Effects of Lithium on Different Membrane Components of Crayfish Stretch Receptor Neurons

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ABSTRACT Unlike several other varieties of input membrane, that of the crayfish stretch receptor develops a generator potential in response to stretch when all the Na of the medium is replaced with Li. However, Li depolarizes the receptor neuron, the soma membrane becoming more depolarized than that of the axon. During exposure to Li the cell usually fires spontaneously for a period, and when it becomes quiescent spike electrogenesis fails in the soma but persists in the axon. These effects are seen in the rapidly adapting as well as the slowly adapting cells. The block of spike electrogenesis of the soma membrane is only partly due to the Li-induced depolarization and a significant role must be ascribed to a specific effect of Li.

INTRODUCTION

It has long been known that Li, unlike other inorganic monovalent cations, can replace Na in spike electrogenesis of those cells that normally require the presence of Na in the bathing medium (Overton, 1902; Hodgkin and Katz, 1949). However, certain types of electrically inexcitable membrane do not respond with depolarizing electrogenesis when Li replaces Na. The excitatory postsynaptic potentials of cat superior cervical ganglion neurons (Pappano and Volle, 1967), of *Tenebrio* muscle fibers (Kusano and Grundfest, 1967), and of crayfish muscle fibers (Ozeki and Grundfest, 1967) as well as the electroretinogram of toad (Furukawa and Hanawa, 1955) and frog (Hamasaki, 1963), all require the presence of Na and cannot be elicited when Li is substituted for Na. The generator potential of *Limulus* photosensitive neurons is abolished after Na is replaced with Li or Tris (Millechia et al., 1966). However, the effect is temporary (personal communication from Dr. A. Mauro). After about 5 to 15 min in the Na-free medium the cells again respond to light, but the generator potential is greatly reduced.

These findings raised the possibility that discrimination between Na and

Li might be a general property of the electrically inexcitable membrane component that generates depolarizing potentials. The present study was undertaken to examine this possibility in still another variety of such a membrane, that of crayfish stretch receptors. As will be shown, the generator potential of this cell can still be evoked by stretch stimuli when all Na is replaced with Li. However, the substitution of Li for Na leads to other effects that are of considerable theoretical interest, since they show that Li exerts different actions on the membrane of different parts of the neuron. A preliminary report of this work has appeared (Obara and Grundfest, 1967).

METHODS

Stretch receptor cells (Alexandrowicz, 1951) were usually prepared from abdominal segments of *Procambarus*, but occasionally thoracic receptors were also used. Almost identical results were obtained with slowly and rapidly adapting receptors. However, the generator electrogenesis was studied mainly in the slowly adapting cells. The isolated neuron was held in an experimental chamber, similar to those described by previous investigators (Eyzaguirre and Kuffler, 1955 *a*; Nakajima, 1964). The amount as well as the rate of stretch was controlled manually and the stimulus was monitored on one trace of a cathode ray oscillograph, with the aid of a displacement transducer (Sanborn 7 DCDT-050; Hewlett Packard Co., Waltham, Mass.). The initial or "resting length" of the receptor was measured under observation with a binocular microscope and the applied stretch, similarly measured, is expressed as per cent increase of the resting length.

The experimental chamber used in the early experiments of this series had a volume of about 5 ml, but in later work the volume was reduced to 2 ml. To eliminate possible retention of the solution by the forceps that fixed the preparation to the stretcher, the spaces within the tips of the forceps were filled with wax. To change the solution, the chamber was flushed continuously with a volume of fluid at least 10 times that of the chamber volume. The perfusion rate was kept low and impaling microelectrodes remained inside the cell during the perfusion.

The control saline was somewhat modified from that described by Van Harreveld (1936) and contained (in mM/liter) 205 NaCl, 5.4 KCl, 13.5 $CaCl_2$, and 1.0 $MgCl_2$. The pH was adjusted to 7.4 with Tris buffer. LiCl was substituted stoichiometrically for NaCl in the experimental solution. When it was desired to study the generator potential without contamination by spike electrogenesis, tetrodotoxin (TTX), supplied by the Sankyo Co. of Tokyo, was used (Loewenstein et al., 1963; Nakajima, 1964). Spikes were abolished when the concentration of TTX was $2\text{–}5 \times 10^{-9}$ g/ml. The experiments were done at room temperatures, which ranged between 22 and 25°C.

The soma of the receptor neurons was impaled with either one, or more frequently, with two microcapillary glass electrodes filled with 3 M KCl, the second electrode being used for injecting current. In one series of experiments a third microelectrode was inserted into the axon. The resistance of the electrodes ranged between 10 and 40 megohms. Recording was differential, against a Ag-AgCl electrode connected to the bath through a KCl-agar bridge. The nerve bundle containing the axon of the

receptor cell was laid on pairs of Ag-AgCl wire electrodes which were embedded in the chamber and were used for extracellular stimulation and recording. The part of the nerve bundle over these electrodes was covered with vaseline. Usually only a few millimeters of the nerve were exposed to the solution bathing the cell body. However, when the control saline was replaced with Tris Cl, choline Cl, or sucrose, spike electrogenesis in this region of the axon as well as in the cell body was rapidly abolished and no spike could be recorded centrally on stimulating with strong intracellularly applied currents (Obara, 1967). The adequacy of exposing only a few millimeters to the test solutions is a consequence of the small effective space constant of the system (Fig. 8).

After penetration of the cell the resting length of the receptor was set by relaxing the stretcher until there was no further increase in membrane potential in response to a small decrease in stretch. With the resting length thus defined, the stretch-response graphs usually intersected the abscissa not at the origin, but at a value several per cent from the resting length.

RESULTS

Control Responses In completely relaxed cells the resting potential averaged 71.8 mv (range 68 to 80 mv). The antidromic invasion of the soma on stimulating the axon gave rise to a spike that averaged 86.6 mv (range 72 to 100 mv). The spike terminated in a depolarizing afterpotential, similar to that which is observed in the cord giant axons (Watanabe and Grundfest, 1961). In the fresh preparations, no hyperpolarizing afterpotential was observed in most cases but such an afterpotential developed when the cells were depolarized (Eyzaguirre and Kuffler, 1955 *b*).

