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Short communication

Sodium and potassium currents recorded during an action potential

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Abstract. A simple method was used to measure directly sodium and potassium currents underlying the action potential in single nerve fibres of *Xenopus laevis*. A short rectangular stimulus under current-clamp conditions elicited an action potential which was digitally stored and later used as command when voltage-clamping the same fibre. The currents thus obtained nearly reproduced the original rectangular stimulus. Adding first 100 n*M* TTX and subsequently 100 n*M* TTX plus 10 m*M* TEA to the extracellular Ringer solution revealed the sodium and the potassium currents during an action potential. They were converted to permeabilities by use of the constant-field equation and are in good agreement with the curves which had been calculated from conventional voltage-clamp data. Thus experimentally determined currents and permeabilities are shown as they are changing during an action potential.

Key words: Action potential, voltage clamp, ionic current, myelinated nerve

Introduction

Ionic currents during an action potential of the nodal membrane have formerly been calculated by Frankenhaeuser and Huxley (1964) who used data which had been obtained in voltage-clamp experiments. We have been fascinated by the idea of voltage clamping an excitable membrane to its own action potential in order to measure directly the underlying ionic currents. To use an action potential as a voltage command can easily be done by means of analog to digital and digital to analog conversions. Cole and coworkers (1980) demonstrated that such operations can be deceptive since limited resolution in frequency and amplitude results in distorted currents. An action potential as voltage command in the voltage-clamp mode has also been used in order to test the accuracy of the space clamp in squid axons (Starzak and Starzak 1978) or to show propagation of excitability in transverse tubules of muscle fibres (Bastian and Nakajima 1974). During the course of an action potential Bezanilla et al. (1970) switched to potential control at different times and obtained membrane currents. We continuously measured Na^+ and K^+ currents underlying the action potential by use of a modern signal processing system with high resolution in frequency and amplitude. Our currents were in good agreement with the calculated ones of Frankenhaeuser and Huxley (1964).

Methods

Single nerve fibres were isolated from the sciatic nerve of *Xenopus laevis* and were current and voltage clamped by the method of Nonner (1969). The node of Ranvier was superfused in a trough with Ringer's containing (in mmol/l) 110 NaCl, 2.5 KCl, 2 $CaCl_2$, and 5 BES buffer (*N,N-bis*-(2-hydroxyethyl)-2-aminoethane sulfonic acid), pH 7.4. Both ends of the fibre were placed in side pools containing 105 KCl, 13 NaCl, and 5 BES and cut off as short as possible. A personal computer (De Haas 1987) was used to stimulate the fibre and to store the digitized signal (Fig. 1 A and B). Twelve bit digital to analog and analog to digital converters reduced the digital noise to about 65 dB of the signals processed. 200 kHz sampling frequency combined with the use of a four pole Bessel low pass filter with a cut-off frequency of 40 kHz (−3 dB) resulted in an adequate frequency resolution of the signal processing unit.

Results and discussion

The nodal membrane was stimulated by short rectangular pulses in the current clamp (Fig. 1 A). After digitizing and averaging the action potentials (Fig. 1 B) the amplifier setup was switched to the voltage-clamp

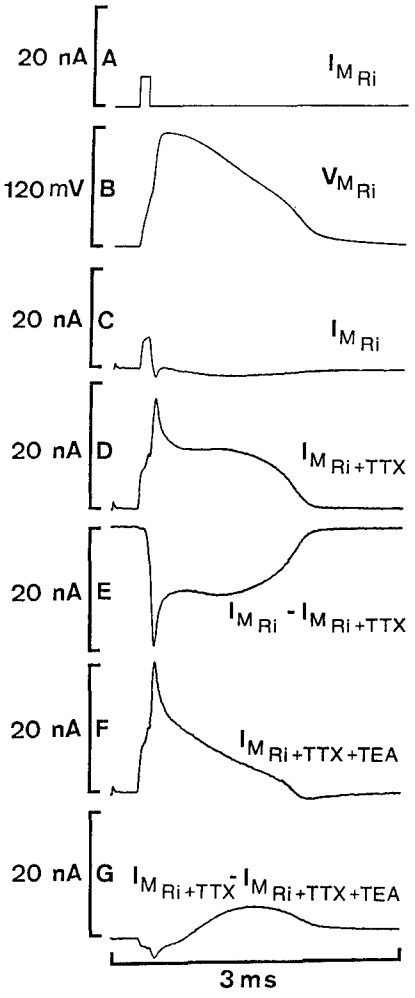

Fig. 1. Stimulus (*A*) and recordings in current clamp (*B*) and voltage clamp (*C* to *G*). In the current clamp the fibre was stimulated with ten current pulses as shown in *A* triggering action potentials (*B*) which were digitally averaged and stored by a personal computer. After switching to the voltage-clamp mode the action potential was used as voltage command eliciting the membrane current in *C*. *A*, *B* and *C* were recorded in Ringer's. Deviation of recorded current in *C* from the current trace in *A* was due to shortcomings of the voltage-clamp amplifier. After addition of 100 n*M* TTX current *D* is recorded. Subtraction *C*−*D* results in the TTX-sensitive current *E* (Na^+ current). After additional application of 10 m*M* TEA^+ current *F* is taken. *G* shows the TEA^+-sensitive current as a result of the subtraction *D*−*F*. The fictitious initial inward current in *G* was due to an instability of the access resistence between recordings *D* and *F*. Vertical calibration is 20 nA except in *B* where it is 120 mV. All recordings from the same fibre, 12 °C, membrane potential adjusted to give 18% steady-state inactivation of P_{Na} ($h_\infty = 0.82$). Plots shown are digitized traces with a resolution of 12 bit and 200 points per ms

