows the distribution of m.e.j.p. amplitudes obtained from a single fibre in Na^+ before and after Li^+ exposure, and below, that obtained in the same fibre after 10, 30 and 60 min in Li^+. Potentials < 100 μV in amplitude were often obscured in noise and were discarded. Comparison of the histograms obtained shows that the relative frequency of small m.e.j.p.s increased slightly while that of larger m.e.j.p.s slightly decreased during Li^+ exposure Nevertheless, the peak value in both Na + and Li + occurs in the 200–300 μV range. The mean m.e.j.p. value obtained in Li + was reduced by an average of 12·3 ± 2·5 S.D. % from control (Na^+) values in this experiment. The results of three experiments are summarized in Table 1. The mean m.e.j.p. amplitude was reduced by 3–22 % (mean 13·2 ± 6·3) during Li^+ exposure and returned to near control levels upon re-exposure to Na^+. It is interesting to note that in Expt 1, in which evoked activity was monitored, while the intracellular e.j.p. diminished steadily and failed at ~ 60 min in Li^+, the mean m.e.j.p. amplitude declined only slightly during this period.

In general, the frequency of m.e.j.p.s increased progressively during Li^+ exposure (Table 1 Expts 1 and 2; Fig. 4B). Upon returning to Na^+ the m.e.j.p. frequency did not return to normal but instead usually underwent a significant rise, sometimes as much as 1·5–2·0 fold over that seen in Li^+ just prior to the bath exchange. As mentioned in the Introduction, these results are consistent with the rise in spontaneous transmitter release during Li^+ exposure observed in other preparations.

L-*Glutamate iontophoresis.* Convincing evidence supports the candidacy of L-glutamate as an excitatory transmitter in the crayfish opener muscle (Takeuchi & Takeuchi, 1964; Taraskevich, 1971; Takeuchi & Onodera, 1973). Thus, microiontophoretic application of glutamate at junctional regions provides a method of assessing the responsiveness of the postsynaptic membrane independent of presynaptic events. The basic question underlying these experiments was: is the normal depolarizing response

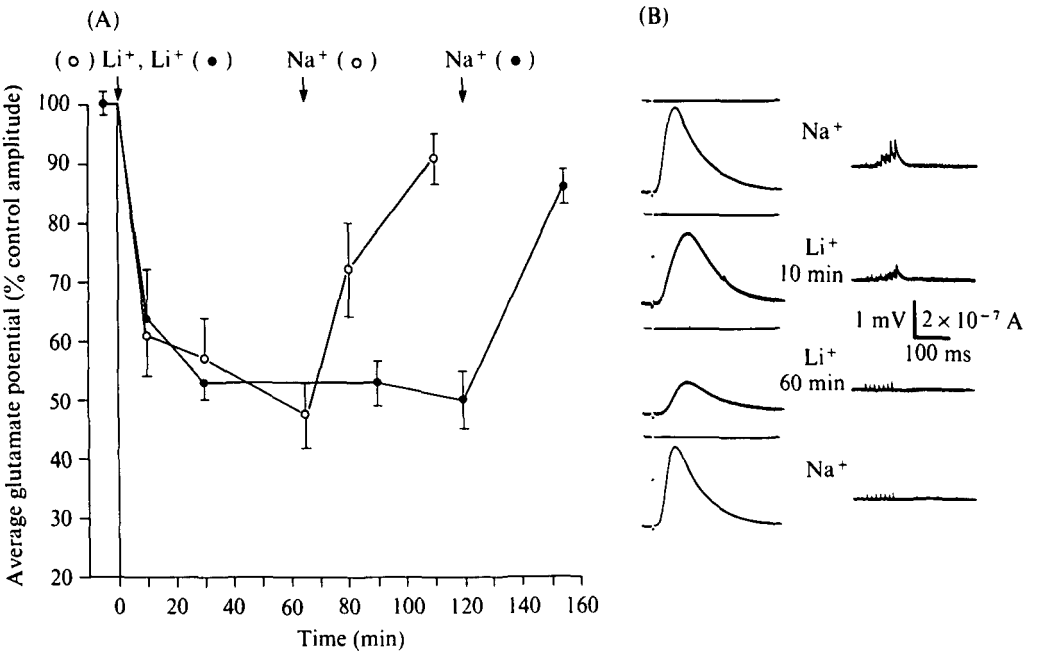

Fig. 5. Effect of Li^+ replacement on L-glutamate potentials. (A) Time course and reversibility of Li^+ effect on L-glutamate potentials. Points represent the percentage change in L-glutamate potentials from control values (±S.D.) in two experiments. Arrows indicate reintroduction of Na^+ medium in each experiment. (B) Depolarization produced by identical pulses of L-glutamate in Na^+ and Li^+ medium. Traces to the right are the responses of the fibre to a brief train of stimuli delivered to the excitatory axon 4 s before L-glutamate pulse. Note continued L-glutamate response after transmission failure at 60 min in Li^+. Nerve train stimulus frequency 100 s^{-1} for 75 ms.

