Relation of Membrane Properties of the Giant Muscle Fiber of a Barnacle to Internal Ionic Composition

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Giant muscle fibers 1 to 2 mm in diameter and 4 to 5 cm in length are found in the barnacle Balanus nubilus. The preparation offers an excellent opportunity for studying membrane mechanisms of excitation by controlling the internal ionic composition of the fiber. The internal ionic composition was altered by intracellular injection through a glass pipette longitudinally inserted into the fiber. A test solution of a given ionic composition was injected over the whole length of the fiber until the diameter increased by a factor of 1.5 to 2.0. This should make the internal ionic composition of the treated fiber close to that of the injected solution. The final concentration of K or Na inside each treated fiber was determined by flame photometry after each experiment. The internal Cl concentration was also determined by the mercuric nitrate method. To obtain a given concentration of the internal Ca++ or Mg++ concentration the injection was made with a solution containing EDTA or EGTA and Ca or Mg salts in varying proportions.

The resting potential of the fiber decreased approximately linearly with the logarithm of the external K+ concentration when an increasing amount of the external NaCl was replaced with KCl, but the slope was smaller than 58 mv for a tenfold change of the concentration. The slope became 58 mv when the K+ concentration was altered by keeping the product of [K+]out and [Cl−]out constant. This result indicates that the resting potential of the barnacle muscle fiber is determined by the concentration gradient of K+ and Cl− across the membrane. When the internal K concentration was decreased below the normal value (150 to 200 mM) by replacing a given amount of K in the injecting solution with sucrose, the resting potential decreased approximately linearly with the logarithm of the internal K concentration. The increase of the internal Cl concentration above the normal value (40 to 60 mM) always decreased the resting potential. These results, therefore, agree with the conclusion obtained in experiments with change of the external ionic composition. However, the result deviated when the internal K concentration was raised.
above 200 mM. The resting potential was rather insensitive to an increase of the internal K concentration above 200 mM, and tended to show a decrease with increasing internal K concentration when the latter exceeded 300 mM.

The fiber membrane was normally incapable of initiating an all-or-none spike potential. However, the membrane became capable of initiating all-or-none spikes when the internal Ca\(^{++}\) concentration was smaller than 5 \times 10^{-8} M. The overshoot of the spike potential increased linearly with the logarithm of the external Ca\(^{++}\) concentration and the slope was approximately 29 mV for a tenfold increase of the external Ca\(^{++}\) concentration. The total removal of the external Na\(^{+}\) altered neither the overshoot nor the time course of the spike potential. The external Mg\(^{++}\) did not restore the spike potential if the total external Ca\(^{++}\) was removed. A similar spike potential was obtained with internal Mg\(^{++}\) concentrations of 10^{-4} to 10^{-3} M if the internal Ca\(^{++}\) concentration was below 10^{-7} M. The results indicate that the spike potential of the barnacle muscle fiber is produced by the permeability increase of the membrane to Ca\(^{++}\). This was confirmed by the observation of Ca influx during the spike potential as measured with radioactive Ca\(^{45}\). Therefore, Ca ions in the barnacle muscle fiber play a role similar to that of the Na ion in the squid giant axon. A similar dependence on Ca\(^{++}\) and independence of Na\(^{+}\) has been observed with spike potentials in some other crustacean muscle fibers in which all-or-none spike potential occur in the normal condition (Parnas and Abbott, 1964). Therefore, the Ca dependence of the spike potential does not seem to be an artefact due to the lowering of the internal Ca\(^{++}\) concentration but one of the physiological properties of the crustacean muscle membrane.

As the external Ca concentration was increased various critical membrane potentials in the current-voltage relation, such as the threshold membrane potential for the spike, shifted in the positive direction along the potential axis. This has been found in the squid giant axon (Frankenhaeuser and Hodgkin, 1957) and called the stabilizing action of Ca\(^{++}\). Thus a common stabilizing action of Ca\(^{++}\) is present in both the barnacle muscle fiber and the squid giant axon.

The decrease of the internal K concentration increased the overshoot of the spike potential as well as the duration of the spike. The result is similar to that obtained with the squid giant axon when the internal K concentration is lowered by adding sucrose to the perfusing solution (Narahashi, 1963; Baker, Hodgkin, and Meves, 1964). In the squid giant axon the critical membrane potential for the spike potential shifts to a more positive membrane potential with decreasing internal K concentration. This phenomenon, however, was not observed in the barnacle muscle fiber. The result indicates that the overshoot of the spike potential depends not only on the external Ca\(^{++}\) concentration but also the internal K\(^{+}\) concentration. Finally, a linear relation was obtained between the overshoot of the spike potential and the logarithm of
the ratio between \([Ca^{++}]_\text{out}\) and \([K^+]_\text{in}\) with a slope of approximately 29 mV for a tenfold increase in the ratio.

REFERENCES