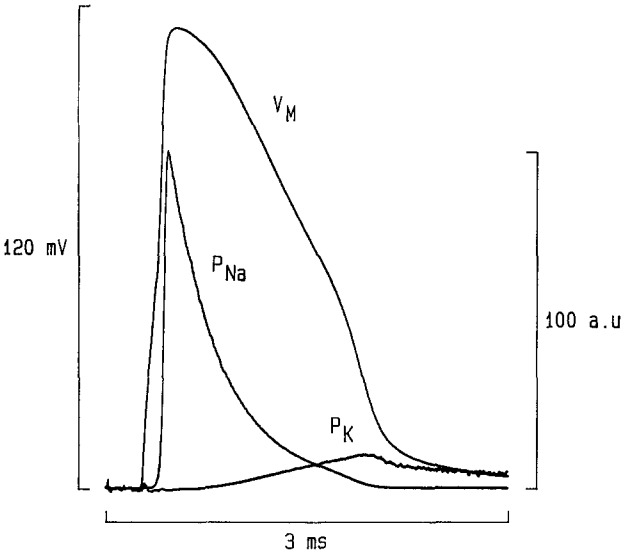

Fig. 2. Action potential and ionic permeabilities. The action potential is identical with that of Fig. 1 B. Na^+ and K^+ permeabilities are derived from currents in Fig. 1 E and G using the constant-field equation ($[Na^+]_i = 13$ m*M*, $[K^+]_i = 105$ m*M*). Since the membrane area cannot reliably be determined arbitrary units are prefered for the permeability. From average fibre geometry the peak Na^+ permeability is estimated to be $4-13 \cdot 10^{-3}$ cm/s. In order to correct for the unstable access resistence (see Legend Fig. 1), traces D and F were scaled to get equal amplitudes of the initial peaks before calculation of I_K and P_K

mode and the stored action potential was imposed on the membrane. This resulted in a current (Fig. 1 C) that resembled the current stimulus which had been used before.

Our intention was to record the Na^+ and K^+ currents passing the membrane during an action potential. Therefore we selectively blocked Na^+ and K^+ channels and determined the eliminated currents. After application of 100 n*M* tetrodotoxin the voltage-clamp amplifier has to add the current previously carried by Na^+ ions to load the membrane capacity to the voltage course of the action potential (Fig. 1 D). Subtraction of this current from the current measured in Ringer's reveals the TTX-sensitive Na^+ current (Fig. 1 E). It shows a first peak at the time when the peak of the action potential is nearly reached and a second lower peak. This agrees with the calculations of Frankenhaeuser and Huxley (1964) and also with calculations of Na^+ currents in squid axons (Hodgkin and Huxley 1952). The peak Na^+ current recorded from the same fibre during voltage clamp with conventional step changes in potential (not shown) was more than four times larger. Frankenhaeuser and Huxley (1964) calculated a maximum Na^+ current of -6.3 mA/cm^2 as opposed to -30 to -40 mA/cm^2 measured in voltage-clamp experiments with rectangular step commands. From this observation they suggested that the membrane can be loaded considerably without too obvious a change in the action potential.

Addition of 10 m*M* tetraethylammonium chloride to the TTX-containing extracellular solution alters the time course of the current as shown in Fig. 1 F. Now the voltage-clamp amplifier has also to supply the current carried by K^+ ions before application of TEA^+.

Subtracting the current shown in Fig. 1 F from that in Fig. 1 D results in the TEA^+-sensitive K^+ current (Fig. 1 G).

The measured Na^+ and K^+ currents were converted to the equivalent permeabilities with the constant-field equation (Fig. 2). The Na^+ permeability quickly reaches its peak value and then decays continuously. The peak K^+ permeability is about ten times smaller.

Our measured current curves deviated somewhat from the classical calculated ones of Frankenhaeuser and Huxley (1964) probably due to imperfect stability of experimental conditions during the time needed to carry out the measurements in the various solutions as described above. Also when switching between voltage- and current-clamp mode, small changes in holding potential will introduce some artifacts.

By using an action potential as command voltage in voltage-clamp experiments we could show that the experimentally determined ionic permeabilities are in fair agreement with the ones computed from voltage-clamp data obtained with rectangular pulses. Since they were obtained from straightforward experiments Figs. 1 and 2 also might be helpful when it comes to the demonstration of the ionic currents and permeability changes underlying action potentials.

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