of a muscle fibre to iontophoretically applied L-glutamate altered when extracellular Na^+ is replaced by Li^+?

Fig. 5 B (left traces) are examples of intracellularly recorded depolarizing potentials produced by identical pulses of L-glutamate applied to a junctional region of a single muscle fibre during exposure to Na^+ and Li^+. The trace to the right of each L-glutamate potential is the response of the same fibre to a brief train of stimuli delivered to the excitatory axon about 4 s before the glutamate pulse (note transmission failure at 60 min in Li^+, when the glutamate response is still present). After transmission failure the glutamate response is restored to near-control level by returning Na^+ to the bath while the e.j.p. remains blocked. The most obvious effect of Li^+ exposure is a proportional reduction in the amplitudes of the L-glutamate potentials produced at all intensities of iontophoretic current. The time course of this effect and its reversibility in two other experiments is seen in Fig. 5 A. The depolarization produced by a given dose of L-glutamate is reduced 35–40 % from its control (Na^+) value within 10 min after Li^+ exposure. Thereafter, L-glutamate potentials continue to decline slightly, reaching their maximum reduction (45–55 %) after about 30 min in Li^+. When preparations were re-exposed to Na^+ medium, L-glutamate potentials returned to 80–90 % of the pre-Li^+ control level within 30 min.

Fig. 6 shows the responses of a single muscle fibre to iontophoretically applied

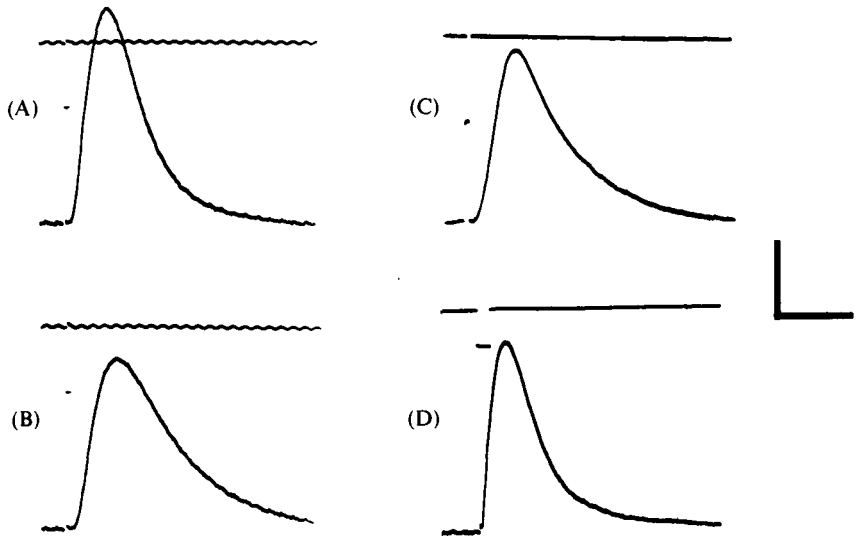

Fig. 6. Glutamate response of single muscle fibre after prolonged Li^+ exposure. (A) Response in Na^+ Ringer prior to Li^+ exposure. (B) Same glutamate-sensitive area after 4 h in Li^+. (C) Same muscle fibre (not same spot as A and B) after soaking in large volume of Li^+ medium for 24 h at 4 °C. (D) Same fibre after continued soaking, total Li^+ exposure approximately 48 h. Photos demonstrate continued postsynaptic response in Li^+ Ringer for up to 2 days. Top trace, iontophoretic current; bottom traces, membrane potential. Calibration: vertical, 1 mV and 1×10^{-7} A (A, B); 1 mV and 5×10^{-7} A (C, D); horizontal, 100 ms (A, B); 200 ms (C, D).