The responses to stretch, either with the spike generator intact or eliminated by TTX, were similar to those reported by earlier workers (Eyzaguirre and Kuffler, 1955 *a*; Terzuolo and Washizu, 1962; Loewenstein et al., 1963; Nakajima, 1964). The properties of the receptor muscle may be changed irreversibly at more than 50% stretch (Krnjevic and van Gelder, 1961). For this reason the maximum amount of stretch in the present work was kept below 50%.

Effect of Li on the Generator Potential Most of the experiments of this series were done in the presence of TTX, so as to eliminate the distortion of the generator potential that is introduced by the presence of spike electrogenesis. Whether in the presence of Na or Li, the cells responded with a generator potential of characteristic form, an initial peak depolarization, and a subsequent gradually decreasing plateau (Fig. 1). The plateau depolarization persisted as long as the stimulus was applied. In the Li saline the cells did not develop the hyperpolarization that is normally observed following the release of stretch (Eyzaguirre and Kuffler, 1955 *a*). In general, the response to a given stretch was somewhat smaller in the presence of Li, but as is seen in the records and graph of Fig. 1 the difference could be quite small.

However, when the cell was bathed in the Li saline it underwent a slow depolarization which decreased the apparent size of the generator potential. This is seen clearly in the graph of Fig. 1, which represents responses to stretches of different amplitudes randomly applied during the course of the experiment. The filled circles without attached arrows represent measurements made early during the exposure of the cell to Li. Both the peak values (large circles) and those during the quasi-steady state of the plateau (small circles) fall close to the corresponding values obtained in control measurements with the cell in the normal Na saline bathing medium (open circles).

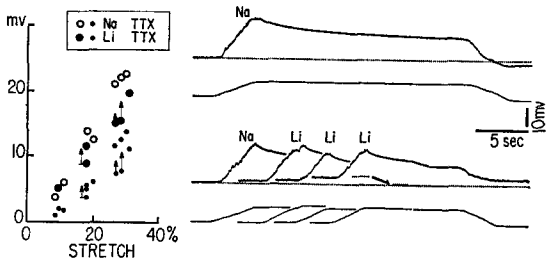

FIGURE 1. Persistence of the generator potential in Li. Slowly adapting stretch receptor. Preparation treated with TTX to eliminate spike electrogenesis. Upper record, control response in the presence of Na. Top trace shows the generator depolarization induced by a stretch that is monitored on the lower trace. The dotted line indicates the resting potential; note afterhyperpolarization. Lower records, generator potentials recorded during exposure of cell to Li. The first record was made before substitution of the Li for the Na. Note gradual depolarization in the Li saline. The short dotted line under the last record shows the resting membrane potential when the cell had been exposed to Li for 15 min. The arrow points to the return of the resting potential to the original value when Na was again introduced. Graph shows the complete data of this experiment, relating the amplitude of the generator potentials to different degrees of stretch in the Na saline and in Li. The large circles show the peak of the generator depolarization and the smaller circles denote the depolarization at a fixed time during the quasi-steady state. Stretches were applied in random order. The data obtained after the cell had begun to depolarize in Li are indicated by an accompanying arrow whose length denotes the amount of depolarization.

The values with arrows attached were measurements which were made late during the exposure to Li, after the cell had depolarized. The lengths of the arrows represent the amounts of depolarization. Thus, when the magnitude of the depolarization is added to the generator potential the total is substantially the same as for the earlier values of the amplitude of the generator potential in the presence of Li. The depolarization occurred also in rapidly adapting receptor neurons. The rate and magnitude of the gradual depolarization induced by Li varied greatly from cell to cell. The cause of the variation was not investigated.

Effects of Li-Induced Depolarization on Spike Electrogenesis Two varieties of experiments were used to test the effects of the depolarization induced by Li on the spike electrogenesis evoked by stretch. Experiments like those shown in Fig. 2 did not require penetration of the cell with microelectrodes and thus obviated that possible source of damage to the cell. A steady stretch was applied to a slowly adapting receptor, so as to elicit discharges that could be recorded with the external electrodes placed on the nerve bundle. The rate of the discharge, which was steady in the control saline, increased markedly shortly after replacing the Na with Li. However, this higher frequency was not maintained, but declined during the next 10 or 15 min and, rather abruptly, the cell ceased to fire at all, although a spike could still be elicited

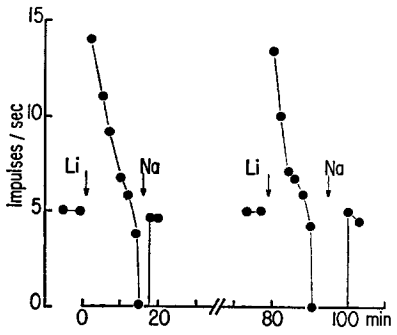

FIGURE 2. Effect of Li on firing frequency of a slowly adapting cell under stretch. Extracellular recording from the axon. A steady stretch (20% above rest length in the first experiment and 32% in the second) was applied, while the bathing medium was changed from the control saline to the Li saline and back again. The second experiment was done on the same cell about 1 hr after the first. Further description in text.

in the axon by stimulating the nerve bundle. The original discharge rate developed again after returning the cell to the control saline. As is to be seen in the second experiment of Fig. 2, which was made on the same cell after an interval of about 1 hr, the time courses of the effect of substituting Li for Na and the reverse were essentially similar. Cells that were not subjected to stretch began to discharge spontaneously within a few minutes after the introduction of Li (cf. Fig. 5 A_2). This discharge lasted for only some 10–15 min. After it had stopped spikes could still be evoked in the axon by stimulating the nerve bundle.

It seemed likely that depolarization of the neuron in Li, like that observed in Fig. 1, had augmented the frequency of the discharges (Fig. 2) and might also have caused the unstretched cells to discharge spontaneously. However, the rapid cessation of discharges was surprising, since the slowly adapting receptor neurons continue to generate spikes for a long time when the soma

is depolarized with an injected current (Nakajima, 1964; cf. also below, particularly Fig. 7).

