Potential, Structure, and Excitability of Giant Axon Membrane

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Intracellular perfusion of squid giant axons has unveiled certain membrane properties which were rather unexpected from the classical concept of electrophysiology and those which were otherwise difficult to study. One of the most surprising findings was that an axon, when perfused internally with low K sucrose solutions, was still able to give rise to full sized action potentials in the face of the drastically reduced resting potential (Narahashi, 1963; Baker, Hodgkin, and Meves, 1964). Another finding worthy of note was that the axon membrane proved symmetrical with respect to the action of potassium on its resistance (Narahashi, 1963), whereas asymmetrical with respect to the action of certain blocking agents (Tasaki and Takenaka, 1964; Tasaki, Watanabe, and Takenaka, 1962). In the present paper, electrical and structural aspects of the giant axon membrane are discussed with special reference to the two problems mentioned above.

Membrane Potential and Excitability

Full sized (about 100 mv) action potentials could still be produced when the resting potential was lowered to −16 mv by substituting a 6 mM K sucrose solution for the standard internal solution which contained 538 mM K (Fig. 1). By adjusting both external and internal potassium concentrations, it was possible to lower the resting potential even to zero without impairing excitability. However, further depolarization by passing a cathodal current through the membrane caused a block of action potential as in intact axons or in axons having high K solutions inside. Critical depolarization for firing remained unchanged by internal perfusion with low K sucrose solutions (Fig. 1). Thus it is concluded that the relation of sodium conductance to membrane potential is greatly shifted along the potential axis under this condition (Narahashi, 1963). Voltage clamp experiments with perfused axons definitely demonstrated the validity of this notion (Moore, Narahashi, and Ulbricht, 1964). Baker, Hodgkin, and Meves (1964) and Chandler and Meves (1964) have shown that low ionic strength rather than low potassium concentration of the internal solution is responsible for this shift.

The relationship between membrane resistance and membrane potential was also shifted along the potential axis to lower membrane potentials by internal perfusion with low K sucrose media (Narahashi, 1963). It can therefore be said that the conductance-potential relations, both for sodium and for potassium, undergo a considerable shift along the potential axis with low K sucrose media inside.

The Na inactivation curve is also affected by the external concentration of divalent ions such as calcium and barium. Increasing calcium or barium shifted the curve to lower membrane potentials in squid (Frankenhaeuser and Hodgkin, 1957), lobster (Narahashi, 1964), and cockroach giant axons (Fig. 2); that is to say, the axon in high Ca can be depolarized to a considerable extent without losing excitability. It has indeed been demonstrated that this

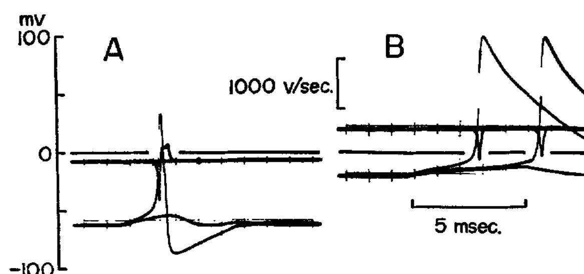

FIGURE 1. Superimposed records of action potentials (lower tracings) and their derivatives (upper tracings) in a perfused squid giant axon. Both subthreshold and suprathreshold cathodal currents of 5 msec. duration were applied. External medium, artificial sea water; internal medium, 538 mM K (A) and 6 mM K plus sucrose (B).

holds true for K depolarization as well as for cathodal depolarization, explaining at least partly the well known K-Ca antagonism. The dependence of the Na inactivation curve on the external calcium suggests that some molecular level structural changes are involved in the phenomenon. Another way of shifting the Na inactivation curve is to expose the axon to a blocking agent such as cocaine, high K media (Narahashi, 1964), or the insecticide allethrin which is a derivative of pyrethrins (Fig. 3).

