EFFECTS OF ANESTHETICS ON NERVE FIBERS AND CELLS: A MACROMOLECULAR INTERPRETATION

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SUMMARY: The conditions of suspension of impulse conduction by anesthetics in myelinated nerve fibers are discussed. The anesthetic action of alcohols is attributed to the enhancement of attraction between the macromolecular strands in the superficial protoplasm layer in its Ca-rich, compact state.

INTRODUCTION

The advancement of our knowledge concerning the mechanism of action of local anesthetics on nerve fibers and cells is linked closely with the progress of the study of the process of nerve excitation and conduction. In classical neurophysiology, local anesthetics were used primarily as a tool for suppressing nervous activities for the purpose of gaining an insight into the physico-chemical processes that are reversibly suppressed by the drug (see e.g. ref.1). In recent years, on the other hand, great efforts have been made to clarify the mechanism of anesthetic action by examining the nature of interaction of various structural constituents of nervous tissues with drug molecules (2). A serious problem confronting these studies is that the role of the constituent under study in the process of excitation is not always clearly understood.

At present, it is well established that nerve fibers and cells undergo rapid reversible structural changes when they are excited (3-7,8). Simultaneously with the generation of an action potential, there is rapid swelling followed by shrinkage of the superficial layer of nerve fibers and cells. We have studied, during the past decade, a number of physiological and pharmacological agents that affect nerve swelling associated with excitation. The interpretation of the anesthetic action proposed in this paper is based on the results of our analyses of the non-electrical manifestations of the process of nerve excitation.

METHODS

The materials employed in the studies described in this paper include isolated myelinated fibers of the toad, bundles of olfactory nerve fibers of the garfish, synthetic polymer gels, etc. Under Results, a brief explanation will be given of the experimental setup and the procedure of each of the observations described.

RESULTS

(1) *Effects of local anesthetics on conduction in myelinated nerve fibers.*

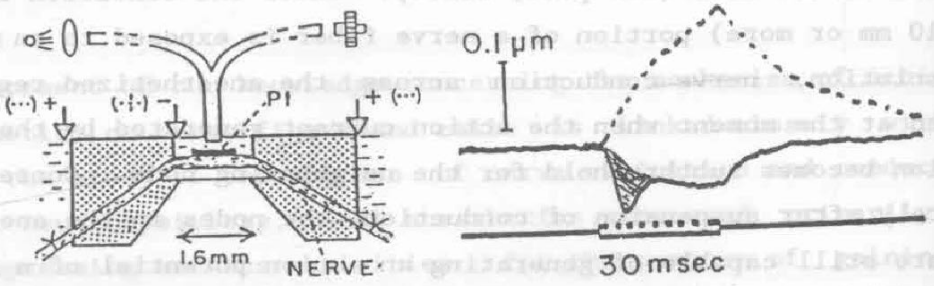
Electrophysiological studies of single Ranvier node preparations of the toad have revealed that, at relatively low concentrations, local anesthetics (such as urethane, cocaine, etc. dissolved in normal physiological saline) depresses the action potential amplitude and raises the threshold for excitation of the node (see p.80, ref.4). Under the condition that a long (about 10 mm or more) portion of a nerve fiber is exposed to an anesthetizing solution, nerve conduction across the anesthetized region is suspended at the moment when the action current generated by the node in the region becomes subthreshold for the neighboring node. Consequently, immediately after suspension of conduction, the nodes in the anesthetized region are still capable of generating an action potential of a sizeable amplitude in response to a strong stimulating current pulse.

