Propagation of Action Potentials in Squid Giant Axons

Repetitive Firing at Regions of Membrane Inhomogeneities

F. RAMÓN and J. W. MOORE

From the Department of Physiology, Duke University Medical Center, Durham, North Carolina 27710

ABSTRACT   Effects of reduction in potassium conductance on impulse conduction were studied in squid giant axons. Internal perfusion of axons with tetraethylammonium (TEA) ions reduces G_K and causes the duration of action potential to be increased up to 300 ms. This prolongation of action potentials does not change their conduction velocity. The shape of these propagating action potentials is similar to membrane action potentials in TEA. Axons with regions of differing membrane potassium conductances are obtained by perfusing the axon trunk and one of its two main branches with TEA after the second branch has been filled with normal perfusing solution. Although the latter is initially free of TEA, this ion diffuses in slowly. Up until a large amount of TEA has diffused into the second branch, action potentials in the two branches have very different durations. During this period, membrane regions with prolonged action potentials are a source of depolarizing current for the other, and repetitive activity may be initiated at transitional regions. After a single stimulus in either axon region, interactions between action potentials of different durations usually led to rebound, or a short burst, of action potentials. Complex interactions between two axon regions whose action potentials have different durations resembles electric activity recorded during some cardiac arrhythmias.

INTRODUCTION

Changes in membrane action potentials have been observed after application of many drugs and toxins. In particular, it is well known that internal perfusion of squid axons with tetraethylammonium (TEA) ions decreases potassium conductance and prolongs the action potential (Lorente de Nó, 1949; Tasaki and Hagiwara, 1957; Armstrong and Binstock, 1965). However, effects of such changes on conduction of impulses have not been studied. We have chosen the squid axon for studies of propagation of long-duration action potentials obtained by internal perfusion of TEA ions. Membrane inhomogeneities were obtained by perfusing only one of the two main axon branches with TEA.

METHODS

Experiments were conducted on giant axons excised from squid, *Loligo pealii*, available at the Marine Biological Laboratory at Woods Hole, Mass. Axons were excised and dissected beyond the main bifurcation region and cleaned before placing them in a chamber filled with artifical seawater (ASW). Most experiments were conducted at room temperature (about 21°C) except where stated otherwise. Axon trunks were ~ 350-600 μm in diameter and 2-3 cm long and axon branches ~ 230-380 μm in diameter and 3 cm long. Axons were internally perfused following a procedure designed by Baker et al. (1962). Briefly, this procedure consists in extruding out the axoplasm by gently squeezing the axon with a small roller made of rubber and then reinflating it through a canula filled with a standard internal solution.

The standard internal solution (SIS) consisted of (in millimolar): K-glutamate 320; sucrose 300; NaF 50; and phosphate buffer 30, at a pH of 7.8. Internal solutions which also contained TEA (20 mM) will be referred to as SIS-TEA. The external solution was artificial seawater of the following composition (in millimolar): NaCl 450; $CaCl_2$ 40; KCl 10; and Hepes 5, at a pH of 7.4.

For experiments requiring two membrane regions of different properties, axons were prepared as follows. After dissection, cleaning, and squeezing out the axoplasm as described above, the whole axon was reinflated with SIS, tied off, and transferred to the experimental chamber. There a cut was made in only one axon branch placed in a small separate compartment that prevented SIS from mixing with ASW (Fig. 1 B). Thus, when normal perfusion solution was replaced by SIS-TEA, it flowed only through the axon trunk and one branch, while the unperfused branch remained filled with normal SIS. Diffusion of TEA into the static internal solution of the unperfused branch was slow, and changes in shape of action potentials recorded at this branch appeared after 30-60 min. Most of our experiments could be completed before this time; however, some experiments were performed after a small amount of TEA had already diffused in (e.g., Fig. 5).

Action potentials were recorded with microelectrodes pulled from glass tubing containing glass fibers and filled with 2.5 M KCl with resistances of 10-15 MΩ. Microelectrodes were connected to DC preamplifiers, and potential traces were displayed on an oscilloscope for photographic recording. Resting membrane potentials averaged about −63 mV and appeared independent of the effect of TEA. Action potentials were initiated by stimulating the axons with short electrical pulses delivered through a pair of small hook platinized-platinum external electrodes.

RESULTS

Membrane Action Potential

Fig. 2 A shows membrane action potentials produced by the space-clamp region of an axon as obtained before and after internal application of TEA. Action potentials have similar rate of rise. Fig. 2 B, at a slower sweep speed, shows a complete action potential under these conditions of space clamp.

