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EFFECTS OF LITHIUM IONS ON ELECTRICAL ACTIVITY IN SYMPATHETIC GANGLIA OF THE BULLFROG

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1 The mode of action of lithium on electrical activity in the sympathetic ganglia of the bullfrog has been studied by recording extracellular and intracellular potential changes. Changes in nerve conduction and various types of synaptic transmission were studied when sodium ions in the external solution were totally replaced by equimolar concentrations of lithium ions and also when lithium ions were added to the external Ringer solution.

2 Nerve conduction and nicotinic transmission in sympathetic ganglia were completely blocked in sodium-free sucrose solution, but were restored when the preparations were transferred to a sodium-free lithium solution.

3 In the sodium-free lithium solution, the slow excitatory postsynaptic potential (e.p.s.p.) and muscarinic acetylcholine-depolarization were restored while the slow inhibitory post-synaptic potential (i.p.s.p.) and the muscarinic acetylcholine-hyperpolarization were not restored. Furthermore, the early after-discharges were accelerated and the inhibition of after-discharges was eliminated. These results support the hypothetical concept that the slow i.p.s.p. is generated by an activation of the electrogenic sodium pump.

4 In the sodium-free lithium solution, restoration of nerve conduction and synaptic transmission were transient phenomena; both conduction and transmission were gradually blocked when preparations were soaked in the solution for long periods. The blockade appeared to be due to membrane depolarization.

5 When lithium ions (20 mM) were added to the Ringer solution, nicotinic transmission was depressed. The slow e.p.s.p. was also depressed, but less so than the slow i.p.s.p. The early after-discharge was, however, accelerated; presumably due to the marked depression of the slow i.p.s.p. in this solution.

6 Changes in synaptic transmission in Ringer solution containing lithium ions could be explained by membrane depolarization, a reduction of acetylcholine release and a depression of the electrogenic sodium pump.

7 All results obtained in the present experiments could be explained by supposing that lithium ions are able to substitute for sodium ions in passive ionic membrane transport dependent on electrochemical energy but not in active ionic membrane transport dependent on metabolic energy.

Introduction

Lithium ions are known to be effective substitutes for sodium ions in maintaining membrane excitation (Hodgkin, 1951; Huxley & Stämpfli, 1951). Nevertheless, it has been reported that lithium ions do not act as effective substitutes for sodium ions in the membrane excitation processes and consequently impede synaptic transmission in tissues, such as sympathetic ganglia (Klingman, 1966; Pappano & Volle, 1966; Pappano & Volle, 1967). The present experiments were designed to re-examine these phenomena and to analyse the mechanism underlying the actions of lithium ions on various types of synaptic transmission in bullfrog sympathetic ganglia.

Methods

Paravertebral sympathetic chains were carefully isolated from bullfrogs *(Rana Catesbiana)* and used exclusively in these experiments.

Records of changes in membrane potential

Extracellular recordings. Action potentials in the preganglionic nerve trunk (which represent nerve conduction) and those in the postganglionic nerve trunk (which represent monosynaptic nicotinic transmission), were recorded extracellularly by applying single stimuli (0.5 ms pulses) to the preganglionic nerve fibre. The recording method is illustrated schematically in Figure 1a. To record postganglionic after-discharges, the arrangement shown in Fig. 1b was used. Bursts of repetitive stimuli (30 Hz) were applied to the preganglionic nerve fibre for periods indicated in the text.

The membrane potential changes in ganglion cells were also recorded by use of the sucrose-gap method (Koketsu & Nishi, 1967; Kosterlitz, Lees & Wallis, 1968). The method is shown diagrammatically in Figure 1c. Membrane potential changes in ganglion cells were induced either by applying single or repetitive stimuli to the preganglionic nerve or by adding one drop of acetylcholine (ACh) solution (0.1 M) to the bathing solution, flowing through a channel (4 x 3 x 50 mm) at the rate of 0.2 ml/second.

Records of changes in membrane potential by the sucrose-gap method were made in Ringer solution, sodium-free lithium solution or Ringer solution containing lithium chloride (up to 20 mM): no potential records were made in sodium-free sucrose solution. Single and repetitive stimuli were applied at intervals of approximately 3 s and 3 min, respectively, and ACh was applied at an interval of 5 minutes.

Intracellular recordings. Glass capillary microelectrodes filled with 3 M KCl (15-25 MΩ) were used for intracellular recording and stimulating electrodes. As shown in Fig. 1d, a single microelectrode was used both for recording and also for applying a direct stimulus to a ganglion cell (Nishi & Koketsu, 1960). In order to study the ACh-sensitivity of a single cell, ACh was applied electrophoretically by use of a glass microelectrode filled with acetylcholine chloride (2 M), as shown in Fig. 1e (Blackman, Ginsborg & Ray, 1963; Koketsu, Nishi & Soeda, 1968).