The block of spike electrogenesis by Li was studied further in the experiments described in the remainder of this section. The records of Fig. 3 A represent antidromic spikes registered with an intracellular electrode in the soma while the axon was stimulated through the external wire electrodes. A_1 and A_2 show the antidromic spikes recorded in the control medium, while the records of A_3 to A_8 register the changes that took place in the course of some

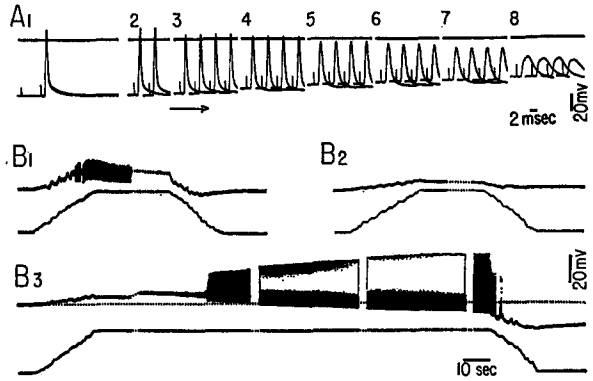

FIGURE 3. Changes induced by long lasting exposure to Li. A, gradual depolarization and block of invasion of the soma by an antidromic spike from the axon. A_1 and A_2, control responses recorded intracellularly from the soma. The records in A_3 to A_8 were made approximately 5, 12, 22, 27, 32, and 41 min after start of the perfusion. Upper trace is the reference zero. Note the immediate decrease in spike amplitude (A_3). The cell was still capable of developing a graded response (A_8) when it was depolarized by about 19 mv. B, responses to stretch. B_1, Na saline. During the prolonged stretch spike electrogenesis stopped. B_2, Li saline, recording after block of antidromic invasion of the soma. The generator depolarization appears to be considerably smaller because the cell is depolarized. This is shown in B_3, in which recording begins with the cell in Li. During the first break perfusion of the chamber with Na was started. The second and third gaps indicate intervals of 5 sec. The last gap was considerably longer. The dotted line shows the membrane potential in the Li saline. At the end of the recording, about 30 sec after the stretch was terminated, the membrane was repolarized by about 18 mv.

40 min exposure to Li. This particular experiment was chosen for illustrative purposes because the rate of depolarization in the presence of Li was very slow.

Even before there was a marked depolarization the amplitude of the spikes decreased (A_3). Subsequently, the overshoot disappeared, the spikes became broader, and the afterdepolarization which is characteristic of the soma spikes in the unstretched cell (A_1, A_2) became an apparent undershoot which in actuality represents a temporary return toward the base line of the original resting potential. This behavior of the afterpotential indicates that the depolarization induced by Li is due to an emf that comes from a battery in parallel with, but distinct from the one that causes the afterpotential.

Some electrogenic activity induced in the soma by the stimulus of the antidromically invading spike is still evident in the records of Fig. 3 A_8. Elimination of this activity is shown in later figures (e.g., Fig. 5 A_3). After soma electrogenesis is eliminated, however, spike electrogenesis persists in the axon. Thus, the cessation of discharges in response to stretch of the cells in the presence of Li (Fig. 2) must have been due to block of spike electrogenesis not only in the soma, but also at the trigger zone some distance from the cell body which is the site where the impulses normally originate in response to the generator potential (Edwards and Ottoson, 1958).

Cells which no longer could develop spikes in response to stretch stimuli nevertheless still produced generator potentials. The latter appeared to be smaller (Fig. 3 B_2) than in the control (B_1). The smaller size, however, was largely due to the steady depolarization induced by the Li, as has already been seen in the graph of Fig. 1. The record of Fig. 3 B_8 was made after long exposure of the preparation to Li. The cell remained impaled and subjected to constant stretch for another 5 or 6 min while the chamber was being perfused with the control saline. The beginning of the replacement of the Li with Na occurred at the first break in the record. The further depolarization was probably the result of several effects, e.g., further depolarization caused by the Li and/or increase of the generator potential in Na. Small oscillations then appeared which gradually increased in amplitude and finally gave rise to impulses. These spikes grew in amplitude while the mean membrane potential between the spikes seemed gradually to become more negative, as judged from the bottoms of the potentials after the impulses. In the last section of the record the spikes had attained a constant amplitude. When the stretch was released the potential became strongly negative. At the end of the record, about 30 sec after the stretch had been terminated, the membrane was about 17 mv negative to the level at the beginning of the recording.

Effects of Li on Soma and Axon Membranes The different effects of Li on the membrane of the soma and the axon were studied in experiments like those illustrated in Figs. 4–6. The nerve bundle from the receptor was dissected centrally as far as possible, sometimes approaching close to the ventral nerve cord. The bundle contained several larger and smaller motor fibers to adjacent skeletal muscles and also sensory fibers from receptors in the carapace, in addition to the nerves of the stretch receptors (Alexandrowicz, 1951). Maximum lengths ranged between 10 to 12 mm. Extreme care was required so as not to injure this length of the sensory axon. The axons of the receptors could be followed centrally under observation with the dissecting microscope, but final identification of the axon of the receptor had to be made electrophysiologically, by stimulation of the cell body.

The experimental arrangement used is shown in the diagram of Fig. 4. Two microelectrodes (I, V_1) were inserted into the receptor cell and a third micro-

electrode (V_2) was then inserted into the axon at varying distances from the soma. The axon is thin close to the soma, but some tenths of a millimeter from the cell body it enlarges, although its diameter is then no more than 20 μ. Thus, penetration of the axon close to the soma was less successful than at some distance from the cell body. In fibers that were impaled successfully a large axon spike could be maintained for a considerable period of time. The records of Figs. 4–6 were all made from a cell that continued to respond for more than 1 hr, during which time the cell was exposed to Li for some 30 min and was then returned to the control medium for another 30 min.

In the first column of Fig. 4 (A_1, A_2) are records obtained while the preparation was in the control saline. The soma and axon had identical resting potentials, but the spike of the axon, whether elicited by stimulation of the axon (A_1) or by propagation from the soma (A_2), was briefer than the soma spike which was initiated either by antidromic invasion (A_1) or by direct stimulation (A_2) as had been observed earlier by Eyzaguirre and Kuffler (1955 *b*).