Propagation in Homogeneous Axons

Perfusion of an axon by TEA also increases the duration of a propagated action potential but has no effect on its speed of propagation. Fig. 2 C and D shows marked increase in duration of propagated action potentials in axons internally

perfused with TEA. Although action potentials become very prolonged, the rising phase does not change significantly (from 282 to 228 V/s in Fig. 2 C, lower trace). This prolongation of action potentials is uniform along a single axon.

The duration of a second action potential depends upon the time interval between two stimuli. When the interval between stimuli is long, a second potential has similar duration as the first one. As the interval between stimuli is decreased, the duration of a second action potential also decreases (Fig. 3).

The shortening in duration of secondary action potentials is further exagger-
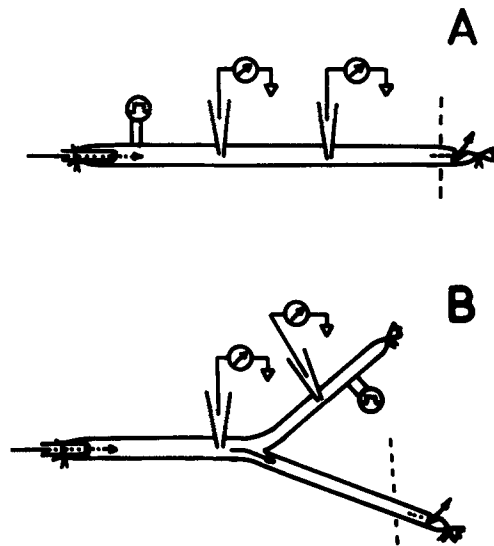

FIGURE 1.   Diagram of the experimental setup used for perfusion experiments in squid giant axons. (A) A single homogeneous axon perfused with solutions flowing from the internal canula and out through the cut. Membrane potentials were measured at different points by microelectrodes. (B) Method used to obtain two axon regions with different internal concentrations of TEA. The perfusion solution flows through the canula into the axon trunk and one of the axon branches. No flow occurs in the other axon branch initially filled with TEA-free SIS. Microelectrode recordings are made in the tied axon branch and at the axon trunk near the bifurcation region. In both cases the cut end of the axon is placed in a small separate compartment to prevent SIS from mixing with ASW that fills the rest of the chamber. External electrodes are used for stimulation.

ated by a train of stimuli. Fig. 4 A and B shows action potentials elicited with a train of stimuli with a frequency of 17 and 23 cycles/s, respectively. Each stimulus elicits a single action potential; however, the duration of successive action potentials is further reduced with each additional stimulus. As the frequency of stimulation is increased to 32 cycles/s (Fig. 4 C), the second stimulus occurs during the falling phase of a first action potential and causes a small increase in duration. All subsequent stimuli produce action potentials which decrease in duration successively. Fig. 4 D shows action potentials

resulting from a stimulus train with a frequency of 37 cycles/s. A second stimulus fails to elicit a response, and later action potentials increase in amplitude as duration decreases.

Propagation in Inhomogeneous Axons

Fig. 5 shows action potentials recorded at the axon trunk perfused with SIS-TEA after a small amount of TEA had diffused into the branch originally perfused with SIS only (see Fig. 1). Action potentials were initiated in the axon
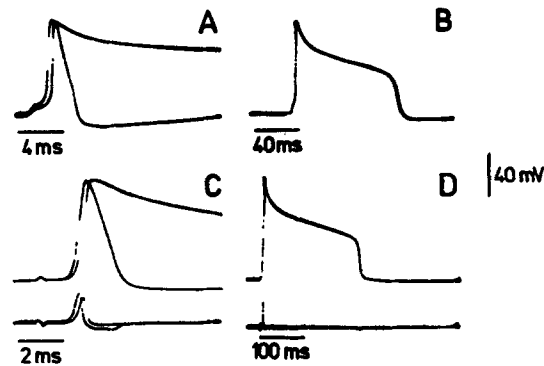