Solutions and drugs

The ionic composition of the solutions used was as follows: Ringer solution (mM): NaCl 112, KCl 2, $CaCl_2$ 1.8 and $NaHCO_3$ 2.4; sodium-free sucrose solution (mM): sucrose 224, $CaCl_2$ 1.8 and $KHCO_3$ 2; sodium-free lithium solution (mM): LiCl 112, $CaCl_2$ 1.8 and $KHCO_3$ 2. Drugs used: acetylcholine-chloride (Daiichi-seiyaku), atropine sulphate (Nakarai-Kagaku), nicotine sulphate (Katayama-Kogyo) and (+)-tubocurarine chloride (Wako-Junyaku).

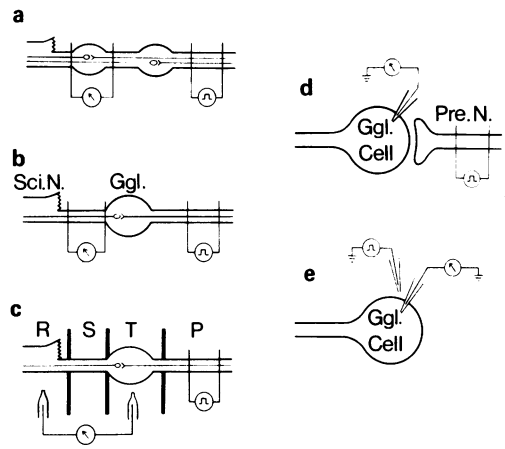

Fig. 1 Schematic drawings of experimental arrangements for recording electrical activity in the sympathetic nervous system of the bullfrog: (a) and (b) are for extracellular recordings and (c) is for the sucrose-gap method; (d) and (e) are for intracellular recording. The 9th or 10th ganglion (Ggl.) and sciatic nerve (Sci. N.) are shown in (a), (b) and (c); a ganglion cell (Ggl. Cell) and a preganglionic nerve terminal (Pre. N.) are shown in (d) and (e). Note the positions of recording and stimulating electrodes in each drawing; R, S, T and P shown in (c) are Ringer, sucrose, test and paraffin solutions, respectively.

Results

I. Sodium-free lithium solution

1. Nerve conduction. Two successive action potentials could be recorded by use of the experimental arrangement shown in Fig 1a, when a single supramaximal stimulus was applied to the preganglionic B nerve (Koketsu, 1969) fibres (Figure 2a). The initial and following diphasic action potentials were those of preganglionic and postganglionic B nerve fibres, respectively. Changes in nerve conduction in sodium-free lithium solution were studied by observing the changes in the initial action (spike) potential in this solution.

In order to ensure that extracellular sodium ions were removed, preparations were not transferred from Ringer solution directly to sodium-free lithium solution. Instead, preparations (six experiments) were first soaked in sodium-free sucrose solution for at least 30 min before being

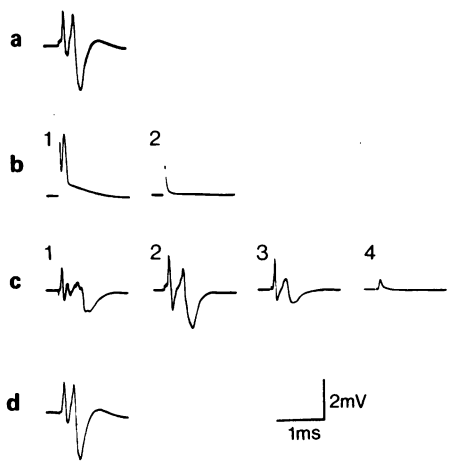

Fig. 2 Restoration of nerve conduction and nicotinic transmission in a bullfrog sympathetic nerve-ganglion preparation: (a) an action potential in the preganglionic nerve fibre (initial spike potential) and subsequent diphasic action potential in a postganglionic nerve fibre. These action potentials were produced by a single supramaximal stimulus applied to preganglionic B nerves; (b) after 20 min (1) and 30 min (2) in the sodium-free sucrose solution. Note the stimulus artifact in record 2, in which no action potential was produced; (c) after 5 min (1), 10 min (2), 30 min (3) and 90 min (4) in the sodium-free lithium solution; (d) restoration of action potential in Ringer solution.

transferred to the sodium-free lithium solution. It was presumed that extracellular sodium ions would be almost completely washed out by this procedure.