The interval between the pairs of spikes that originated from the two modes of stimulation differed significantly. This is evident from the separation of the peaks of the spikes in A_1 and A_2. However, a more precise estimate could be obtained from the capacitative coupling artifact between the two recording electrodes that appeared in the two traces of intracellular recording. The peak of this coupling artifact at one recording site coincides with the maximum rate of rise of the spike at the other site. The difference between the pairs of peaks in A_1 and A_2 (dotted vertical lines) is about 20%, the interval being larger in A_1. This difference is readily explained by the earlier finding (Edwards and Ottoson, 1958) that a stimulus at the soma initiates a spike at a trigger zone some distance along the neurite, from which site there is centripetal (orthodromic) propagation along the axon (conduction time = t_1) and antidromic (centrifugal) propagation to the soma (conduction time = t_2). The elapsed time in (A_2) is $t_1 - t_2$, while the conduction time in the antidromic direction is $t_1 + t_2$. It is not possible, however, to calculate the site of origin of the orthodromic impulse from these data, since the neurite is not uniform and is considerably smaller at the region of the cell body than further away. Thus, it is likely that the distribution of the intracellularly applied currents and the conduction velocities of the propagating impulses were far from uniform. The spike could be evoked after the threshold stimulus had been terminated (A_2). The latency probably reflects not only the time for the impulse to propagate from the trigger zone to the soma (t_2), but also the time for a slowly developing and sustained local response.

The records of the second and third columns of Fig. 4 were made after the preparation had been bathed in the Li saline for 24 min. The soma had depolarized by about 16 mv, while the depolarization at the axonal recording site was only about 4 mv. The spike recorded from the axon became smaller

and broader. Activity induced by antidromic invasion was now abolished (B_1, C_1), a small depolarization reflecting the electrotonic spread of current from activity at a distant region.

An intracellularly applied current, however, could still elicit a spike in the

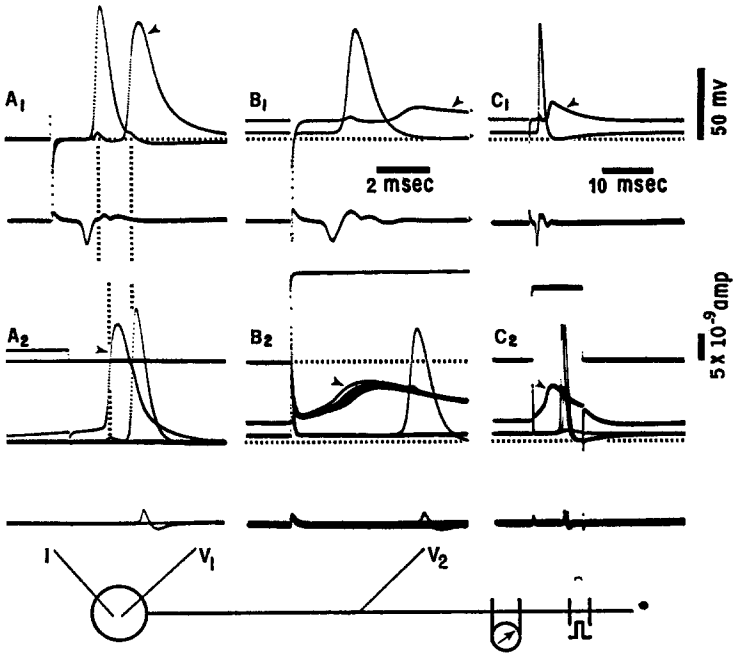

FIGURE 4. Simultaneous recording from soma and axon in slowly adapting stretch receptor cell. Diagram shows the experimental arrangement, with the cell body impaled also with an electrode for passing current. The distance between sites V_1 and V_2 was 3.8 mm. A_1–C_1, responses to stimulation of the axon. A_2–C_2, responses to stimulation of the soma. A, records in the control saline, B and C, in the Li saline for approximately 24 min. Records in C were made before those in B. Lowest trace in each record shows external recording from the nerve bundle. Note that the recording in response to soma stimulation differs from that due to external stimulation, since in the latter case it is contaminated with responses from other axons. The soma recordings are marked by arrows. The sweep was expanded fivefold in A and B. The currents injected into the soma are monitored on the upper traces in A_2–C_2. The stimulus was a 10 msec pulse, shown in full in C_2. The expanded sweep in A_2 shows the end of the threshold current. The soma spike arose about 1.5 msec after the stimulus and the axon spike followed about 1 msec later. In B_2 and C_2 the axon spike arose about 5 msec after the pulse was initiated.

axon (B_2, C_2), but the current had to be very large and the threshold increased progressively. The records of column C had been made earlier than those of column B and the current needed to excite a spike was smaller in C_2 than in B_2.

In the soma the intracellularly applied current evoked a larger depolariza-

tion (B_2) than did the spike that propagated toward the cell from the axon. This larger depolarization must be a graded local response. It is likely that the long term depolarization induced by Li in the region of the soma had caused Na inactivation as well as some K activation (Hodgkin and Huxley, 1952), so that the smaller conductance change induced by the applied current could develop only a small depolarization inadequate to evoke an all-or-none spike in the immediate region of the soma. Nevertheless, the axon could still develop a spike albeit one smaller and more slowly rising than that of the control.

The interval between the peak of the axon spike and the peak of the electrotonic potential (B_1, C_1) or the peak of the graded response (B_2, C_2) in the soma was longer than the intervals between the peaks of the spikes in the control (A_1, A_2), indicating, as may be expected, that the smaller, more slowly rising spikes must have propagated in the axon at a slower rate. The difference indicates a slowing of conduction by about 50%.

After the soma spike is abolished in the Li saline it can be restored by applying inward current so as to repolarize the soma membrane (Fig. 5). The records of the upper row were made in the control condition (A_1); during the phase of spontaneous discharge which lasted from the 4th to the 10th min in the Li saline (A_2); after 32 min in Li, well after propagation into the soma had been abolished (A_3); and 20 min after return of the preparation to the control saline (A_4). In the lower row, the first record was made when the preparation had been in Li for 20 min, just after full spike electrogenesis of the soma had been abolished, but while the soma was still capable of generating a graded response, as in Fig. 4 B_2. Repolarization by inward current (Fig. 5 B_2) restored spike electrogenesis in the soma, although the amplitude of the spike overshoot was considerably smaller than in the control (A_1) and also less than during the phase of spontaneous repetitively firing spikes (A_2). The overshoot in B_2 was larger, however, than that recorded later, after the fiber had been returned to the control saline (A_4).

