Single sodium channels from human ventricular muscle in planar lipid bilayers

Abstract  Sodium channels from human ventricular muscle membrane vesicles were incorporated into planar lipid bilayers and the steady-state behavior of single sodium channels were examined in the presence of batrachotoxin. In symmetrical 500 mM NaCl the averaged single channel conductance was 24.7 ± 1.3 pS and the channel fractional open time was 0.85 ± 0.04. The activation midpoint potential was –99.5 ± 3.1 mV. Extracellular tetrodotoxin blocked the channel with a \( k_{1/2} \) of 414 nM at 0 mV. In 7 out of 13 experiments subconductance states were observed (9.2 ± 1.2 pS). When sodium chloride concentration was lowered to 100 mM, single channel conductance decreased to 19.0 ± 0.9 pS, steady-state activation shifted by –17.3 ± 5.1 mV, tetrodotoxin sensitivity increased to 324 nM, and sub-conductance states were invariably observed in single channel records (7.9 ± 0.7 pS).

In the planar lipid bilayer system the properties of cardiac sodium channels from different species are not very different, but there are significant differences between sodium channels from human heart and from human CNS.

Key words  Conduction (block) – membrane potential – Na-channel – single channel currents – ventricular function

Introduction

Voltage-dependent sodium channels play an essential role in the propagation of action potentials not only in the heart but also in the entire central and peripheral nervous system. They provide a pathway for a positive sodium ion flux into the cell and thereby transiently reverse the negative resting membrane potential. Malfunction of cardiac sodium channels may have serious clinical implications, such as long QT syndrome (15, 33) and cardiac arrhythmia (18). Consequently, cardiac sodium channels are targets for therapeutic interventions, such as in the treatment for cardiac arrhythmia (2, 16).

The lipid bilayer techniques has provided stable experimental conditions needed for pharmacological studies (10, 11, 34). Individual sodium channels can be observed literally for hours, making it possible to distinguish steady-state properties (e.g. an intrinsic variability) from a natural run-down of the preparation (28), a consideration that is particularly important in the study of drug actions (10, 35). The main advantages of the bilayer system are that the protein is kept in a well characterized lipid environment, that the protein is remaining intact and that the complete channel with all its native subunits and native posttranslational modifications can be investigated.

In expression systems usually only the alpha sub-unit is expressed or the beta sub-unit from another species is
used for co-expression. In most cases, a foreign cell machinery is used to perform the post-translational modification. Functional and pharmacological differences in the behavior of different sodium channel could thus be distorted as they may result not only from variations in the amino acid sequence of the native ion channel (14, 21, 32) but also from alterations involving the additional attachment of other non-native sub-units or non-native posttranslational modification (4, 19, 20, 24, 27, 29).

Major drawback and limitations of bilayer experiments involve the use of batrachotoxin with the consequence that general properties are modified from those that sodium channels would exhibit normally in an intact cell and that inactivation is inhibited. Therefore, the choice of experimental technique will not only depend on the functional property of the sodium channel to be investigated, but different experimental techniques may even complement each other.

In the study presented, sodium channels from human ventricular muscle were fused with planar lipid bilayers, and single channel conductances and subconductances, single channel fractional open times, the voltage dependence of tetrodotoxin (TTX) block and the steady-state activation behavior of single sodium channels were examined in the presence of batrachotoxin (BTX). Fractional open times are related to overall conductances provided by sodium channels and influence the upstrokes of action potentials, while the midpoint potentials of activation determine the threshold of action potential generation. While it may eventually prove to be a regulatory site of the protein (25), the physiological significance of the TTX binding site remains uncertain; however, TTX block has routinely been used in the structural characterization and identification of different sodium channels (14). These parameters and the experimental conditions were chosen in order to allow a detailed comparison of our findings with data published in the bilayer literature. By combining these data with data from patch-clamp studies of native cells and of expression systems, a more complete molecular and functional picture of human voltage-gated sodium channels and their response to pharmacological agents may be obtained.

**Methods**

### Preparation

With the approval of the local Committees on Human Rights in Research (the investigation conforms with the principles outlined in the Declaration of Helsinki), human ventricular muscle samples were obtained from 4 patients with congestive heart failure undergoing heart transplantation. The source of tissue was the patient’s heart, which was removed prior to the transplantation and was considered surgical waste. Samples were extracted from macroscopically unaffected parts of the left ventricular muscle directly after explantation. Samples were immediately frozen at –80 °C. Membrane preparation was as described for canine heart (14) and stored at –80 °C.

A short description of the preparation procedures is given here: Transmural samples of the left ventricular muscle were taken from hearts removed for heart transplantation. Histologic examinations resulted in the diagnosis of cardiac heart disease (CHF) for all four hearts, but no other pathology was confirmed. In each heart there were areas which macroscopically seemed unaffected by CHF, and there were subsequently used for our preparations. They were minced and lightly homogenized in sucrose buffer, then centrifuged and re-homogenized in several successive steps. Finally plasma membrane banding at a 0.3/0.7 M sucrose interface were collected.

#### Bilayer procedures

Most materials and experimental methods are described elsewhere (6, 26); a brief description is given below. All experiments were conducted at room temperature (22–24 °C) in either symmetrical 500 mM NaCl or symmetrical 100 mM NaCl buffered at pH 7.4 with 10 mM HEPES (United States Biochemical); no corrections were made for temperature differences between experiments. Planar bilayers were formed from neutral phospholipid solutions containing (4:1) 1-palmitoyl-2-oleyl-phosphatidylethanolamine and 1-palmitoyl-2-oleoyl-phosphatidylcholine (Avanti Polar Lipids, Birmingham, AL) in decane (5 % wt/v, 99.9 % pure; Wiley Organics, Columbus, OH). Tetrodotoxin (TTX) was purchased from Sigma Chemical Co., St. Louis, MO. BTX was a gift from Dr. J. Daly, NIH (Bethesda, MD). Teflon chambers were prepared and used as previously described (26); the chambers were divided by a Teflon partition into 2 cis compartment to which the preparation was added, and a trans compartment. The partition had an approximately 300 µm hole in its center; planar bilayers were formed over this aperture. Sodium channels were incorporated into the bilayers in the presence of 0.5 µM BTX; the electrophysiological sign convention in bilayer papers was used in the presentation of results (i.e. the side to which TTX binds is the reference for the potentials).

Channel currents were recorded under voltage-clamp conditions and filtered at 50 Hz. Time-averaged conductances were measured by computer. After incorporation of a sodium channel into the bilayer, control currents were measured for at least 60 minutes and the sidedness of the channel experimentally determined. In some