As seen in Fig. 2b, the initial spike potential of the preparations completely disappeared within approximately 30 min in the sodium-free sucrose solution. When the preparations were transferred to the sodium-free lithium solution, the initial spike potential was restored within 5 min and their amplitudes returned almost to normal within 10 min (Figure 2c).

The restoration of nerve conduction in the sodium-free lithium solution was a transient phenomenon; the initial spikes of these preparations were gradually depressed until they had completely disappeared within 90-120 min (Figure 2c). The spike potentials were completely restored by reimmersion in Ringer solution (Figure 2d).

2. Synaptic transmission.

A. Nicotinic transmission.

a) Fast excitatory postsynaptic potentials (e.p.s.p.) – Monosynaptic nicotinic transmission from pre- to postganglionic neurones is mediated by ACh producing the fast e.p.s.p. (Koketsu, 1969; Libet, 1970). The second diphasic spike potential seen in Fig. 2a is the action potential in postganglionic nerve fibres, being mediated by the fast e.p.s.p. Thus, changes in the fast e.p.s.p. can be determined by observing changes in these spike potentials in the sodium-free lithium solution.

As seen in Fig. 2b, the second spike potentials of the preparations (six experiments) disappeared in sodium-free sucrose solution within about 20 min, which was quicker than the disappearance of the initial spikes. When preparations were transferred to the sodium-free lithium solution after soaking in sucrose solution for 30 min, second spikes were restored within 5 min and their amplitude became almost normal within approximately 10 min (Fig. 2c); they then gradually diminished until they completely disappeared after about 90 minutes. Second spikes disappeared quicker than initial spikes. The failure of nicotinic transmission was reversible in Ringer solution (Figure 2d).

b) Nicotinic acetylcholine-depolarization – There is a possibility that the failure of nicotinic transmission observed in sodium-free sucrose solution is simply due to failure of nerve conduction, particularly at preganglionic nerve terminal branches. In other words, the restoration of the second spikes in sodium-free lithium solution could be simply due to the restoration of nerve conduction. Thus, it must be clarified whether or not lithium ions are able to substitute for sodium ions in the production of the fast e.p.s.p. itself.

To this end, nicotinic ACh-depolarization was produced in the presence of atropine by direct application of ACh to ganglia. Changes in the depolarization produced in sodium-free lithium solution were studied. The transient ganglionic depolarization (Fig. 3a), which can be recorded by the sucrose-gap method in the presence of atropine (0.14 mM) when a drop of ACh solution (0.1 M) is added to the perfusate, is a membrane potential change which corresponds to the fast e.p.s.p. (Koketsu, 1969; Libet, 1970). Whether or not the fast e.p.s.p. itself could be restored in the sodium-free lithium solution, was determined by observing changes in the ACh-depolarization in this solution.

Five preparations were perfused with sodium-free lithium solution after being perfused for approximately 30 min with sodium-free sucrose solution. The amplitude of the nicotinic ACh-depolarization of these preparations was very small immediately after transfer to the sodium-free lithium solution, but gradually increased during the next 20-40 min (Figure 3b).

The nicotinic ACh-depolarization was gradually depressed when these preparations were soaked in sodium-free lithium solution for more than 30-40

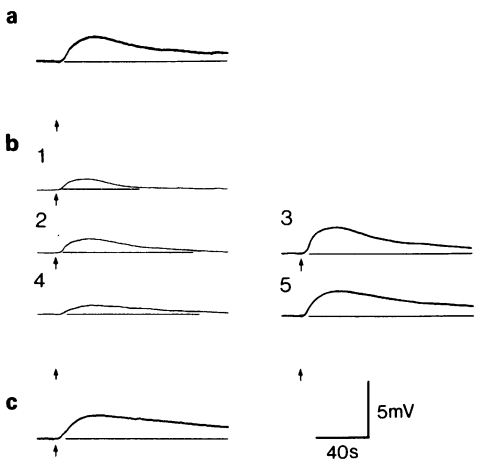

Fig. 3 Restoration of the nicotinic acetylcholine (ACh)-depolarization recorded by the sucrose-gap method: (a) in Ringer solution; (b) after 20 (1), 40 (2), 50 (3), 90 (4) and 100 min (5) in sodium-free lithium solution; the preparation was previously soaked in sodium-free sucrose solution for 30 minutes. A constant anodal current was applied in records 3 and 5; (c) restoration in Ringer solution. One drop of ACh (0.1 M) solution was added to the perfusion fluid at the arrow in each record.