L-glutamate in Na^+ medium (A) and during 48 h exposure to Li^+ medium (B–D). Between (B), (C) and (C), (D), the preparation was removed from the experimental chamber and immersed in a large volume of Li^+ medium at 4 °C. After soaking, it was again placed in the experimental bath and tested for L-glutamate sensitivity. The responses are presented solely to demonstrate the continued L-glutamate sensitivity of the postsynaptic membrane after prolonged Li^+ exposure, and are not intended for quantitative comparison.

Reversal potential. Decreased postsynaptic responsiveness as measured by the diminished average m.e.j.p. amplitude, average unit response, V_1 and L-glutamate responses might reflect a change in transmitter reversal potential towards more hyperpolarized levels. To test this possibility we determined L-glutamate reversal potentials in Na^+ and Li^+ by the method of Taraskevich (1971). The results of three experiments are summarized in Table 2. In only one case was any substantial reduction of the reversal potential seen in Li^+ solution. Since the magnitude of the synaptic current depends on the difference between the resting potential and the reversal potential, it seems unlikely that the observed changes could account for the decrease of more than 50% which we found for the glutamate responses.

Effects of Li^+ on muscle membrane potential (Em). Recorded resting potentials almost always exceeded -75 mV; the typical value was close to -80 mV. In 31 experiments, substitution of Li^+ for Na^+ hyperpolarized muscle cells an average of $2{\cdot}3 \pm 1{\cdot}7$ S.D. mV within 5 min. In no instance was depolarization observed during this period. With prolonged exposure (greater than 60 min), fibres were observed to depolarize gradually by 1–15 mV. Such a biphasic response to Li^+ has also been

Table 2 *Reversal potentials of* L-*glutamate responses in sodium and lithium media (mV)*

Fibre	Na^+	Li^+ early (min)	Li^+ late (min)	Na^+ recovery
1	−26	−24 (17)	—	−26
2	0	—	−3 (60)	0
3	+12	+6 (8)	−10 (42)	+8

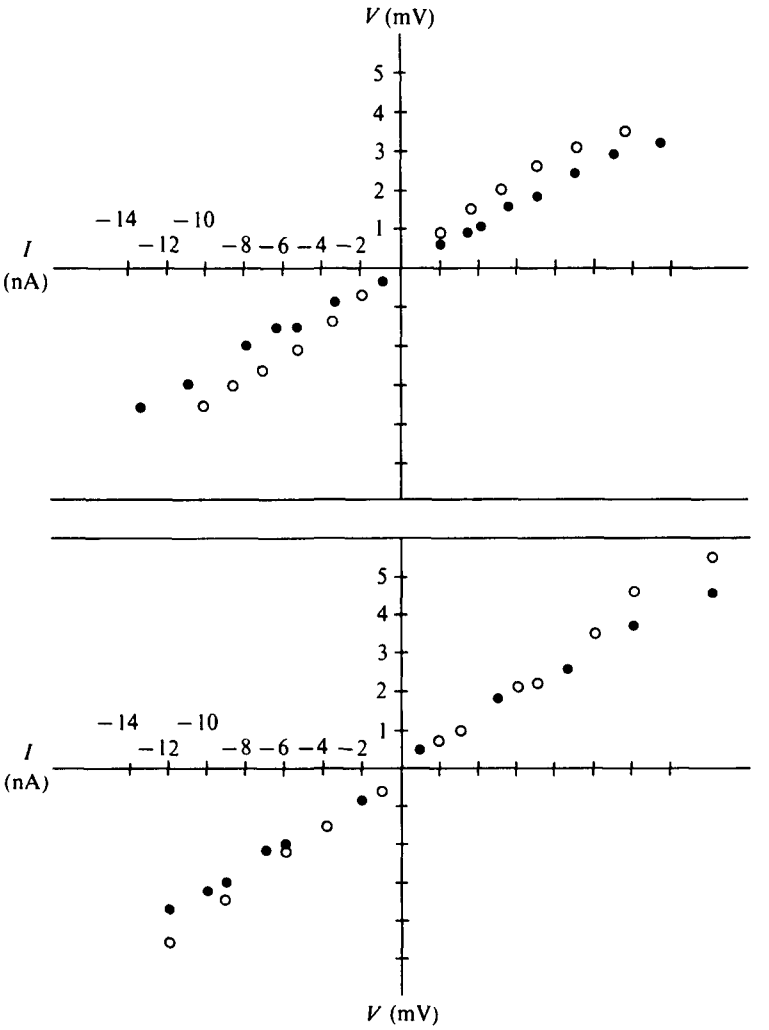

Fig. 7. *I-V* relation in Na^+ and Li^+. Steady-state *I-V* curves in Na^+ (closed circles) and after 1 h exposure to Li^+ (open circles) in two experiments. Data points for Na^+ include both pre- and post-Li^+ measurements. Experiments performed with both *I* and *V* electrodes within 200 μm of fibre centre. Electrode position unchanged during entire experiment.

observed in *Aplysia* neurones (Ono, Sato & Maruhashi, 1974). Clearly, these changes were too small to account for the observed decrease in postsynaptic sensitivity.

