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Excitability of Squid Axon Membrane in the Absence of Ion-concentration Gradient across the Membrane

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ABSTRACT

A squid axon membrane separating two solutions of the same chemical composition can exhibit electrical excitability. Passage of a constant inward current through such a membrane induces oscillatory responses in membrane potential. The salts of cobalt, manganese, nickel, and barium are suitable as a constituent in the solution for demonstrating this oscillatory response.

INTRODUCTION

In order to construct a physicochemical theory of nerve excitation, it is advantageous to start the reasonings on the basis of experiments carried out under a simple ionic condition. One successful approach along this line was the demonstration of bi-ionic action potentials, namely, excitation processes which involve only two cations (7-9, 15-17). I present here an alternative approach, a study on membrane excitability involving only one species of cation. The absence of ion-concentration gradient under mono-ionic conditions may reduce ionic theory for nerve excitation into a simple form, thus greatly facilitate understanding of nerve excitation from physicochemical points of view.

MATERIALS AND METHODS

A giant axon of the squid (Loligo pealei) was excised and mounted in a Lucite chamber containing natural sea water. Two glass cannulae for internal perfusion were introduced into the axon. Next, the natural sea water in the chamber was replaced with a continuously flowing solution which contained a divalent-cation salt at the concentration of 1 or 2 mM. Subsequently, the axoplasm was replaced with the same solution as that used externally. Finally, both potential recording and current-supplying electrodes were inserted into the perfusion zone through the outlet cannula (see Fig. 1).

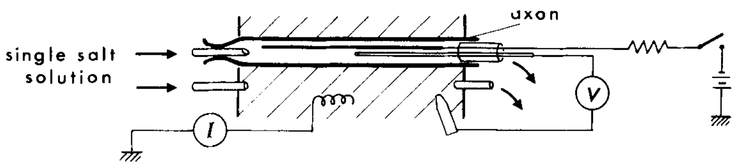

Fig. 1 Schematic diagram of the experimental set up used.

The solution was prepared by adding a small amount of a concentrated divalent-cation solution to a 12% (v/v) glycerol solution. The electrode used for recording the internal potential was a combination of Ag-AgCl wire and a thin glass pipette filled with 0.6 M KCl-agar. The electrode used for measuring the external potential was a calomel half cell. Platinum wire electrodes were sometimes used instead of these KCl-containing electrodes. The current-supplying electrodes were pieces of platinized platinum wire placed inside and outside of the axon. A constancy of the membrane current was assured by a 10MΩ resistance placed in the circuit in series to the axon membrane.

RESULTS

When axons were perfused intracellularly and extracellularly with solutions containing a cobalt salt only, the potential difference across the membrane remained quiescent in the range of -20 to +15 mV. Upon application of a constant electric current through the membrane, oscillatory variations of the transmembrane potential were observed. The response shown in Fig. 2 (upper trace) was obtained with using solutions containing 1 mM cobalt citrate and 12% glycerol internally and externally. These responses could be induced only when the direction of current was inward. An abrupt rise (an upward deflection in Fig. 2) in internal potential was accompanied by a large (7-fold) increase in membrane conductance which was measured by superposing small pulses of current on the sustained inward current.

The shape and the amplitude of these oscillatory responses varied widely

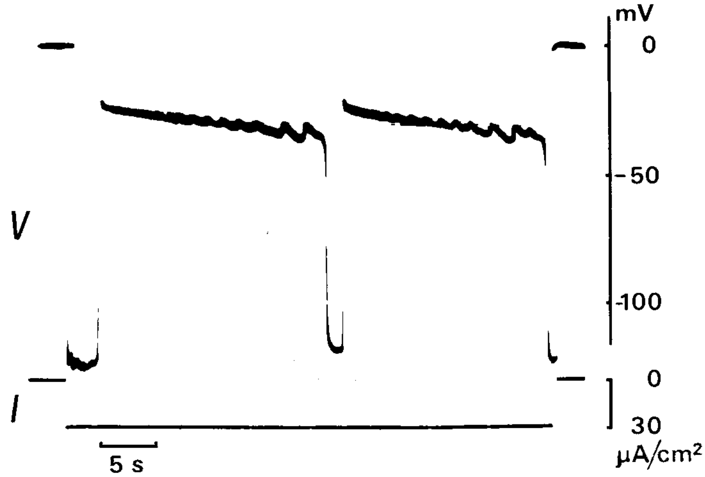

Fig. 2 Oscillatory response obtained with 1 mM cobalt citrate internally and externally.