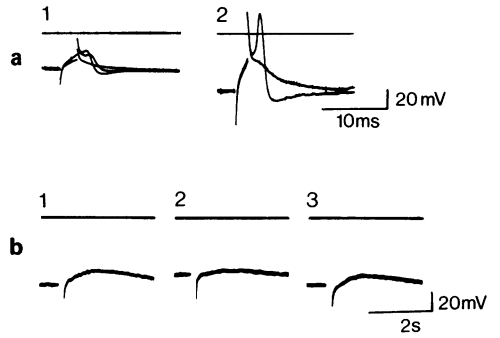

Fig. 4 (a) intracellular potential changes recorded from a preparation perfused with the sodium-free lithium solution for approximately 2 hours. The membrane was strongly depolarized and produced no action potentials when stimulated directly (1); an action potential, however, was produced when the membrane was hyperpolarized by applying a constant anodal current to the cell (2); (b) nicotinic acetylcholine (ACh)-depolarizations recorded from a single cell by iontophoretic application of ACh in the presence of atropine (0.14 mM). The nicotinic ACh-depolarization was first recorded in Ringer solution (1) and record 2 was taken approx. 90 min after the perfusate was changed to the sodium-free lithium solution; note membrane depolarization and depression of the response. Record 3 was taken immediately after record 2 while the membrane was hyperpolarized by applying a constant anodal current.

minutes. This seemed to be due to membrane depolarization in ganglion cells. Indeed, the depression was compensated when ganglion cell membranes were hyperpolarized by applying a constant artificial anodal current (see Figure 3b, 3 & 5). This suggested that the ACh-sensitivity of the ganglion cell was not depressed even when preparations were soaked for long periods in the sodium-free lithium solution.

c) Nicotinic acetylcholine-sensitivity – Changes in the nicotinic ACh-sensitivity in sodium-free lithium solution were studied by recording the intracellular potential changes of a single ganglion cell. The changes were recorded by the iontophoretic application of ACh (see methods section).

In this experiment (see Fig. 4b), ACh-depolarization was recorded from the same cell first in Ringer solution and then in sodium-free lithium solution. When the perfusion fluid was changed to the sodium-free lithium solution, the cell membrane was gradually depolarized and the size of nicotinic ACh-depolarization was decreased. Reduction of the ACh-depolarization seemed to be due to the membrane depolarization. Indeed, if the resting membrane potential was maintained at a normal level by applying a constant anodal current, the size of the ACh-depolarization remained unchanged. These results were confirmed in five different cells.

d) Action potential – Changes in the resting and action potentials of 12 ganglion cells from three ganglia in sodium-free lithium solution were studied by the intracellular recording method. As before, the ganglion cell membrane was gradually depolarized and the resting membrane potential became generally less than −50 mV within 2 h (the resting membrane potential in Ringer solution ranged between −60 and −65 mV). In this condition, no action potentials were produced by either antidromic (stimulation of postganglionic nerve trunk) or orthodromic stimulation. Action potentials, however, could be produced by direct stimulation through an intracellular electrode. The peak level of these action potentials was low and the threshold for their initiation was high, by comparison with those produced in Ringer solution. Some cells which were depolarized produced no action potentials even after powerful direct stimulation (Figure 4a,1). However, these cells could produce action potentials provided the resting membrane potential was maintained at

normal level by applying a constant artificial anodal current (Figure 4a,2).

B. Muscarinic transmission.

a) Positive potential (P-potential) and late negative potential (LN-potential) – In preparations in which nicotinic transmission was completely blocked by the presence of nicotine (0.24 mM), the P-potential and LN-potential (see Fig. 5a) could be recorded from ganglia when repetitive preganglionic stimulation was applied (Koketsu & Nishi, 1967; Nishi & Koketsu, 1968a). Both the P-potential and LN-potential are muscarinic responses which represent the slow inhibitory postsynaptic potential (i.p.s.p.) and the slow e.p.s.p., respectively (Koketsu & Nishi, 1967; Nishi & Koketsu, 1968a).

In six nicotinized preparations perfused with the sodium-free sucrose solution, both the P- and LN-potentials recorded by the sucrose-gap method were completely blocked within approximately 30 min (Figure 5b). When the perfusion fluid was changed to sodium-free lithium solution, the LN-potential was restored within 5 min and its amplitude became almost normal within 10 min (Figure 5c). The P-potential, by contrast, was never restored in this solution (Figure 5c).

