PRELIMINARY NOTES

BBA 71029

Tetrodotoxin interaction with squid nerve membrane lipids

Recent experiments on the non-electrolyte permeability of the axon excitable membrane (axolemma), carried out in resting and stimulated squid axons, have revealed that during conduction of the nervous impulse a transient increase in the number and size of the axolemma aqueous pores occurs[1,2], and also a transient opening of pathways through the membrane non-polar regions[2]. It has been suggested that at least part of these aqueous and lipidic routes may be used by Na^+ and K^+ to cross the axolemma during activity[1,2].

Tetrodotoxin appears at the present to be the finest tool available to deal with the problem of identifying the membrane structures associated with the sodium pathways. This substance has a highly selective ability to block the pathways normally used by Na^+ to move through the axolemma during the nervous impulse and during the transient early phase of conductance increase in voltage-clamped axons[3–6].

The present note is a preliminary report of results obtained in experiments designed to investigate the interaction of tetrodotoxin with lipid molecules isolated from squid nerve fiber membranes. The effect of tetrodotoxin on the surface pressure–area (π–A) diagrams of monolayers of membrane total lipids and its polar and non-polar fractions, were studied at a liquid–air interface.

Membranes were obtained from nerve fibers of the giant squid *Dosidicus gigas*. The membranes were prepared by a slight modification of the method of AUTILIO, NORTON AND TERRY[7]. At the end of the preparative procedure, only membranes were observed with the electron microscope in negatively stained samples of the last pellet.

Total lipids were extracted from the squid nerve membrane preparations by the method of FOLCH, LEES AND SLOANE-STANLEY[8], and fractionated by preparative thin-layer chromatography into a non-polar and a polar fraction. The non-polar fraction contained cholesterol, fatty acids, hydrocarbons and trace amounts of fatty alcohols and triglycerides. The polar fraction contained the phosphatides. The lipids were identified by thin-layer chromatography against appropriate standards. The percentage composition of the best characterized preparation is given in Table I. This preparation was made of 33.2 % of proteins and 66.8 % of lipids.

The surface pressure was measured by the Wilhemy plate method[9], using a rectangular platinum plate with a perimeter of 2.02 cm, suspended from a Cahn RG automatic recording balance. The Kel-F coated tray of 100 ml capacity of the Cahn surface tension attachement was used. The temperature of the trough was kept constant at 25°. As liquid subphase a 0.15 M NaCl solution prepared with 0.001 M phosphate-buffered tridistilled water at pH 7.5 was used. The lipids dissolved at concentrations of 0.5–1.2 mg/ml in chloroform–methanol (85:15, v/v) were spread in successive 1-μl additions on the liquid surface (71.08 cm^2) by means of a 10-μl Hamilton microsyringe.

TABLE I

PERCENTAGE COMPOSITION OF SQUID NERVE FIBER MEMBRANE LIPIDS

"Non-polar" fraction (50.2 %)		*"Polar" fraction (49.8 %)*	
Cholesterol	35.0	Sphingomyelin	5.5
Fatty acids	5.7	Choline phosphatides	23.5
Hydrocarbons	9.5	Ethanolamine phosphatides	17.9
		Others	2.9

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

Fig. 1a shows the π–A diagrams of monolayers of nerve membrane total lipids spread on $6 \cdot 10^{-7}$ M tetrodotoxin and on tetrodotoxin-free subphases. Each diagram is the average of 14 experimental curves. The results reveal that tetrodotoxin interacts with the membrane lipids and produces expansion of the monolayer.

Figs. 1b and 1c show the π–A diagrams of the monolayers of the non-polar and polar lipid fractions, respectively, spread on $5 \cdot 10^{-7}$ M tetrodotoxin and on tetrodotoxin-free subphases. Fig. 1b shows that the π–A diagram of the non-polar lipids spread on the subphase containing $5 \cdot 10^{-7}$ M tetrodotoxin is strikingly different from that of the same fraction spread on the tetrodotoxin-free subphase. Each π–A diagram in Fig. 1b is the average of 7 experimental curves.

The expansion of the monolayers of the non-polar lipid fraction caused by tetrodotoxin clearly indicates that this substance interacts with one or more of the components of this lipid mixture. It is not possible at the present to indicate the molecule or molecules to which tetrodotoxin is bound. However, it should be pointed out that at the aqueous side of the monolayer only two different functional groups of the "non-polar" lipids are present. Thus, it is tempting to suggest that the functional groups of tetrodotoxin could be bound to the hydroxyl and/or carboxyl groups of the lipids by means of coulombic forces, charge–dipole or dipole–dipole bonds.

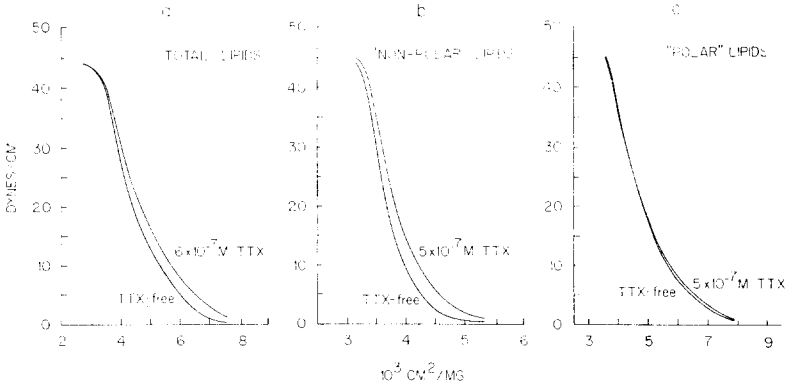

Fig. 1. Surface pressure–area curves of monolayers of lipids isolated from squid nerve fiber membranes, spread on tetrodotoxin-free subphases (TTX-free), and on subphases containing either $5 \cdot 10^{-7}$ or $6 \cdot 10^{-7}$ M tetrodotoxin (TTX). (a) Membrane total lipids; (b) "non-polar" fraction of membrane lipids; (c) "polar" fraction.

In order to establish which molecule or molecules and which of these types of associations are responsible for the expansion caused by tetrodotoxin to the total lipid and non-polar lipid monolayers, experiments with the components of this latter lipid fraction are under progress in our laboratory.

The interaction between tetrodotoxin and the membrane "non-polar" lipid fraction herein described is relevant to the identification of the membrane lipids associated with the sodium permeability mechanism underlaying the conduction of the nervous impulse.

The authors are deeply indebted to Ing. José Whittembury from the Laboratorio de Biofísica U.P.C.H.–I.V.I.C., Universidad Peruana Cayetano Heredia (U.P.C.H.), Lima-Perú, for his generous supply of the squid nerve fibers used in the present work.

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Received August 15th, 1968