I-V relation in Na^+ and Li^+. The current–voltage characteristics of muscle fibres exposed to both Na^+ and Li^+ were determined in 11 experiments. The procedure consisted of recording the change in E_m produced by a square pulse of current, of known amplitude and duration, delivered to the cell interior by a K-citrate microelectrode. Fig. 7 typifies results obtained. The input resistance usually increased slightly within 1–2 min after Li^+ exposure; however, no further changes were observed during continued exposure up to 4 h. Return to Na^+ quickly shifted the slope of the *I-V* curve to within ± 10% of its pre-Li^+ values. These results cannot explain the decrease in the glutamate response or the e.j.p.; in fact, an increase in membrane resistance would be expected to increase the response to a given amount of postsynaptic current.

Effect of Li^+ at low concentration. When Li^+ is used as a psychotherapeutic agent, the plasma concentration is usually maintained at $\leqslant$ 2 m-equiv/l (Gershon & Yuwiler, 1960). It seemed reasonable to ascertain whether any obvious effect(s) on transmission at the crayfish neuromuscular junction was apparent at a similarly low Li^+ concentration. The intracellular e.j.p. was monitored in normal Na^+ medium and then during exposure to normal medium +5 mM-LiCl. In three experiments the presence of 5 mM-Li^+ in the bath for up to 70 min produced no obvious change in either the amplitude or time course of the e.j.p. The amplitude and frequency of spontaneous m.e.j.p.s were similarly unaffected. Extended periods of exposure may be required to produce detectable effects. It is interesting to note that the therapeutic action of lithium in the treatment of mania may require days to several weeks to develop when serum concentrations are maintained at standard sub-toxic levels, e.g. $\leqslant$ 2·0 m-equiv/l (Gershon & Yuwiler, 1960).

DISCUSSION

Blockade of synaptic transmission by replacement of external sodium with lithium has been ascribed both to presynaptic (Kelly, 1968; Benoit *et al.* 1973; Crawford, 1975) and postsynaptic action (Ozeki & Grundfest, 1967; Pappano & Volle, 1967). In the crayfish neuromuscular junction, Ozeki & Grundfest (1967) concluded that the observed decline and failure of transmission during Li^+ exposure was solely attributable to its postsynaptic action. This conclusion was based mainly on two experimental findings: (1) continuation of axon spikes in Li^+ during the decline and disappearance of the intracellular e.j.p. and (2) rapid decline and disappearance of L-glutamate potentials after Li^+ exposure. The former is not unexpected since any effect of intraxonal Li^+ accumulation (e.g. decreased spike amplitude and eventual conduction block) occurs more rapidly in small axon terminals than in larger trunks due to the larger surface-to-volume ratio. Thus, continued conduction in large axon trunks during Li^+ exposure does not infer similar conditions at synaptic terminals. The latter effect may reflect receptor desensitization induced by leak of glutamate from the pipette (Obata, Takida & Shinozaki, 1970).

Two factors satisfactorily explain nearly all the decline in peak amplitude of the intracellular e.j.p. and the subsequent failure of transmission which we observed