The restoration of the LN-potential, like that of nicotinic transmission, was a transient phenomenon; its amplitude gradually decreased when preparations were soaked in sodium-free lithium solution for more than 30 minutes. Both the P- and LN-potentials were reversible in Ringer solution (Figure 5d).

b) Muscarinic acetylcholine-responses – Hyperpolarization followed by depolarization can be recorded by the sucrose-gap method when ACh is directly applied to nicotinized ganglia (Figure 6a). The ACh-hyperpolarization and ACh-depolarization are muscarinic responses and correspond to the P-potential and LN-potential, respectively (Koketsu, 1969). Six nicotinized preparations were perfused with sodium-free lithium solution after they had been perfused with sodium-free sucrose solution for at least 30 minutes. In the sodium-free lithium solution, the muscarinic ACh-depolarization was gradually restored whereas the muscarinic ACh-hyperpolarization was never restored (Fig. 6b); the restoration of ACh-depolarization was a transient phenomenon.

The muscarinic ACh-depolarization could be produced even after the LN-potential was completely blocked in sodium-free lithium solution. This indicated that the cause of the disappearance of the LN-potential was the depression of ACh

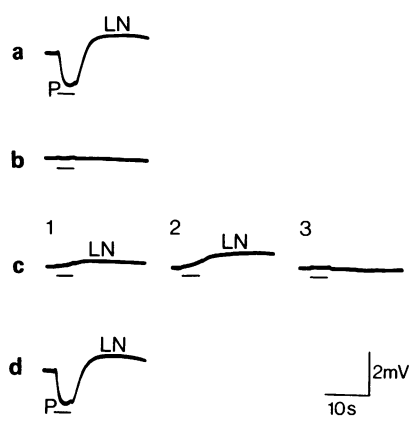

Fig. 5 Restoration of muscarinic synaptic transmission: (a) the positive-potential (P) and late negative-potential (LN) recorded by the sucrose-gap method in Ringer solution containing nicotine (0.24 mM); (b) after 30 min in sodium-free sucrose solution; (c) after 5 (1), 10 (2) and 45 min (3) in the sodium-free lithium solution; (d) again in Ringer solution containing nicotine. These potential changes were produced by applying repetitive preganglionic B nerve stimulation (30 Hz for 4 s); the duration of stimulation is shown by the horizontal line under each record.

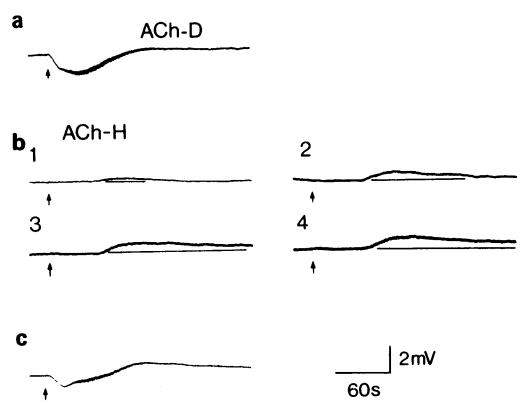

Fig. 6 Restoration of the muscarinic acetylcholine (ACh)-depolarization and disappearance of the muscarinic ACh-hyperpolarization in sodium-free lithium solution: (a) the muscarinic ACh-hyperpolarization (ACh-H) and the muscarinic ACh-depolarization (ACh-D) recorded by the sucrose-gap method in Ringer solution containing nicotine (0.24 mM); (b) after 5 (1), 15 (2), 25 (3) and 45 min (4) in the sodium-free lithium solution, following immersion in sodium-free sucrose solution for approximately 30 minutes. Note the gradual restoration of the muscarinic ACh-depolarization; (c) return to Ringer solution containing nicotine. One drop of ACh (0.1 M) solution was applied at the point marked by an arrow in each record.

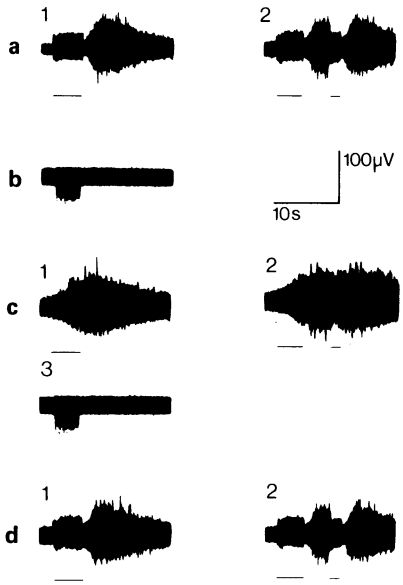

Fig. 7 Restoration of the early after-discharge and depression of the inhibition in the sodium-free lithium solution: (a) the early after-discharge (1) and its inhibition (2) in Ringer solution; repetitive preganglionic B nerve stimulation (30 Hz) was applied for 4 s to initiate the early after-discharge and it was applied for 2 s to inhibit it. The duration of stimulation is shown by horizontal lines under each record; (b) after 30 min in the sodium-free sucrose solution; (c) after 15 (1 and 2) and 45 min (3) in the sodium-free lithium solution. Note the augmentation of discharges and disappearance of its inhibition; (c) return to Ringer solution containing nicotine.