Large inward currents carried the membrane into considerable hyperpolarization (B_3, B_4), but even with a current sufficient to hyperpolarize the membrane by some 50 mv (B_4) the peak of the overshoot showed only a very small decline. Thus, the conductance increase that must have occurred during the spike electrogenesis must have been quite large. It is also noteworthy that the maximum amplitude of the spike was attained on repolarization to the original resting potential. Also noteworthy is the fact that hyperpolarization by as much as 50 mv (B_4) did not block spike electrogenesis. The data suggest that the repolarization had restored many initial current (Na) channels that had been inactivated by the Li-induced depolarization. However, the degree of restoration varied in different preparations. In some, spike electrogenesis in the soma induced by antidromic invasion was blocked when the cell was

hyperpolarized by some 30 mv beyond its original resting potential. In others, hyperpolarization by about 100 mv did not block spike electrogenesis of the soma.

Restoration of spike electrogenesis by the repolarization was rapid (Fig. 6).

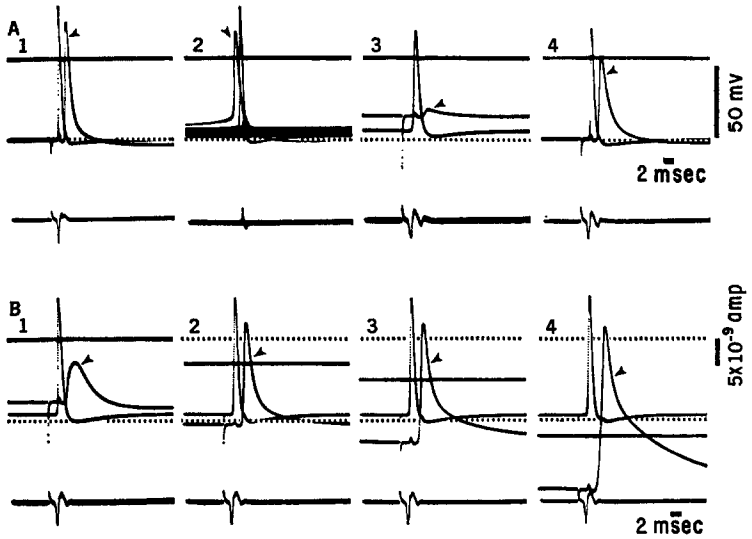

FIGURE 5. Block of spike electrogenesis in the soma by Li and its restoration on applying inward currents. Same cell as in Fig. 4. A_1, responses evoked by stimulating the nerve bundle in the control saline. Soma recording is marked with an arrow. A_2, during the period of spontaneous repetitive activity of the cell which was induced during the 4th through 10th min of exposure to Li. Superimposed sweeps, during one of which the spike originated near the soma and preceded the axon spike. Note the corresponding change in form of the external recording. A_3, after 32 min in Li the soma was depolarized by about 17 mv while the axon had depolarized by only 6–7 mv. The axon spike caused only a small electrotonic potential in the soma. A_4, after 20 min in the control saline. The resting potential had returned to its initial level and the axon spike invaded the soma, but the response of the latter was depressed. B_1, this record was made when the cell had been exposed to the Li saline for 20 min. The soma had depolarized by about 15 mv while the axon was depolarized by about 3–4 mv. The soma responded only with a graded response. B_2–B_4, steady inward currents were applied to the soma, their magnitude being monitored as deflections of the uppermost trace from the initial reference level (upper dotted line). The initial resting potential is shown by the lower dotted line. Repolarization to the original potential (B_2) restored spike electrogenesis in the soma. Further hyperpolarization did not affect the peaks of the spikes, although the overall amplitudes increased in proportion to the hyperpolarization.

Control responses are shown in A. In B are superimposed responses (at 1/sec) after 12 min exposure to Li, shortly after spontaneous repetitive firing had ceased. The soma was depolarized by about 10 mv and the soma spike was markedly decreased. At this stage repolarization had little effect on the peak

amplitude. After 20 min (C) and 32 min (D) in Li, when the spike electrogenesis had been abolished, the response that developed within 1 sec after repolarization commenced was markedly larger and within the next 2 or 3 sec it attained its maximum amplitude. This was smaller not only in relation to the control (A), but also to earlier stages in Li (B). However, even after 32 min recovery in the Na saline (E) the soma spike was still small. Prior to replacement of the Na with Li the same cell had also been subjected to applied

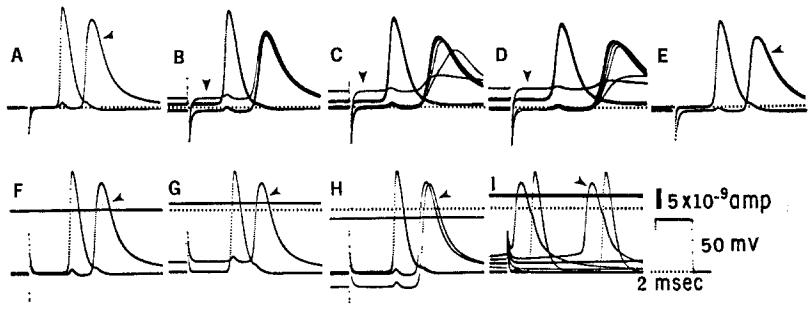

FIGURE 6. Effects of polarizing currents. Same cell as in Figs. 4 and 5. A, control response evoked by stimulation of the nerve bundle. B, after 12 min in Li, soon after the spontaneous activity shown in Fig. 5 A_2 had ceased. The soma had depolarized by about 10 mv and the spike induced by antidromic invasion was small. Repolarization approximately to the resting potential was produced while the nerve was being stimulated at a rate of 1/sec. The amplitude of the soma spike was not greatly affected by the change in membrane potential. C, after 20 min in Li the soma was depolarized about 15 mv and produced only a small graded response (seen in Fig. 5 B_1 on a slower time base and at higher amplification). Repolarization of the cell caused an increase in the soma response that was marked within the first second and increased further during the next few seconds of repolarization. D, after 32 min exposure to Li. The soma had depolarized by about 17 mv (cf. Fig. 5 A_3) and developed only an electrotonic potential. A large graded response was evoked within the first second of repolarization and the maximal amplitude of a spike was evident after about 3 sec repolarization. E, recovery of spike electrogenesis in the soma 32 min after return to the Na saline. F-I, an earlier series on the same cell, prior to substitution of the Li for Na. F, control. G, the soma was subjected to steady depolarization and H to hyperpolarization. Note the very small effect on the resting potential of the axon. I, a larger depolarization initiated repetitive orthodromic firing. The changes in membrane potential had little effect on the soma spike.

currents, but for relatively brief periods (Fig. 6 F-I). The repetitive spikes which were induced by depolarization of the soma (I) were of about the same amplitude as those evoked by antidromic invasion (F, H).