from axon to axon. Usually at the beginning of observation on one axon, the amplitude of responses was large, rise and fall of the responses were very quick and the period in which the membrane potential stayed in high level was long. Afterward, this period became shorter and the fall of the membrane potential became slow. When the current applied to the membrane lasted for more than 1 min, the responses gradually became smaller in amplitude and shorter in duration. However, large and long responses reappeared after the current had been interrupted and then re-established, suggesting the existence of a kind of refractoriness. Later, the frequency decreased again, and eventually oscillatory responses failed to appear upon simple application of inward current. Responses could be induced further by superposing small and short outward current pulses on the sustained inward current. In this case, the amplitude of the small pulses had to be larger than a certain level which might be called a threshold. The conductance change, the refractoriness, and the presence of threshold suggest that the oscillatory variation of the membrane potential is a phenomenon similar to a repetitive firing of action potentials. Very similar responses in membrane potential described above could be observed with the use of manganese salts in the place of the cobalt salt.

In addition to manganese and cobalt salts, barium chloride and nickel chloride could be used to demonstrate oscillatory responses. When a barium chloride solution was used, rise and fall of membrane potential repeated at high frequency (Fig. 3). Sometimes, the response appeared as a burst of spikes; the duration of each spike was as short as 100 msec. On the contrary,

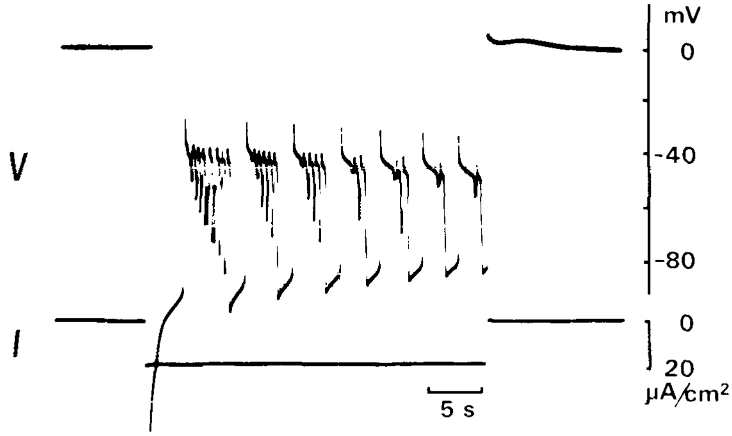

Fig. 3 Oscillatory response obtained with 2 mM barium chloride internally and externally.

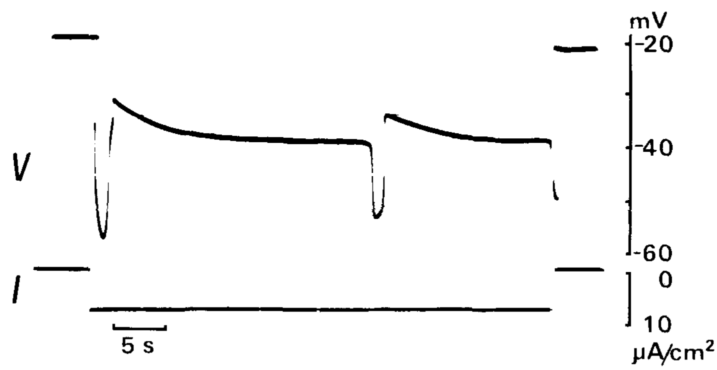

Fig. 4 Oscillatory response obtained with 2 mM nickel chloride internally and externally.

when nickel chloride was used, the duration of oscillatory responses was long and the frequency of them was low (Fig. 4). It was difficult to obtain responses of large amplitude by using nickel chloride.

It was extremely difficult to obtain oscillatory responses when a solution of calcium chloride, magnesium chloride, or strontium chloride was used. In 10 axons examined with each solution, fall of the membrane potential was not large in spite of a strong inward current. Very small responses shown in Fig. 5 were barely obtained with a 2 mM calcium chloride solution. It was also

