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Tetrodotoxin interaction with cholesterol

Tetrodotoxin has been shown to specifically block the pathways normally used by Na^+ to cross the axon excitable membrane (axolemma) during the nerve impulse and during the transient early phase of conductance increase in voltage-clamped axons[1-4]. This finding suggests that tetrodotoxin could be used to identify the chemical constituents of the axolemma associated with these sodium pathways. As a preliminary step in this direction, lipids from nerve-fiber membranes of the squid *Dosidicus gigas* were isolated and the effect of tetrodotoxin on the surface pressure–area (π–A) diagrams of their monolayers at a liquid–air interface was investigated. Total lipid fractions of the nerve membrane and its polar and nonpolar fractions were studied. Preliminary results indicated that tetrodotoxin in the liquid subphase produced expansion of the monolayers of the total lipids of the membrane and of its nonpolar fraction[5]. The percentage composition of the best-characterized nonpolar lipid fraction used in these experiments (see Table I, ref. 5) was: cholesterol, 70; fatty acids, 11; hydrocarbon (*n*-pentacosane), 19; and trace amounts of fatty alcohols and triglycerides.

To investigate further the component of the nonpolar lipid fraction interacting with tetrodotoxin, experiments with its constituents were carried out. The present note is a preliminary communication of the results obtained.

The various constituents of the nonpolar lipid fraction of *D. gigas* nerve-fiber membranes were separated by thin-layer chromatography. The same technique as described in our previous communication[5] was used to study the lipid monolayers. The surface pressure was measured by the Wilhelmy plate method[6], using a rectangular platinum plate (perimeter, 2.02 cm) suspended from a Cahn RG automatic recording balance. The Kel-F-coated tray (100 ml capacity) of the Cahn surface-tension attachment was used. The temperature of the trough was kept constant at 20 or 25°. A 0.15 M NaCl solution, prepared with 0.001 M phosphate-buffered tridistilled water at pH 7.5, was used as the liquid subphase. The lipids dissolved in chloroform–methanol (85:15, v/v) at concentrations of 0.4 to 1.0 μg/μl were spread on the liquid surface (71.08 cm^2) by successive 1-μl additions using a Hamilton microsyringe.

To investigate the effect of tetrodotoxin on the π–A diagrams, tetrodotoxin was added to the subphase before spreading the different lipids.

Fig. 1 shows the π–A diagrams of purified cholesterol spread on $5 \cdot 10^{-7}$ M tetrodotoxin and tetrodotoxin-free subphases at 25°. Each curve in Fig. 1 is the average of 7 experiments. The π–A diagrams reveal that $5 \cdot 10^{-7}$ M tetrodotoxin in the subphase causes expansion of the cholesterol monolayer. Similar results were obtained in experiments carried out at 20°. Experiments with tetrodonic acid, a biologically inactive derivative of tetrodotoxin[7], show that at the same concentration ($5 \cdot 10^{-7}$ M) and temperatures (20 or 25°), tetrodonic acid does not change the corresponding π–A diagram of cholesterol. Further experiments indicate that tetrodotoxin does not produce expansion either of the monolayers of the fatty acids or of the fatty acid–hydrocarbon mixture of the nerve-membrane nonpolar lipid fraction. When cholesterol was added to the fatty acid–hydrocarbon mixture, the tetrodotoxin effect described in our previous communication for the nonpolar fraction of the squid nerve membrane lipids, was observed[5].

These results, which show that tetrodotoxin interacts with cholesterol, one of the main components of nerve membranes, indicate that this interaction is probably the one responsible for the expansion to the monolayers of the nerve membrane total lipids and its nonpolar fraction[5] caused by tetrodotoxin.

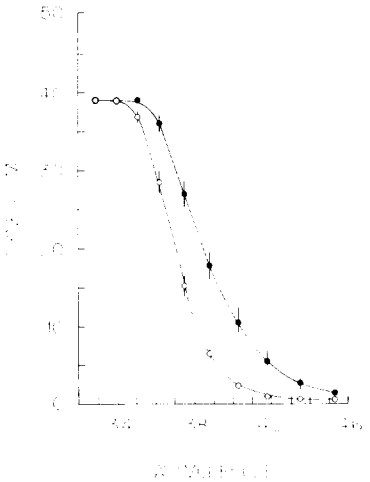

Fig. 1. Surface pressure–area diagrams of cholesterol monolayers, spread on tetrodotoxin-free subphases (○), and on subphases containing $5 \cdot 10^{-7}$ M tetrodotoxin (●). Each value represents the mean ± S.E. of 7 experimental measurements. Temp., 25°.

In the axon-excitable membrane, cholesterol should be arranged in a similar way to its orientation at the liquid–air interface[8]. Tetrodotoxin, through a specific interaction with cholesterol, may restrain the transient increase in Na^+ entry during the nerve impulse. Our results suggest that cholesterol is possibly part of the structure of the pathways used by Na^+ to cross the axolemma during the nerve impulse, or part of the mechanism controlling the Na^+ permeability.

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