FIGURE 2. Action potentials recorded from homogeneous squid axon during internal perfusion with SIS-TEA. (A) Superimposed traces of membrane action potentials recorded before and after initiating axon perfusion with TEA. Note that the slope of the rising phase of the action potentials is similar. (B) Final shape of the membrane action potential. (C) Propagated action potentials recorded before and after perfusion with TEA. Note the similarity with membrane action potentials (A). Lower trace in C (dV/dt) shows small change in spite of the marked prolongation of the action potential. (D) Propagated action potential at a lower sweep speed. Bath temperature in A and B is 8°C, in C 11°C, and in D 5°C. Traces have been retouched. Calibration bar for dV/dt in C and D is 400 V/s.
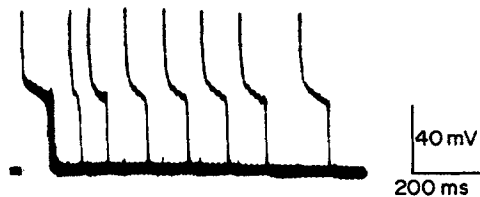

FIGURE 3. Changes in action potential duration due to different intervals between pairs of stimuli in an axon uniformly perfused with TEA. Traces have been retouched.

trunk by a single stimulus pulse. The longer duration action potential, recorded at the axon trunk, travels to the axon branch where a spike of shorter duration is recorded (Fig. 5 A). As SIS-TEA perfusion is continued, the long duration action potential in the trunk elicits a second spike from the initially TEA-free branch (Fig. 5 B). That second spike acts as a stimulus to prolong the action potential in the trunk in a manner consistent with observation in Fig. 4 C. Later

still, in Fig. 5 C and D, the action potential duration in the axon trunk is much longer, producing a short train of action potentials at the SIS-perfused branch which appears to give reinforcements to the plateau in the trunk. After this, the duration of the plateau in the trunk is reduced enough (as if following a train of
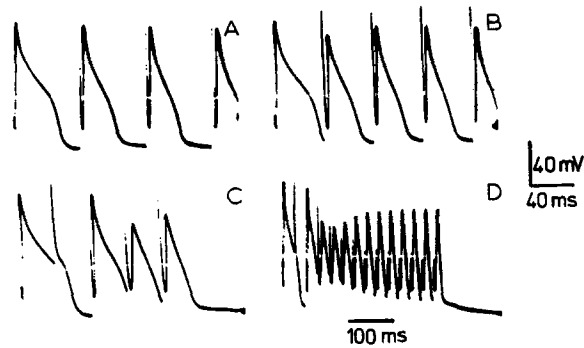

FIGURE 4. Changes in action potentials with a train of impulses in a uniformly TEA-treated axon. (A) Action potential shapes at a train frequency of 17 cycles/s. When the frequency of stimulation is increased to 23 cycles/s (B), the second and consecutive action potentials are of shorter duration and amplitude. At train frequencies of 32 cycles/s (C), the second stimulus does not elicit a response, but consecutive stimuli elicit action potentials. A train of 37 cycles/s (D) demonstrates the shape of consecutive action potentials after the second stimulus fails to elicit response.
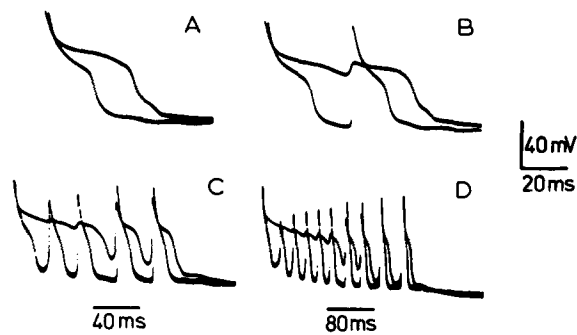

FIGURE 5. Action potentials recorded at two axon regions affected differently by the internal perfusion with TEA. In all parts of the figure the longer action potential is recorded at the axon trunk, where a single stimulus is applied, and the short duration action potential is from the axon branch where the internal solution initially TEA-free is static. As the action potential at the axon trunk increases in duration, the axon branch fires a second (B), and then multiple action potentials (C-D). In all action potentials the direction of propagation is from the axon trunk towards the branch.

impulses as in Fig. 4) so that it has almost recovered before the next action potential is generated in the branch. The sequence terminates spontaneously when the spikes are in synchrony and when the duration of the action potential in the trunk becomes almost as brief as that in the branch.