It might therefore be said that, although membrane excitability is voltage-dependent, the apparent membrane potential values that are necessary for excitability to persist are very variable, depending on the internal and external environments. It is naturally supposed that the apparent membrane potential is the sum of several potentials (Tasaki, Watanabe, and Takenaka, 1962), and that only some of them are responsible for maintaining excitability. The presence of fixed negative charges at the inner surface of the nerve membrane with low ionic strength inside would yield in the membrane a potential gradient which integrates the molecular level structure in such a way as to

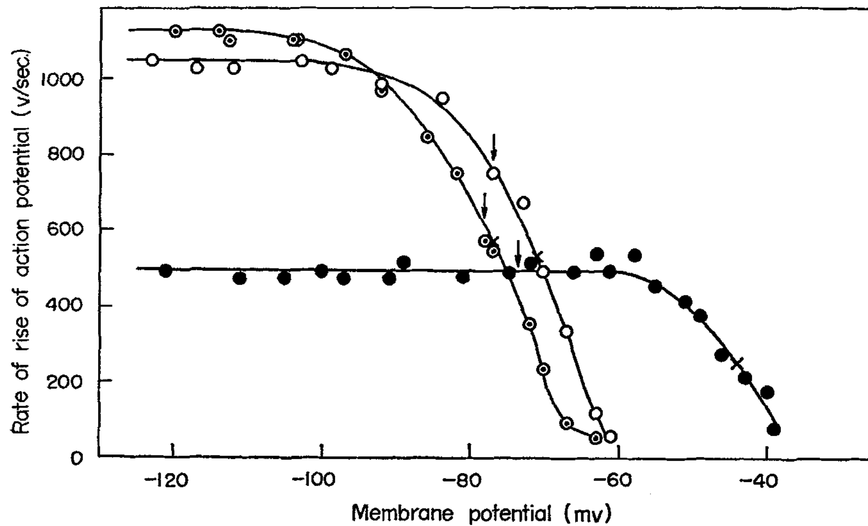

FIGURE 2. Shift of the Na inactivation curve by changes in external calcium concentration in a cockroach giant axon. The maximum rate of rise of the action potential was measured at various membrane potentials displaced by polarizing currents. Arrows indicate the resting potentials. Crosses on the curves show the half-inactivated membrane potentials. ○, 1.8 mM Ca (normal cockroach Ringer); ◉, 0.18 mM Ca; ●, 54 mM Ca. The tonicity and Na concentration were kept constant by replacing Ca with choline.

maintain excitability intact (Baker, Hodgkin, and Meves, 1964; Hodgkin in this conference).

It should be noted that during the course of low K sucrose perfusion the resting potential continues to decline progressively, while the active membrane

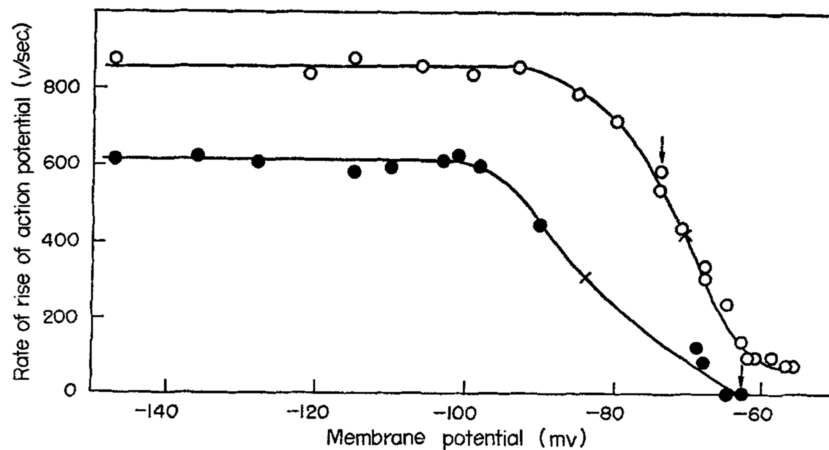

FIGURE 3. Shift of the Na inactivation curve by exposure to allethrin 10^{-6} gm/ml in a cockroach giant axon. Arrows indicate the resting potentials. Crosses on the curves show the half-inactivated membrane potentials. ○, normal cockroach Ringer; ●, allethrin Ringer.

potential remains rather constant. Although this type of experiment was not quantitative enough to permit extensive analyses, available data indicate that this amount of depolarization would be enough to decrease the active membrane potential, if it were caused by cathodal polarization. It seems then probable that the membrane potential component that is actually decreased slowly during the perfusion has no direct bearing on maintaining excitability.