Nerve conduction can not be suspended when the anesthetic solution is applied only to a single Ranvier node (p.47, ref.4). Under the circumstance that the neighboring nodes remain unaffected by the anesthetic, the action current generated by the normal node on one side of the anesthetized node spreads, by the virtue of the cable property, to the normal node on the other side and evokes a normal response of the node. When two successive nodes are heavily anesthetized, nerve conduction across the anesthetized region is suspended in some prepartations and remains unhindered in others; under these conditions, the action current generated by the normal node on one side of the anesthetized region is reduced, on account of the cable property, to the threshold level for the node on the other side of the region. Note that the internodal distance is 1.5 - 2.5 mm in large myelinated nerve fibers; it decreases linearly with the fiber diameter.

(2) *Mechanical changes in nerve fibers associated with excitation processes.*

The discovery of rapid mechanical changes associated with excitation of nerve fibers and cells has led us to a better understanding of the process of nerve excitation and of the nature of anesthetic action. One of the records showing this readily-detectable, non-electrical manifestation of the excitation process is presented in Fig. 1. Here, using a bundle of olfactory nerve fibers of the garfish in conjuction with a dual light-guide, movements of the nerve surface were recorded during the period in which a rectangular stimulating current pulse was delivered to the site of optical recording (9). It is seen that an inwardly-directed current through the superficial layer of the fibers brought about a gradual increase in the distance between the optical marker (Pt) on the nerve surface and the tip of the light-guide (see the broken line), indicating that the current induced

Fig. 1



gradual shrinkage of the fibers. A pulse of outwardly-directed (stimulating) current evoked, on the contrary, a decrease in the distance, indicating that the applied current induced rapid swelling of the nerve. It is to be noted that there are two phases, rapid and slow, in the time-course of the swelling induced by a stimulating current. When Na-ions in the external medium was replaced with tetramethylammonium (or other polyatomic univalent cations with hydrophobic side-groups), the rapid phase of swelling (marked by cross-hatching) was suppressed, leaving the slow phases virtually unaffected. Elimination of Ca-ions in the medium was found to suppress both the rapid and slow phases of the mechanical changes.

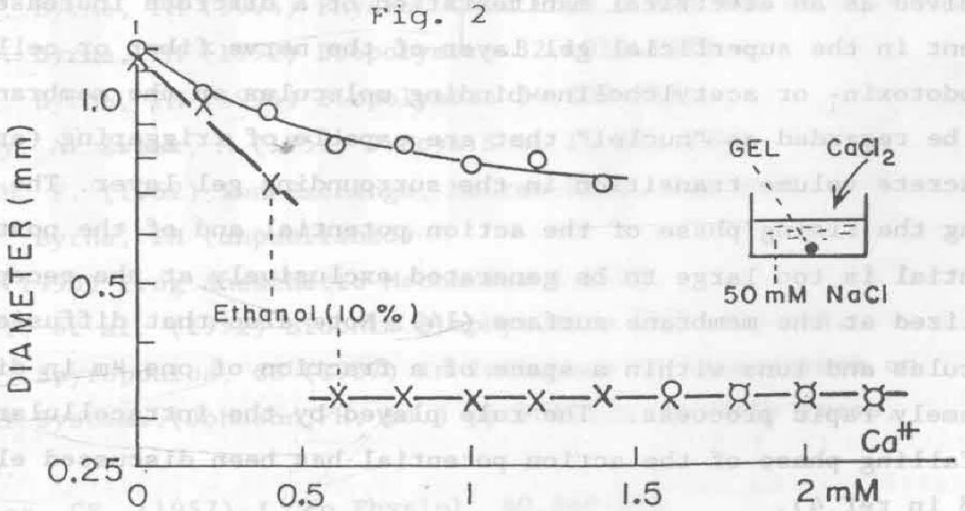
The experimental finding just mentioned is quite consistent with our previous experimental finding (p.232-254, ref.4), indicating that a cooperative Ca^{2+}-Na^{+} exchange process in the superficial layer of nerve fiber is at the base of the process of action potential production. Here, we emphasize that alcohols, tetrodotoxin, and other agents capable of blocking nerve conduction also suppresses the rapid phase of the mechancial changes.

(3) Effect of local anesthetics on cation-exchange process in inanimate anionic gels.