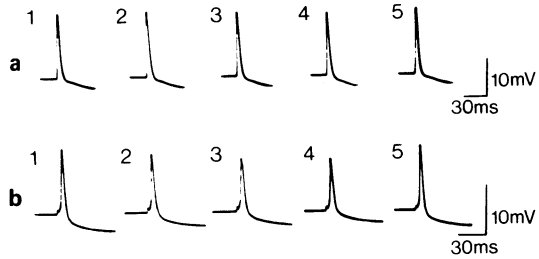

Fig. 8 Action potentials of postganglionic nerve fibres produced by single supramaximal preganglionic B nerve stimulation recorded in Ringer solution containing lithium ions (20 mM): (a) and (b) were in the absence and the presence of (+)-tubocurarine (0.007 mM), respectively. In both (a) and (b), record 1 was in Ringer solution containing nicotine (0.24 mM) and records 2, 3 and 4 were 3, 5 and 10 min after the addition of lithium ions (20 mM), respectively. Record 5 was obtained on returning the preparation to Ringer solution containing nicotine.

release from preganglionic nerve terminals. Both muscarinic ACh-hyperpolarization and ACh-depolarization returned to normal in Ringer solution.

c) Early after-discharge and its inhibition – The early after-discharge can be recorded from postganglionic nerves when repetitive stimuli are applied to preganglionic B nerve fibres (Nishi & Koketsu, 1968a). The early after-discharge is produced by the slow e.p.s.p. and can be inhibited by applying repetitive preganglionic stimuli which produce the slow i.p.s.p. (Koketsu & Nishi, 1967) (Figure 7a).

The early after-discharge recorded in four nicotinized preparations was completely eliminated when these preparations were soaked in sodium-free sucrose solution for approximately 30 min (Figure 7b). When the preparations were transferred to sodium-free lithium solution, the early after-discharge was not only restored, but also markedly augmented (Figure 7c). When the early after-discharge was augmented, the inhibitory effect of preganglionic nerve stimulation had almost completely disappeared (Figure 7c). These results parallel the observation that the slow e.p.s.p. was restored whereas the slow i.p.s.p. was not restored in sodium-free lithium solution. The restoration of the early after-discharge was a transient phenomenon and was reversible in Ringer solution.

II. Ringer solution containing lithium

1. Nerve conduction. The effects on nerve conduction of lithium ions added to the external Ringer solution, were studied. No detectable changes were obtained in the presence of lithium ions up to 20 mM; in three experiments the action potentials of preganglionic nerve fibres showed no changes during 120 min in these solutions.

2. Synaptic transmission.

A. Nicotinic transmission. Nicotinic transmission was depressed when lithium ions were added to the external Ringer solution. When the concentration of lithium ions was less than 10 mM, the effect could be observed after more than 2 h contact. When lithium ions (20 mM) were applied (three experiments) a slight depression of nicotinic transmission was detected within 10 min by use of the sucrose-gap method (Figure 8a). As seen in Fig. 8b, the depression could be demonstrated more clearly by using preparations (three experiments) in which nicotinic transmission was

partially blocked by (+)-tubocurarine (0.007 mM).

The cause of the depression of nicotinic transmission was studied by recording intracellular potential changes in a single cell in the presence of lithium ions (20 mM). Changes in the fast e.p.s.p. were investigated by use of a preparation in which the fast e.p.s.p. was depressed by the presence of tubocurarine (0.007 mM). No detectable depolarization of the resting membrane was observed for 10 min after lithium was added to the Ringer solution (five cells). As shown in Fig. 9, however, the amplitude of the fast e.p.s.p. in these cells was markedly decreased and the action potential of the ganglion cell was finally blocked within 10 min of the addition of lithium (20 mM). The depression of fast e.p.s.p. was quickly restored in Ringer solution (Figure 9c).

B. Muscarinic synaptic transmission. The effects of the addition of lithium to Ringer solution on the P-potential, LN-potential and muscarinic ACh-responses were investigated. In six experiments, nicotinized preparations were perfused with Ringer solution containing 20 mM lithium ions and the P-potential was markedly depressed in about 5 min (Figure 10a). The change in LN-potential was difficult to determine, since it was always superimposed on the P-potential. As seen in Fig. 10b, the muscarinic ACh-hyperpolarization was also depressed under these experimental conditions.