Thus, it seems likely that the presence of Li itself affects the spike amplitude. Since the restored spike in the repolarized cell is accompanied by a large increase in conductance (Fig. 5 B), the gradual decrease of the spike in the presence of Li (Fig. 6 B-D) and the persistence of this diminution long after replacement of Na (E) indicates that the inside-positive emf for the spike

generator had decreased. Such diminution might be caused by the accumulation of Li in the cell, so that the diffusional emf across the membrane is decreased. The accumulation might result from the block of the Na pump by Li (Nakajima and Takahashi, 1966). However, spike electrogenesis involves the interplay of several processes (Hodgkin and Huxley, 1952). The interplay may be sufficiently complex (Grundfest, 1961, 1966) so that the factor(s) causing the decrease in amplitude cannot be specified precisely with the data available.

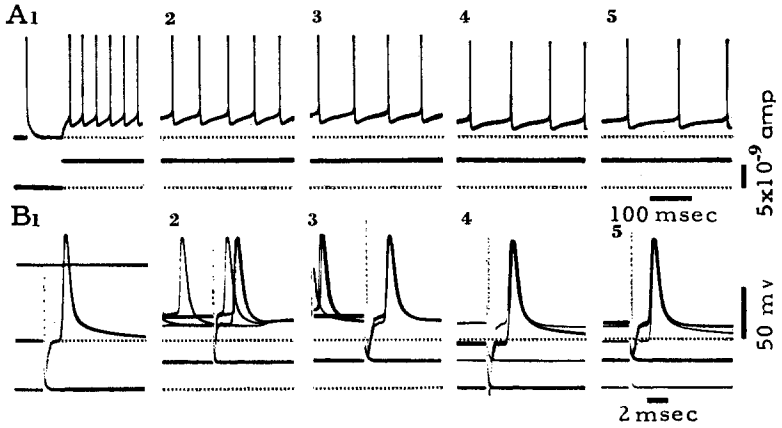

FIGURE 7. Persistence of spike electrogenesis during prolonged depolarization of a slowly adapting neuron. The current (monitored on the lower trace) was approximately 7×10^{-9} amp, twice the threshold for spike initiation. A, slow sweep registration. (1) An antidromic spike preceded the onset of the current. The frequency of the repetitive spikes was about 30/sec. (2) After 10 min the frequency decreased to about 15/sec. (3) After 22 min the frequency was about 12/sec. (4) After 30 min, frequency was reduced to 10/sec. (5) At 40 min, the frequency was 8/sec. B, fast sweep registrations at the corresponding times, and with stimulation of the axon evoking antidromic spikes. (1) Before the current was applied. Upper trace shows the zero reference. (2)–(5) Repetitive sweeps. In (4) and (5) the current was turned off briefly to show that the antidromic spikes evoked with the cell near the resting potential were of the same amplitude as the spikes of the depolarized cell. The peaks of the spikes had fallen from the initial value of 97 mv to 93 mv after 45 min of depolarization.

Nevertheless, because the effect of Li on spike electrogenesis of the soma developed slowly, there was a possibility that block of spike production in the soma was the result of the concomitant long lasting depolarization. This was ruled out by experiments illustrated in Fig. 7. The cell was depolarized with a current twice the rheobasic value and continued to develop spikes throughout the stimulation which lasted 45 min. At the beginning of record A, a response to an antidromic stimulus serves as the control. During the first 22 min of stimulation the spike amplitude was essentially unchanged, although the frequency of the discharges had fallen from almost 30/sec to about 12/sec