Membrane Structural Aspects of Excitability

Two problems related to the membrane structure will be discussed here: (*a*) Is the nerve membrane symmetrical in nature? (*b*) What is the relation between the structure of the nerve membrane and the maintenance of excitability? The first problem is the one for which the internal perfusion technique particularly proves its usefulness. There would be several approaches to the second problem; here some hypothetical considerations deduced from the experiments on anodal restoration will be mentioned.

SYMMETRICAL AND ASYMMETRICAL PROPERTIES OF THE NERVE MEMBRANE

The membrane resistance decreased when a high K medium was applied to either side of the perfused nerve membrane (Narahashi, 1963). Thus, the membrane resistance was highest with low K media on both sides, lowest with high K media on both sides, and intermediate with a low K medium on one side and a high K medium on the other (Fig. 4).

Unlike the symmetrical property of the nerve membrane with regard to the K action on the resistance, an asymmetrical property was found in conjunction with delayed rectification (Narahashi, 1963). The rectification, upon moderate changes in membrane potential, occurred only in the normal direction, and only when high K and low K were present inside and outside respectively (Fig. 4); that is to say, the membrane resistance decreased upon depolarization. However, it should be pointed out that anomalous rectification was seen with greater hyperpolarization, and this was independent of the potassium concentration gradient across the nerve membrane.

Other asymmetrical properties of the nerve membrane are observed in connection with the actions of divalent ions such as calcium, magnesium, and barium; these ions were toxic to the membrane only when applied to the inside (Tasaki, Watanabe, and Takenaka, 1962; Hodgkin and Keynes, 1956; Grundfest, Kao, and Altamirano, 1954). Certain drugs and enzymes also act selectively only on one side of the nerve membrane. Tetraethylammonium (TEA) causes a marked prolongation of the action potential when applied to the inside of the squid axon, but has no effect when applied to the outside (Tasaki and Hagiwara, 1957). Trypsin and alpha chymotrypsin block the action potential when applied internally (Tasaki and Takenaka, 1964; Rojas and Luxoro, 1963), but have no effect when applied externally (Narahashi

and Tobias, 1964; Tobias, 1955, 1960). On the contrary, tetrodotoxin acts only externally (Narahashi, Moore, and Scott, 1964), the inert internal action being mentioned in detail by Moore in this conference. However, phospholipase C blocks the action potential both internally and externally (Tasaki, and Takenaka, 1964; Narahashi, 1964; Narahashi and Tobias, 1964; Tobias, 1955, 1960).

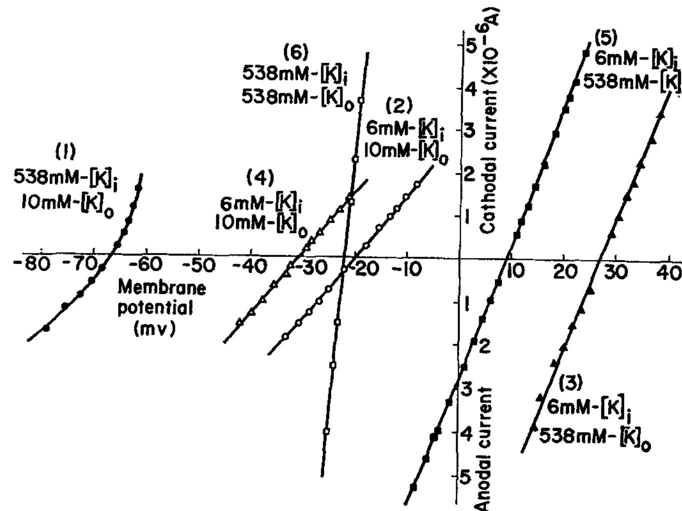

FIGURE 4. Current-voltage relation in a squid giant axon perfused with various potassium media. For 6 mM $[K]_i$, sucrose was substituted for K. For 538 mM $[K]_o$, K was substituted for Na. Measurements were made in the order numbered in parentheses (Narahashi, 1963).