There is little doubt that the superficial layer of the nerve fiber is a gel with an appreciable density of negative fixed charges. The potential difference across the superficial layer is totally insensitive to changes in species of the anions in the external salt solution (as long as the Ca-ion concentration remains unaffected by anion substitution). Under physiological conditions, the action potential amplitude varies with the cation concentration in the external salt solution. As is well known, the presence of Ca-ions (or their substitute) in the external medium is essential for the maintenance of nerve excitabilty.

Quite recenetly, we have examined variations in the degree of swelling of synthetic, negatively charged gels associated with exchange of the counter ions (10,11). Most of our observations were carried out on poly(acrylic acid) gels donated by Kao Corporation in Japan. However, similar results have been obtained by using polymethacrylate gels which were investigated previously by Katchalsky and Zwick (12). An example of our results is furnished in Fig. 2. Here, poly(acrylic acid) gel beads of 0.25 mm in diameter were placed in a series of small cuvettes containing 50 mM NaCl solution (pH about 7.7), usually one bead in each cuvette. Then, small aliquots of a Ca-salt solution were added to the medium in the cuvettes. After equilibrium was reached, the diameters of the beads were measured under a microscope.

Fig. 2



The circles in the figure show the result of such measurements. We see that, in aqueous media containing both Ca- and Na-salts, the anionic gel is in either one of the two discrete states, swollen or compact (shrunken). Transition from one state to the other is discontinuous. In solutions with low Ca-ion concentrations, the gel has a high water content, its volume being more than 30-times the initial value. In the shrunken state, the gel loses the major portion of its water, its volume being only slightly larger than that in the completely dried condition. At the threshold for transition from the shrunken state to the swollen state, a nearly 20-fold increase in the water content can be induced in association with an extremely small rise in the Na^{+}-Ca^{2+} mole ratio in the gel.

The thick lines connecting the crosses in the figure show the diameters of the same gel beads measured after addition of a small quantity of ethanol to each of the salt solutions in the cuvettes (10% by volume). In a wide range of Na^{+}-Ca^{2+} mole ratio in the solutions, gel beads are now stabilized in the shrunken state. [Note that a 10% increase in water in the cuvettes does not bring about any detectable change in the gel sizes.] Many other short-chain alcohols were found to exert similar effects on the gel sizes.

DISCUSSION

It is well known that ion mobilities and selectivities for hydrated ions are profoundly enhanced by a rise of the water content in ion-echanger membranes (13). The process of action potential production may therefore be conceived as an electrical manifestation of a discrete increase in the water content in the superficial gel layer of the nerve fiber or cell (10,11). The tetrodotoxin- or acetylcholine-binding molecules on the membrane surface may then be regarded as "nuclei" that are capable of triggering (or suppressing) a discrete volume transition in the surrounding gel layer. The heat produced during the rising phase of the action potential and of the post-synaptic potential is too large to be generated exclusively at the receptor sites localized at the membrane surface (14). Note also that diffusion of small molecules and ions within a space of a fraction of one μm in dimension is an extremely rapid procoess. The role played by the intracellular K-ions in the falling phase of the action potential has been discussed elsewhere (see p.218 in ref.4).

From our macromolecular viewpoint described above, the action of neutral

local anesthetics on nerve fibers and cells may be attributed to the stabilization of the superficial gel layer in its Ca-rich, compact state. Evidently, this stabilization is brought about by enhancing the attractive interaction between biopolymer strands in their hydrophobic state (see Ueda,15; Kaminoh et al,16). The stabilization of the superficial gel layer at rest by ionized local anesthetic molecules appears to involve interactions of these molecules with the phospholipids in the membrane. Qualitatively, the effects of temperature and pressure changes on the anesthetic action on nerve fibers (17,18) may be explained on the basis of changes of the heat content and of the water structure in the superficial gel layer associated with excitation.

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