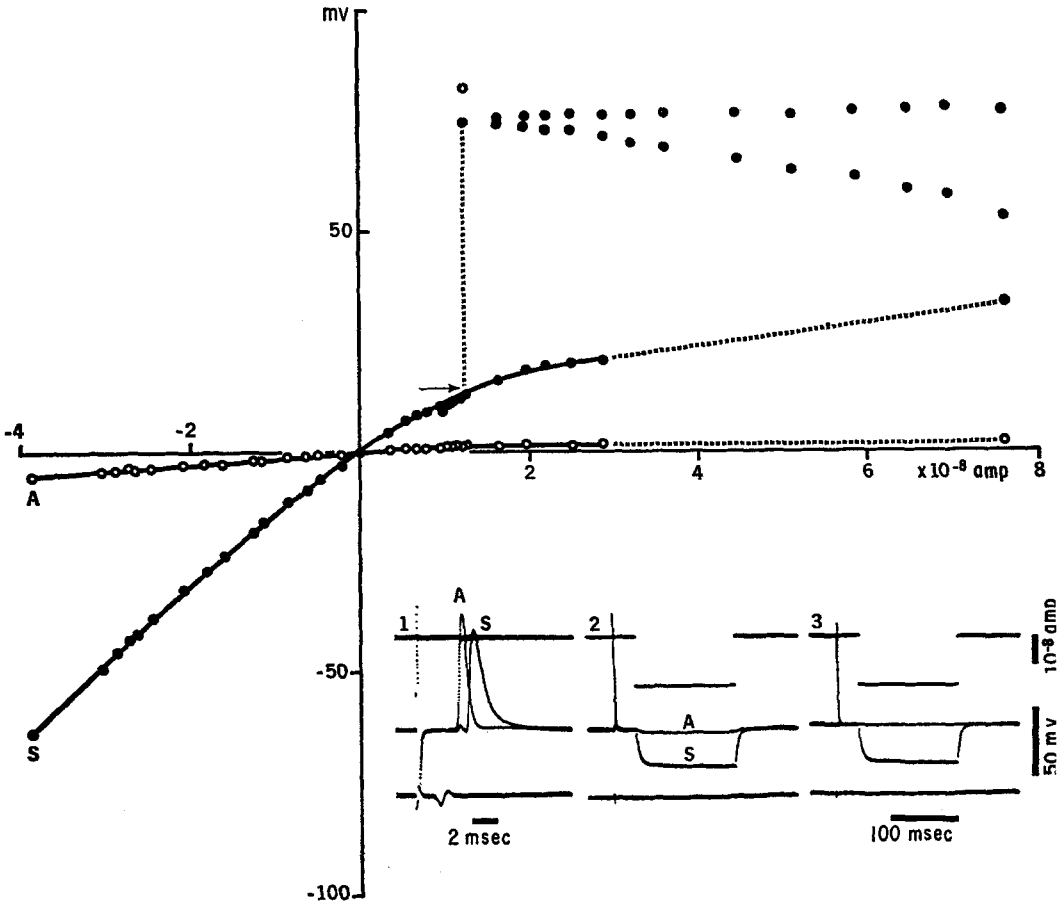

FIGURE 8. Changes in membrane potential of the soma and axon of a rapidly adapting neuron induced by intracellularly applied current. Preparation in the control saline. Inset records show the responses in axon (A) and soma (S) to stimulation of the nerve bundle (1) and on a slower time base in records 2 and 3. The changes in membrane potential induced at the two recording sites by inward currents lasting about 150 msec and applied in the soma are also shown in records 2 and 3. The electrode in the axon was withdrawn prior to record 3. Absence of a deflection of the axonal trace demonstrates that there was no significant extracellular field potential during the applied current. The graph shows that the current-voltage relation is linear in the hyperpolarizing quadrant. The hyperpolarizations induced in the axon are only about 8% of those in the soma. In the depolarizing quadrant the characteristic begins to exhibit a downward curvature for outward currents above about 10 n amp. When the soma was depolarized by about 14–15 mv a train of spikes was initiated (dotted vertical line). The two rows of filled circles show the amplitude of the first and last spike of each train as recorded in the soma. As the current was increased sevenfold the amplitude of the initial spike increased only slightly, indicating that the conductance during the peaks was high. The terminating spikes decreased in amplitude. The amplitudes of the axon spikes recorded at the same time were not altered and are shown by a single open circle above the vertical dotted line. The lower set of filled circles connected by lines was obtained

(A_3). After 40 min (A_5) the frequency had declined to about 8/sec, but the decrease in spike amplitude was very small. Spike electrogenesis could also be evoked in the soma by antidromic invasion throughout this period (B_{1-5}). The neuron continued to discharge spikes for another 5 min when one of the impaling microelectrodes was displaced accidentally.

Sites of Li-Induced Depolarizations The depolarization that Li caused in the soma was considerably larger than that recorded from the axon (Figs. 4–6). A few experiments were done on both types of receptors with regard to the question of whether the Li depolarized only the soma membrane or whether it had a similar though small effect on the axon membrane as well. Similar results were obtained for both. The experiment of Fig. 8 which was done in the control saline on a rapidly adapting cell is the most complete. A current (abscissa) was applied to the soma and the resulting changes in membrane potential (ordinate) were recorded at the soma and in the axon. The current-voltage characteristic of the soma membrane is nearly linear in the hyperpolarizing quadrant and for depolarizations up to about 10 mv. A depolarization of about 15 mv caused spikes (vertical dotted line). The first spike of the train recorded from the soma (upper row of filled circles) was of almost constant height even for currents about sevenfold above the threshold value. However, spike electrogenesis was only maintained for a range indicated by broken lines during the 150 msec depolarizing stimulus. The amplitude of the last spike of the train fell markedly as the current was increased. The plateau of depolarization at the end of the train is shown by the lowest filled circles and line of the graph. The downward concavity denotes considerable rectification due to depolarizing K activation.

The open circles of the graph show the concomitant changes at the recording site in the axon. The spike amplitude remained constant throughout the train of discharges and is shown by the single open circle some 8 mv above the amplitude of the initial soma spike. Occasionally axon spikes were smaller than those of the soma, presumably because of injury to the axon during penetration of the microelectrode. The changes in membrane potential of the axon induced by the currents applied at the soma were rather small, indicating that the length constant (λ) was small. The ratio ΔE axon/ΔE soma for a given current was about 0.08 in the hyperpolarizing quadrant and about 0.06 in the depolarizing one during the plateau phase. When Li had induced depolarization, however, the ratio between axon and soma depolarizations was of the order of 0.2 to 0.3. Thus, it is likely that the depolarization induced

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after the spikes had ceased. The slope resistance at the soma in the dotted portion of the graph was almost as low as that during the peaks of the spikes, indicating that a large conductance increase due to depolarizing K activation must have developed. The depolarization induced in the axon was only about 6% of that in the soma.

in the axon was only in part due to electrotonic spread from the soma, the major part of the depolarization being due to the direct, though smaller, action of Li on the axonal membrane.

As may be expected from the *I-E* relation of Fig. 8, the depolarization induced by Li decreased the slope resistance (dE/dI) of the soma membrane. However, when the membrane was repolarized with a steady inward current the slope resistance was again increased to about the same value as in the control, regardless of the level of the depolarization induced by the Li. Thus, the change in membrane resistance was due mainly or entirely to the depolarizing K activation and not to a direct action of Li.

DISCUSSION

The Absence of a Diffusion Barrier at the Receptive Membrane The persistence of the generator potential in Li and the slow depolarization induced by this ion might suggest that a diffusion barrier of some sort prevents the replacement of Na with Li at the cell membrane. However, it has already been reported (Edwards et al., 1963) that both the generator potential and the spike electrogenesis are abolished immediately after substitution of either sucrose or choline for Na. This was confirmed in the present work. Further evidence demonstrating the absence of a significant diffusion barrier in the crayfish stretch receptor preparation will be presented elsewhere (Obara, this journal, in press).

