

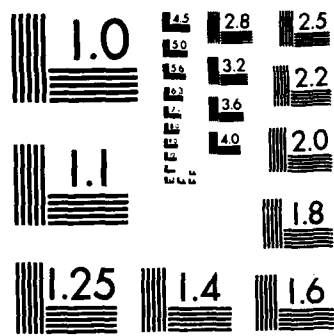
AD-A194 459 IONIC BASIS OF POTENTIAL REGULATION(U) BAYLOR COLL OF 1/1
MEDICINE HOUSTON TX DEPT OF PHYSIOLOGY AND MOLECULAR
BIOPHYSIC S D C CHANG 1988 N00014-85-K-0424

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FINAL REPORT

IONIC BASIS OF POTENTIAL REGULATION

ONR Contract No. N00014-85-K-0424

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The objectives of this contracted research is to understand the mechanisms by which the resting potential of the nerve cell is controlled. The following is a summary of the major findings of this contracted research:

- 1) A new understanding of the physiochemical basis of potential regulation at the resting state. It is generally assumed that the resting potential of a nerve cell is determined by a mixture of chemical potentials of K^+ and Na^+ , as described by Goldman - Hodgkin - Katz (GHK) equation. Using the squid giant axon as a biological model, we studied how the resting potential (V) varied as a function of internal and external ionic concentrations. We found that, although the $[K]_o$ - dependence of V under high external Na could be fitted with the GHK equation, this fitting was fortuitous. When one reduced the external Na^+ , the GHK equation could no longer describe the $[K]_o$ - dependence of the resting potential. Furthermore, the GHK equation failed to describe the effects of $[K]_i$ and $[Na]_o$ on V under the condition of low external K^+ concentration.

One possible explanation for the failure of the GHK equation is that the assumption of equilibrium between the K^+ activities at the outer surface of the membrane and in the external solution may be violated. By modifying this assumption, we are able to derive a new electrodiffusion equation which varies slightly from the GHK equation. This modified equation appears to describe the effects of $[K]_o$, $[K]_i$, $[Na]_o$ and $[Na]_i$ on V satisfactorily.

Our study suggest that the nerve cell has a mechanism of regulating its resting potential. The membrane acts as a potential buffer, which automatically limits the membrane potential to a pre-set

value. The K^+ gradient is required mainly to charge the membrane capacitor. Once the chemical potential of K^+ exceeds the pre-set membrane potential, the resting potential becomes self-regulated and is no longer sensitive to the variation of internal or external concentrations of K^+ .

- 2) A new type of K channel that controls the resting potential of the nerve axon. Unlike the case of the action potential, the membrane pathways responsible for the generation of the resting potential of a nerve cell have not been well understood. It is unclear whether the resting current passes mainly through a small number of excitable K and Na channels that stay open in the resting state, or, as some investigators have suggested, the pathway for resting current may simply be a "leakage" conductance. Using the internally perfused squid axon as a biological model, we studied the effects of various cations on the membrane potential, membrane conductance, and isotope-labelled efflux in the resting state. We found evidence that the semi-permeable property of the resting membrane is controlled by a new class of K channel. This resting K channel has a slight tendency of inward rectification and is activated at a voltage below the normal resting potential. Its ion-selectivity sequence, as determined from our V-clamp studies, $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+ \geq Li^+$, is similar to that of the resting membrane. The pharmacological properties of this resting channel seem to differ from those of the delayed rectifier K channel. For example, this resting channel is less sensitive to TEA (tetraethylammonium). Furthermore, the K efflux through this resting channel responds to a change of the external ionic environment in a

manner different from that of the delayed rectifier. When the external concentration of K^+ increases, the K^+ efflux is actually elevated. These findings suggest that this new class of K channel may be structurally different from the delayed rectifier K channel.