MEMBRANE STRUCTURE AND EXCITABILITY Electrophysiological analyses of the mechanism of anodal restoration of excitability in lobster (Narahashi, 1964) and cockroach giant axons have led us to a hypothesis that it is the reintegrity of the membrane molecular structure that is responsible for restoration of action potential. First of all, the membrane has to be hyperpolarized beyond the normal resting potential level for the applied anodal current to exert its full restorative effect. This means that the simple recovery of membrane potential to its original level is not the sufficient condition for anodal restoration. Secondly, the time course of anodal restoration, whose time constant ranges from tens of milliseconds to several seconds depending on the magnitude of anodal hyperpolarization (Fig. 5), is very much slower than that of the ordinary Na inactivation defined by Hodgkin and Huxley (1952). Hence, anodal restoration cannot simply be ascribed to the removal of the ordinary Na inactivation. Thirdly, in high K media, the action potential is completely restored by anodal polarization despite the membrane

resistance being far short of the normal value. This excludes the possibility that the recovery of membrane resistance is a primary cause of anodal restoration.

The degree of anodal restoration depends, among other things, on the external concentration of calcium, lowering calcium generally improving restoration. Some changes in the integrity of the membrane molecular structure with which calcium is closely associated may occur during the passage of anodal current. It seems reasonable to assume that reorientation of dipoles

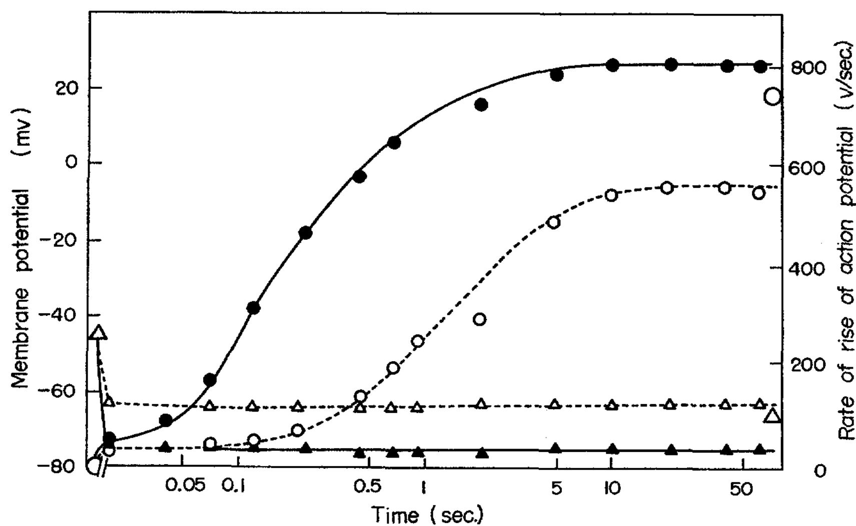

FIGURE 5. Time course of the recovery of the maximum rate of rise of the action potential (circles) during anodal hyperpolarization (triangles) in a lobster giant axon in 50 mM K sea water. The effects of a moderate (open symbols and broken lines) and a strong (solid symbols and solid lines) anodal current are shown. Large symbols at right and left corners in arbitrary time positions indicate the values before exposure to the K-rich medium and those immediately before applying the anodal currents, respectively (Narahashi, 1964).

of the membrane phospholipids under the influence of imposed electric field is a direct cause of excitability recovery (*cf.* Goldman in this conference). Possible metabolic activation by anodal current, though consistent with the slow process, cannot be regarded as a cause of anodal restoration, because excitation does not directly depend on the supply of metabolic energy (Hodgkin and Keynes, 1955).

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