The Generator Membrane As was already mentioned, the electrically inexcitable input membranes of several types of cells discriminate between Na and Li, while spike-generating components do not. However, the finding that the crayfish stretch receptor develops a generator potential after substitution of Li for Na denotes that discrimination between the two cations is not a general characteristic of electrically inexcitable membrane. In fact, in its active state, the input membrane of the crayfish stretch receptor is also rather unspecific for a number of monovalent organic cations (Obara, 1967, and this journal, in press). A few preliminary experiments (by Dr. H. Sugi, cited in Ozeki and Grundfest, 1967) showed that the EPSP's of frog muscle were blocked after the preparation had been exposed for 1 or 2 hr to Li. However, this reversible block must be ascribed to an effect of the Li on transmissional activity, presumably in the presynaptic component, since the end plate is still depolarized by applications of acetylcholine (personal communication from Drs. L. Cohen and R. A. Steinhardt). The generator potentials evoked in the crayfish stretch receptor in the presence of Li are somewhat smaller than those produced in the presence of Na (Fig. 1), presumably indicating that the membrane is somewhat less permeable to Li than it is to Na. The axon membrane of the stretch receptor neuron also appears to be less permeable to Li than to Na, since the amplitude of the axon spike is diminished by the substitution.

During spike electrogenesis of the squid axon, however, the permeability for Li is about the same as, or somewhat greater than that for Na (Chandler and Meves, 1965). The findings on *Limulus* photosensitive neurons described above present still another variant in membrane properties. At first the cells are impermeable to Li, but upon continued exposure to the Na-free medium they become somewhat permeable to this cation.

The Spike-Generating Membrane The depolarization that is induced by Li does not itself appear to be the sole cause of the block of soma spikes. Slowly adapting cells that are depolarized by intracellularly applied currents to the same or to a larger degree continue to respond with spikes for a long time (Fig. 7). However, repolarization of the Li-depolarized cells does restore the spikes of the soma (Figs. 5 and 6), but these spikes are considerably smaller than in the control conditions. Presumably, therefore, the block is due to the combined effects of the rapid decrease in spike amplitude by Li, and of the slower depolarization.

In the experiment of Fig. 7 the depolarization of the soma must have been considerably larger than that of the trigger zone. Yet, the soma membrane continued to generate spikes throughout the period of applied current. The capacity of the slowly adapting receptor neurons to respond to long continued depolarization is a specific property of an electrically excitable membrane component, which accommodates much more slowly than does the membrane of the rapidly adapting cell (Nakajima, 1964). The slow accommodation is confined to a region of the neurite no more than about 0.5 mm distant from the cell body (S. Nakajima, personal communication). The data of Fig. 7 indicate that the membrane of the soma also accommodates slowly, but it differs from that of the trigger zone with respect to electrical threshold (Edwards and Ottoson, 1958). The differences in properties of the different regions of the membrane are presumably due to differences in the kinetics of the various electrically excitable ionic processes which have not as yet been analyzed.

The Li-Induced Depolarization Irreversible depolarization is caused in vertebrate unmyelinated nerve fibers (Ritchie and Straub, 1957) and more or less reversible depolarization also occurs in some other cell types (Schou, 1957) including heart muscle (Trautwein and Schmidt, 1960; Carmeliet, 1964) on exposure to Li. No depolarization was observed in the photosensory neurons of *Limulus* (Millecchia et al., 1966), or in muscle fibers of crayfish (Ozeki and Grundfest, 1967), or frog (Onodera and Yamakawa, 1966; Sugi, unpublished data). In the frog preparation, however, there is a gradual increase in miniature EPP's on prolonged exposure to Li, and this is considered as suggesting that the presynaptic terminals are depolarized in the presence of Li (Onodera and Yamakawa, 1966). It is noteworthy, however, that conduction along the

axon of the stretch receptor remains unimpaired for a long time and that the depolarization of the axon by Li is much smaller than that of the soma.

The differences between the effects of Li on soma and axon are not due to different degrees of accessibility of the Li to the membrane in the different regions of the cell. The axon spike becomes smaller immediately on perfusing the preparation with Li, but does not change significantly further (Fig. 5), until there is marked depolarization. Furthermore, the axon spike is abolished within 30 sec after replacement of Na by Tris, choline, or sucrose (Obara, 1967, and this journal, in press.)

The present work has not established the mechanisms of the Li-induced depolarization. However, it appears likely that the depolarization is linked to an interference with the Na pump system by Li. Such interference has been observed in various tissues (Keynes and Swan, 1959). In the slowly adapting stretch receptor, the posttetanic hyperpolarization (PTH) that is due to an electrogenic component of the Na pump system is abolished by Li (Nakajima and Takahashi, 1966). However, the Li-induced depolarization which occurs in both types of receptor neurons was not observed in that work, very probably because the earlier experiments were of only brief duration (personal communication from Drs. Nakajima and Takahashi).

Only about 20–30% of the Na influx that results from a tetanus or from iontophoretic injection of Na can be extruded by the electrogenic component that causes the PTH of the slowly adapting cell (Nakajima and Takahashi, 1966). It has been suggested that a component of the Na pump system might be asymmetrical and thereby could contribute an emf to the resting potential (Grundfest et al., 1954; Grundfest, 1955). If the rate of pumping of this component were relatively independent of the intracellular concentration of Na there would be no overt electrogenesis, but it would manifest itself as a depolarization when the pump was blocked. If such a "pump emf" does occur it is likely that it would exist in both types of cells. Thus, block of the pump electrogenesis by Li might cause the depolarization that has been observed in the present work in the rapidly as well as in the slowly adapting cells. An alternative possibility is that the depolarization is itself due to accumulation of Li, and a loss of K within the cell by virtue of the block of the electrically neutral pump component.

Experiments on drug-induced interference with the Na pump of crayfish stretch receptors are as yet preliminary and will be reported in detail at a later time. Ouabain caused gradual depolarization of a few millivolts with a time course that is similar to that of the Li-induced depolarization and further depolarization developed still more gradually. However, when applied for more than 1 hr with the medium at pH 7.4, DNP had no effect on the membrane potential (Nakajima and Takahashi, 1966).

Thus, while block of the Na pump system appears to be implicated in the

depolarization, the specific nature of the effect is not yet clear. The present data do, however, demonstrate that there must be differences with respect to permeability to Li in the membrane at the soma and the axon. If, as was suggested above, the Na pump system has several different components, these may be distributed in different proportions in different parts of the same cell, as well as in the two types of stretch receptor neurons.

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