- 3) Ion-selectivity of the delayed rectifier K channel in squid axon. In spite of the fact that the delayed rectifier of the squid axon is one of the most well known examples of excitable K channel, the ion-selectivity of this channel has only been determined qualitatively (Oxford & Adams, Biophys. J. 33:70a, 1981; Matteson & Swenson, J. Gen. Physiol. 87:795-816, 1986) but not quantitatively. We found several complications in making such a determination: A) When we employed an instantaneous I-V method (such as those used by Matteson & Swenson), the reversal potential was often influenced by the accumulation of K ions in the periaxonal space. This was particularly a problem when the test ion in the external solution was Na^+ , Li^+ , or NH_4^+ . B) In some cases the current passing through the delayed rectifier does not obey the so-called "ion-independence" principle. External Cs^+ , Rb^+ and K^+ all interfered with outward K current. We have partially overcome these problems by 1) minimizing the K current when we activated the delayed rectifier for instantaneous I-V measurements (i.e., by decreasing $[K]_i$, lowering the depolarizing potential and shortening the depolarizing time); 2) in addition to measuring the reversal potential when the test ions were placed in the external solution, we also measured the relative amplitude of delayed current when the internal K^+ was replaced by test ions. Our best estimated values for the relative permeabilities of K^+ , Rb^+ , NH_4^+ , Na^+ and Li^+

through the delayed rectifier are 1: 0.25 : 0.08 : 0.05 : 0.04, respectively. Cs^+ appears to be practically impermeable to the K channel. Its permeability is less than 0.01 of that of K^+ .

- 4) Potassium efflux through the resting channel of squid axons. It has been suggested based on electrophysiological studies that the majority of resting potassium current passes through a membrane pathway different than the delayed rectifier K channel (Chang, Biophys. J. 50:1095, 1986). To further examine this hypothesis, we measured the K efflux from squid giant axons under voltage-clamp, with or without blocking the excitable K channel by internal application of TEA (tetraethylammonium). ^{42}K was applied inside the axon by an internal perfusion technique. The axoplasmic concentration of radioisotope thus remained constant throughout the experiment. Labelled K ions passing across the membrane were collected in the external perfusate. This method allows measurement of a steady-state unidirectional flux and avoids complications due to loading and unloading of ^{42}K into the periaxonal space and Schwann cell layer. Our measurements indicate:
- A) When the axon was clamped at the normal resting potential (-65 mV), the ^{42}K efflux was not significantly affected by the internal application of 20 mM TEA, suggesting that very little of the resting flux passes through the delayed rectifier. B) The K efflux increased with depolarization as expected. However, the V-dependence of efflux was less steep than that previously observed using a wash-out method (Hodgkin & Keynes, J. Physiol. 128:61-88, 1955). C) Elevation of the external K concentration from 10 mM to 100 mM stimulated ^{42}K efflux when the axon was clamped at constant voltage (-65 mV). This result

suggests that an ion exchange mechanism may be involved in the functioning of the resting channel.

- 5) Evidence of a Mg-activated channel which appears only in intact squid axons but not internally perfused axons. In the course of studying the resting conductance of intact squid axons, we observed a component of inward current (I_x) which was time-dependent and was activated at potentials more negative than -100 mV. I_x is peculiar because it appears only in the intact axon. Several axons were first studied in the intact state and then internally perfused with standard perfusion solution. The anomalous current disappeared completely following internal perfusion. Furthermore, we found that Mg ions were required to activate I_x . When Mg was replaced by Ca, I_x was greatly reduced or absent. Monovalent ions including Na, K, Rb, or choline failed to activate I_x . Although activation of I_x is strongly dependent on the external concentration of Mg, this anomalous conductance appears to have relatively poor ion selectivity. In the presence of external Mg, I_x is not very sensitive to the principal external cations, among which Na, K, NH_4 , Rb, Cs, Li, Ca, Mg, and choline have been tested. I_x showed inward rectification and is apparently separate from the commonly observed linear leakage current. Adding Mg to the internal perfusion solution or reducing internal Ca could not restore I_x in the perfused axon. It is suspected that activation of I_x may involve the action of cytoplasmic enzymes that require the presence of external Mg.

The publications resulted from this contracted research are:

Chang, D.C., 1986, Is the K permeability of the resting membrane controlled by the excitable K channel? Biophys. J. 50:1095-1100.

Chang, D.C., Hunt, J.R. and Gao, P.Q., 1987, Resting conductance of the squid axon membrane. Biol. Bull. 173:441.

Chang, D.C., 1987, Evidence for a resting K channel in the squid axon. Biophys. J. 51(2):545a.

Zhao, Z.Y. and Chang, D.C., 1987, Ion-selectivity of the delayed rectifier K channel in squid axon. Biophys. J. 51(2):52a.

Hunt, J.R. and Chang, D.C., 1987, Potassium efflux through the resting channel of squid axons. Biophys. J. 51(2):51a.

Fong, C.N. and Chang, D.C., 1988, K^+ -selective microelectrode study of internally dialysed squid giant axons. Biophys. J. (in press).

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