The early after-discharge was significantly augmented 10 min after lithium ions (20 mM) were added to the Ringer solution (Figure 11b). In three cases, the inhibitory effect of repetitive preganglionic nerve stimulation was significantly depressed (Figure 11b). These changes were reversible (Figure 11c).

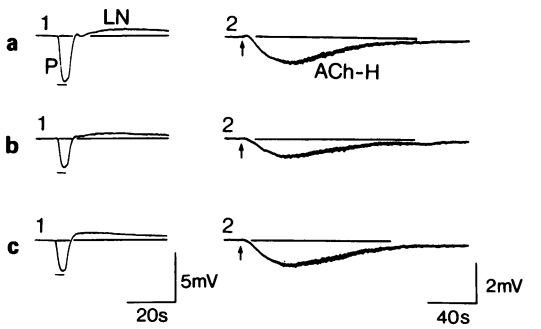

Fig. 10 Effects of lithium ions (20 mM) on the positive-potential (P), the late negative-potential (LN) and the muscarinic acetylcholine-hyperpolarization (ACh-H). All these potential changes were recorded from a single preparation by the sucrose-gap method in the presence of nicotine (0.24 mM); (a) in Ringer solution containing nicotine; (b) 5 min after an addition of lithium ions (20 mM); note the depression of the P-potential and the muscarinic ACh-hyperpolarization; (c) return to Ringer solution containing nicotine.

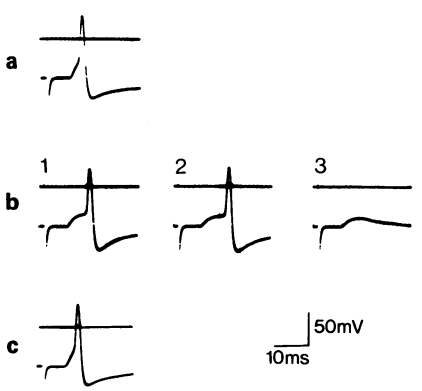

Fig. 9 Intracellular potential changes in a single cell: (a) the fast excitatory postsynaptic potential (e.p.s.p.) and the action potential of the cell-body, produced by a single supramaximal preganglionic B nerve stimulation in Ringer solution containing (+)-tubocurarine (0.007 mM); (b) 3, 5 and 10 min after the addition of lithium ions (20 mM). Note the decrease in size of the fast e.p.s.p. with no detectable membrane depolarization; (c) after returning to Ringer solution containing (+)-tubocurarine.

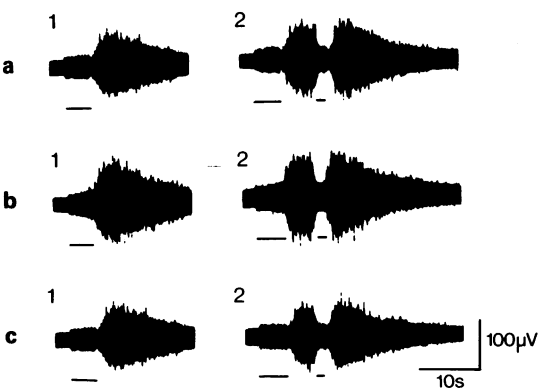

Fig. 11 Effects of lithium ions (20 mM) on the early after-discharge (1) and its inhibition (2): (a) Ringer solution containing nicotine (0.24 mM); (b) 10 min after the addition of 20 mM lithium ions and (c) return to Ringer solution containing nicotine. The early after-discharge and its inhibition were observed by applying repetitive preganglionic B nerve stimulation (30 Hz) for 4 s and 2 s, respectively; the duration of stimulation is shown by horizontal-lines under each record. Note a slight increase in the early after-discharge and depression of its inhibition in B.

Discussion

The results show that lithium ions are able to substitute for sodium ions in maintaining nerve conduction and nicotinic transmission in the sympathetic ganglia of the bullfrog. Thus, the general concept that lithium ions are effective substitutes for sodium ions for maintaining the membrane excitation (Hodgkin, 1951; Huxley & Stämpfli, 1951) can be applied to the present preparation.