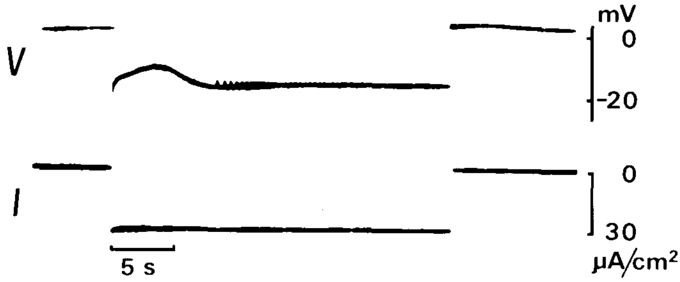

Fig. 5 Oscillatory response obtained with 2 mM calcium chloride internally and externally.

difficult to obtain the oscillatory response when solutions of cupric chloride, cadmium chloride, zinc chloride, and ferrous chloride were used. In these cases, however, a stepwise decrease in the inward current induced a

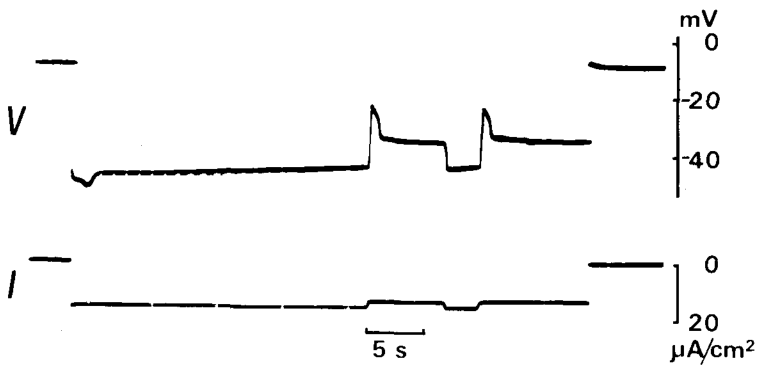

Fig. 6 Response in membrane potential obtained with 2 mM cadmium chloride internally and externally.

single response as shown in Fig. 6. Superposition of a small pulse of outwardly directed current on the sustained inward current also induced a single response. These responses were similar to the deteriorated responses observed in the axons perfused with a cobalt or manganese solution.

The strength of current applied to the membrane affected the amplitude and the interval of oscillatory responses. With higher strength of current the amplitude of responses became larger and the interval between the fall of a response and the rise of the next response became longer. Such a relationship observed from an axon perfused intracellularly and extracellularly with a 2 mM manganese chloride solution is shown in Fig. 7. The thin broken line indicates the weakest possible current which would induce the oscillatory response.

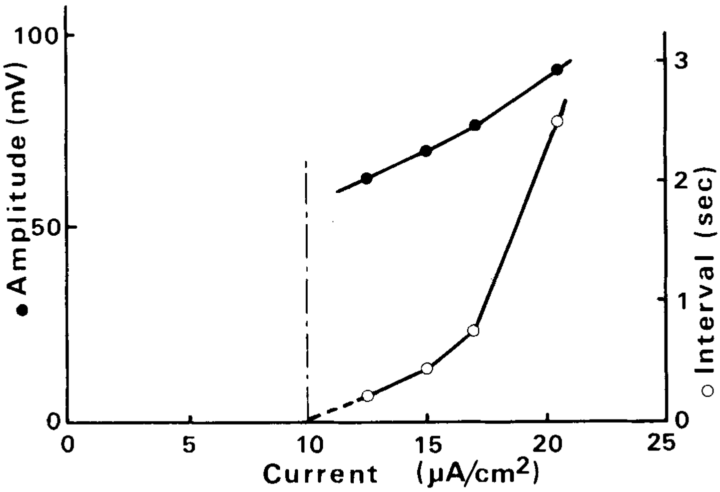

Fig. 7 Dependence of the amplitude and the interval of responses on the strength of current.

DISCUSSION

The squid axon is found to be capable of maintaining electrical excitability under mono-ionic conditions in which the internal and external concentrations of ions are the same. The results obtained and the conditions employed are very similar to those of inanimate membranes such as porous glass membranes (10, 11), Sephadex gel membranes (13), polyelectrolyte membrnaes (5, 6), and lipidic membranes (3, 4). The physicochemical processes underlying oscillatory phenomena in these inanimate membrane systems have been explained satisfactorily by several investigators (1, 2, 12, 14). A slight modification of the theories proposed for inanimate membranes may be enough to account for the phenomena described here, provided that interactions such as electrostatic cross-linkage or coordination bonding between divalent cations and the membrane macromolecules are taken into consideration. The approach presented here probably fills the gap between the studies of artificial membranes and those of biological membranes living under normal ionic conditions. The result of detailed studies will be published elesewhere.

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