When a single stimulus is applied to the SIS-perfused branch a complementary interaction is seen. Fig. 6 shows action potentials recorded in the trunk near the bifurcation (upper trace) and at the branch (lower trace). TEA perfusion of the trunk and a main branch is initiated, and upper traces in Fig. 6 show progressive effects (Fig. 6 A-D) of prolongation of action potentials there. With lengthening of the action potential, a hump develops on the falling phase of action potentials in the TEA-free branch (Fig. 6 B, lower trace). This hump changes into an action potential of small amplitude (Fig. 6 C, lower trace). With continued lengthening of action potentials at the trunk, the TEA-free branch develops two small action potentials (Fig. 6 D). The second, of larger amplitude than the first, reflects back to the axon trunk producing a hump at the end of the repolarization phase. This hump produces a third small action potential in the axon branch (Fig. 6 E). Later, a very complex interaction
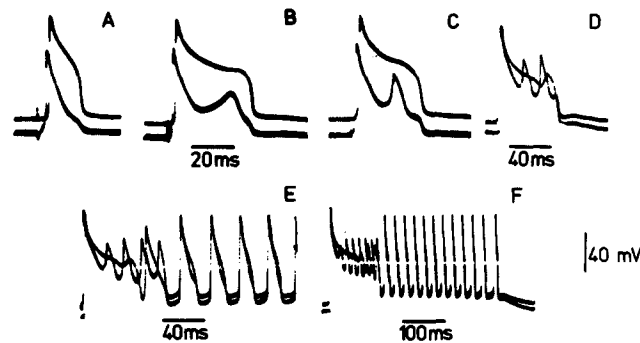

FIGURE 6.   Action potentials recorded at two axon regions affected differently by internal perfusion with TEA. In all parts the upper trace is the recording from the axon trunk and the lower trace is from the axon branch initially TEA-free. A single stimulus is applied at the axon branch to elicit the propagated response. As the action potential at the axon trunk becomes longer it elicits one (C) or several responses at the axon branch (D). The second response from the axon branch reflects back on the action potential from the axon trunk (D). Finally, there is a short train of action potentials following a complex interaction between both axon regions (E-F).

between both axon regions occurs as spikes of different amplitude ride on action potentials recorded at the axon trunk. Finally, both axon regions synchronize their activities in a train of action potentials that terminates spontaneously (Fig. 6 F). Progressive increase in synchronization of falling phases of the spikes in the training can be seen in Fig. 6 E. For the later spikes, synchronization can be observed only at much faster sweep speeds. During the period of interaction between those axon regions, the speed of propagation of initial action potentials remain unchanged (~10 m/s), while that of reflected spike varies (1.5-2.2 m/s).

DISCUSSION

The effect of TEA on excitable membranes is well known. Original observations by Lorente de Nó (1949) about prolongation of nerve action potentials due to

external application of TEA, were followed by several studies on nerves and muscle fibers trying to elucidate its mechanism of action (c.f. Narahashi, 1974). Understanding of the mode of action of TEA was facilitated by the observation that TEA placed inside of squid axons prolongs the duration of action potentials (Tasaki and Hagiwara, 1957). It is now clear that the effect of TEA on excitable membranes can be explained by a blockage of membrane potassium channels (Armstrong and Binstock, 1965; Armstrong, 1975). However, there is also some effect, although comparatively small, on sodium channels (Armstrong and Binstock, 1965).[1]

Earlier experiments conducted to test the effect of TEA on skeletal muscle fibers, resulted in observations and conclusions similar to ours from squid axons. Hagiwara and Watanabe (1955) observed repetitive spikes in response to a single stimulus in muscle fibers treated with external application of TEA. These authors concluded that repetitive firing is an effect not specific to TEA, but that it would be caused by any factor tending to prolong the action potential. Similar prolongation of action potentials and subsequent repetitive firing could be obtained by other blocking agents of potassium channels such as Aminopyridine (Yeh et al., 1976) or by slowing the inactivation of sodium channels with pronase (Armstrong et al., 1973) or N-bromoacetamide (Oxford et al., 1978).

In squid axons the rising phase of action potentials is caused by sodium currents and, as we noted above, effects of TEA on the sodium system are small. Thus, action potentials elicited by stimulus pulses propagate at normal speed for squid axons. However, the conduction velocity of a second and all subsequent action potentials decreases because partial inactivation of the sodium system due to residual depolarizations.

Membrane action potentials in squid axons perfused with TEA have a shape similar to propagating action potentials in axons uniformly perfused with TEA. Therefore, further changes in shape and additional spikes seen in homogeneous axons must be caused by interactions between regions of different membrane characteristics. Membrane regions producing long action potentials act as a prolonged source of stimulation for those regions where spikes are of shorter duration. This source persists throughout the repolarization phase and may initiate action potentials in neighboring axon. If these membrane regions can develop a complete action potential and repolarize before the current source is terminated, they may be excited again. These interactions may last for hundreds of milliseconds during which numerous action potentials are traveling back and forth along the axon.