during exposure to Li^+ (Fig. 1 A, B). These are: (1) a decrease of 25–70 % in the mean quantal output of transmitter from individual nerve terminals and (2) a differential failure rate among the large terminal population of single muscle fibre (Fig. 2 B). Quantitatively similar reductions in quantal content during Li^+ exposure have been reported at the frog NMJ under conditions of partial Mg^{+2} blockage (Onodera & Yamakawa, 1966). The initial drop in transmitter output seen in Li^+ may be explained either by a decrease in nerve terminal action potential amplitude or an inhibition of some subsequent reaction(s) in the transmitter release process. Some decrement in action potential amplitude has been reported for toad myelinated fibres (Uehara, 1962), and rat cervical ganglion (Klingman, 1966) when Na^+ was replaced by Li^+. It has also been reported that the permeability of mammalian C-fibres to Li^+ is only 70 % of the value obtained for Na^+ (Armett & Ritchie, 1963). Hille (1972) has reported that although the Na^+:Li^+ permeability ratio in frog myelinated nerve fibres is 0·93, the currents measured in Li^+ Ringer are as much as 28 % lower than those seen in Na^+ suggesting some block of the Na^+ channel by Li^+. Alternatively, the reduction in action potential amplitude could result from a depolarization of the presynaptic terminals, secondary to blockage of an electrogenic pump mechanism. Such a depolarization following treatment with lithium is known to occur in the crayfish stretch receptor (Sokolove & Cooke, 1971) and *Aplysia* neurones (Junge & Stephens, 1973). Thus, action potential amplitude in presynaptic terminals could be rapidly reduced by (1) a diminution of the resting potential and (2) inactivation of the inward current-carrying mechanism. Small changes in amplitude of the action potential might result in significant changes in transmitter release. Indeed, extremely steep relations between postsynaptic response and presynaptic depolarization produced artificially (Katz & Miledi, 1970) or resulting from increased terminal action potential amplitude (Takeuchi & Takeuchi, 1962; Hubbard & Schmidt, 1963) have been reported. The extracellular recording technique used to measure nerve terminal potentials in our studies may have been insufficiently sensitive to record such small changes

Following the initial decline in transmitter output, release remained relatively stable or declined until transmission failure (Fig. 2 B). Transmission failure at single junctions always occurred simultaneously with the disappearance of the extracellularly recorded nerve terminal potential. Lithium-induced loss of tissue excitability has been reported for frog muscle (Overton, 1902); frog nerve trunks (Gallego & Lorente de Nó, 1947) and *Helix* neurones (Gardner & Kerkut, 1968). This slow blocking effect seems likely to result from accumulation of Li^+ ions in the terminals, decreasing their excitability.

After nerve terminal failure in Li^+, return to Na^+ for up to 48 min failed to restore transmission (Figs. 1 and 2). This result, however, may only indicate that the recovery periods used in the present experiment were insufficient. In other studies, the loss of tissue excitability observed during Li^+ exposure was reversed only after prolonged recovery in Na^+ (Gallego & Lorente de Nó, 1952) but in some cases blockade was irreversible (Overton, 1902; Klingman, 1966).

The increase in frequency of m.e.j.p.s seen during Li^+ exposure may have been due to depolarization of the presynaptic terminals (Del Castillo & Katz, 1954). This could result from block of an electrogenic pump or K^+ loss. Calcium may be implicated in this process, since application of Li^+ is known to stimulate Ca^{2+} entry in the squid

axon (Baker *et al.* 1969; Baker, Hodgkin & Ridgway, 1971). Alternatively, Li^+ might facilitate release of Ca^{2+} from internal stores such as mitochondria (Carmody & Gage, 1973; Crawford, 1975).

Postsynaptic sensitivity to transmitter, measured as mean m.e.j.p. amplitude (Table 1), declined 3–22% when Li^+ replaced Na^+ as the major extracellular cation. Analogous reductions have been observed at the frog NMJ under similar conditions by Onodera & Yamakawa (1966) (12%) and Benoit *et al.* (1973) (18%). Tests of postsynaptic sensitivity by L-glutamate iontophoresis (Fig. 5), however, showed a reduction of up to 45% in Li^+. The most likely explanation of this discrepancy is that glutamate responses were further attenuated due to receptor desensitization resulting from leakage of glutamate from the pipette (Obata *et al.* 1970). Two-pulse iontophoresis experiments (Ortiz, unpublished) showed that glutamate desensitization was always substantially greater in the presence of lithium ion. In a cholinergic synapse, Parsons, Schnitzler & Cochrane (1974) have shown a similar increase of receptor desensitization in the presence of Li^+. It thus appears that the postsynaptic receptors undergo less than 20% reduction in sensitivity when exposed to Li^+ and remain responsive to the transmitter for many hours after transmission failure. This reduction could be due to (1) lower permeability of the postsynaptic ionic channels to Li^+ than to Na^+ (Ozeki & Grundfest, 1967); (2) a direct inhibition of the glutamate receptor, or (3) reduction of second-messenger function of cyclic AMP by inhibition of adenyl cyclase (Forn, 1975).

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