It has been suggested that lithium ions may not be able to substitute for sodium ions in maintaining synaptic transmission in sympathetic ganglia, because of the observation that nicotinic transmission in this preparation was gradually blocked when the external solution was changed from Ringer solution to sodium-free lithium solution (Klingman, 1966; Pappano & Volle, 1966; Pappano & Volle, 1967). The present results agree with the observation, provided that the preparation was soaked in the sodium-free lithium solution for an extended period of time. However, when the nicotinic transmission was completely blocked in sodium-free sucrose solution it was restored by transferring the preparation to a sodium-free lithium solution. This clearly indicates that lithium ions are able to substitute for sodium ions in maintaining nicotinic transmission.

The fact that nicotinic transmission was eventually blocked in sodium-free lithium solution could be explained by: a) blockade of nerve conduction in pre- or postganglionic nerve fibres; b) a reduction in the release of transmitter (ACh) from preganglionic nerve terminals; or c) a depression of the ACh-sensitivity of the subsynaptic membrane of ganglion cells. The second and third explanations seem likely because blockade of nerve conduction was preceded by that of nicotinic transmission. The present experiments show that the nicotinic ACh-sensitivity of ganglion cells was not depressed even when preparations were soaked in sodium-free lithium solution for long periods. This observation leads to the conclusion that the cause of the blockade in nicotinic transmission may be a reduction in ACh release from presynaptic nerve terminals.

The reason for the reduction in ACh release in the sodium-free lithium solution is not completely explained by the present experiments. It was observed, however, that the initial change in the membrane potential in the sodium-free lithium solution was a fall in the resting potential. The action potential was unaffected even when the resting membrane was depolarized provided the resting membrane potential was kept normal by applying a constant anodal hyperpolarizing current. This suggests that the mechanism of ACh release (Katz, 1969) which is associated with the production of an action potential, may not be affected in the sodium-free lithium solution. Thus, the reduction in ACh release appears to be due to the depolarization of the resting membranes of the presynaptic nerve terminals, since the amount of ACh released is proportional to the value of the resting membrane potential (Takeuchi & Takeuchi, 1962).

The present results do not provide any conclusive explanation of the cause of membrane depolarization in the sodium-free lithium solution. It would be expected, however, that lithium ions would accumulate in the intracellular space when they replace sodium ions in the extracellular space because they are pumped out of the cell with difficulty compared with sodium ions (Maizels, 1954; Zerahn, 1955; Keynes & Swan, 1959). Under such experimental conditions, the intracellular potassium concentration would be reduced (Carmeliet, 1964; Araki, Ito, Kostyuk, Oscarsson & Oshima, 1965; see, however, Yonemura & Sato, 1967). Thus, a decrease in the intracellular potassium concentration would lead to membrane depolarization, particularly at presynaptic nerve terminals where the diameter of nerve fibres is extremely small.

Action potentials in the sodium-free lithium solution showed smaller amplitude and higher threshold, compared with action potentials in Ringer solution. This would be explained by the fact that membrane permeability to lithium ions is smaller than that to sodium ions (Keynes & Swan, 1959; Armett & Ritchie, 1963). When the membrane was depolarized in the sodium-free lithium solution, the threshold would become higher because of the partial reduction of permeability. Under these experimental conditions, nerve conduction, particularly at the terminals, would be expected to be blocked (Bjegovic & Randic, 1971).

An interesting finding in the present experiments is the fact that the slow e.p.s.p. or muscarinic ACh-depolarization was completely restored while the slow i.p.s.p. or muscarinic ACh-hyperpolarization was not restored when preparations were transferred from the sodium-free sucrose solution to the sodium-free lithium solution. These results are explained by the hypothetical concept that the slow i.p.s.p. is generated by activation of the electrogenic sodium pump (Koketsu & Nishi, 1967; Nishi & Koketsu, 1968b). The sodium efflux may be accelerated when the extracellular sodium ions are totally replaced by lithium ions (Beauge & Sjodin, 1968; Baker, Blaustein, Hodgkin & Steinhardt, 1969). In any case, the intracellular sodium concentration would be reduced in a preparation soaked successively in

sodium-free sucrose and sodium-free lithium solution. The reduction of the intracellular sodium concentration would lead to a depression of the sodium pump, proportional to the fall in the intracellular sodium concentration (Baker *et al.*, 1969; Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969).

The mode of action of lithium ions added to Ringer solution can easily be explained by the results obtained in the experiments carried out in sodium-free lithium solution. Indeed, there is no reason to assume a specific action of small concentrations of lithium ions on the membrane excitation process. When lithium ions were added to extracellular fluid, they would penetrate to the intracellular space in proportion to the extracellular concentration and the duration of application. The intracellular accumulation of lithium would lead to membrane depolarization and a depression of the sodium pump. In such conditions, synaptic transmission as well as nerve conduction would be impeded in proportion to the concentration of lithium and the duration of its application.

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