Spatial interactions between membrane regions generating action potentials of different duration, by themselves, do not produce repetitive firing. It is also necessary for a membrane to be capable of repetitive excitation; that is, "intrinsic automaticity." However, this automaticity does not require the presence of a specific mechanism such as that proposed for cardiac muscle (Katzung and Morgenstern, 1977), and it can be explained by the normal activation and inactivation of axon sodium and potassium channels (Hodgkin and Huxley, 1952).

[1] Ramon, F., and J. W. Moore. Unpublished observation.

Similarities between cardiac muscle action potentials and those from TEA-treated axons was noted first by Tasaki and Hagiwara (1957). Temporal and voltage dependence of conductance changes in cardiac muscle membrane seem to be different from those in squid axons (McAllister et al., 1975; Beeler and Reuter, 1977). However, similarities in cable properties between those two tissues support the following explanation for initiation of some cardiac muscle arrhythmias: if two cell regions (or two different cells connected by means of low resistance pathways) have action potentials whose duration is very different, they can interact in the manner shown above. Regions with long action potentials would constitute a source of current for excitation of other neighboring connected cells. Prolonged action potentials could be triggered by arrival of a single impulse and such interactions may persist for long periods of time. In axons, spontaneous activity seems to stop when all membrane regions are synchronized, but in a tridimensional tissue it is difficult to predict the duration of these interactions. Action potentials of bizarre shape and complex interactions between different regions of cardiac muscle can be easily found in heart during ischemia (Lazzara et al., 1973) and other pathological conditions (Trautwein, 1970) and are thought to be the source of some cardiac arrhythmias.

REFERENCES

ARMSTRONG, C. M. 1975. Ionic pores, gates and gating currents. *Q. Rev. Biophys.* **7**:179–210.

ARMSTRONG, C. M., and L. BINSTOCK. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. Gen. Physiol.* **48**:859–872.

ARMSTRONG, C. M., F. BEZANILLA, and E. ROJAS. 1973. Destruction of sodium conductance inactivation in squid axons perfused with pronase. *J. Gen. Physiol.* **62**:375–391.

BAKER, P. F., A. L. HODGKIN, and T. I. SHAW. 1962. Replacement of the axoplasm of giant nerve fibers with artificial solutions. *J. Physiol. (Lond.).* **164**:330–337.

BEELER, G. W., and H. REUTER. 1977. Reconstruction of the action potential of ventricular myocardial fibers. *J. Physiol. (Lond.).* **268**:177–210.

HAGIWARA, S., and A. WATANABE. 1955. The effect of tetraethylammonium chloride on the muscle membrane examined with an intracellular microelectrode. *J. Physiol. (Lond.).* **129**:513–527.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* **117**:500–544.

KATZUNG, B. G., and J. A. MORGENSTERN. 1977. Effects of extracellular potassium on ventricular automaticity and evidence for a pacemaker current in mammalian ventricular myocardium. *Circ. Res.* **40**:105–111.

LAZZARA, R., EL-SHERIF, and B. J. SCHERLAG. 1973. Electrophysiological properties of

canine Purkinje cells in one-day myocardial infarction. *Circ. Res.* **33:**722–734.

LORENTE DE NÓ, R. 1949. On the effect of certain quaternary ammonium ions upon frog nerve. *J. Cell. Comp. Physiol.* **33**(Suppl.):1–231.

MCALLISTER, R. E., D. NOBLE, and R. TSIEN. 1975. Reconstruction of the electrical activity of cardiac Purkinje fibers. *J. Physiol. (Lond.)* **251:**1–59.

NARAHASHI, T. 1974. Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54:**813–889.

OXFORD, G. S., C. H. WU, and T. NARAHASHI. 1978. Removal of sodium channel inactivation in squid giant axons by *N*-bromoacetamide. *J. Gen. Physiol.* **71:**227–247.

TASAKI, I., and S. HAGIWARA. 1957. Demonstration of two stable potential states in the squid giant axon under tetraethylammonium chloride. *J. Gen. Physiol.* **40:**859–885.

TRAUTWEIN, W. 1970. Mechanisms of tachyarrhythmias and extrasystoles. In: Symposium on cardiac arrhythmias. E. Sandoe, E. Flensted-Jensen, and K. H. Olesen, editors. AB Astra, Sodertalje, Sweden.

YEH, J. Z., G. S. OXFORD, C. H. WU, and T. NARAHASHI. 1976. Interactions of Aminopyridines with potassium channels of squid axon membranes. *Biophys. J.* **16:**77–81.