TEA-Insensitive K-Channels in the Crab Giant Axon

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Abstract. TTX and TEA-insensitive permeabilities were studied in the crab giant axon under voltage-clamp. Membrane currents in the presence of internal TEA (40 mmol/l) and external TTX (300 nmol/l) may be analyzed as the sum of two components: a linear component, identified as the so-called leakage current, and a non-linear component. indentified as a TEA-insensitive potassium channel. Ion permeability ratio of the TTX and TEA insensitive cation channel calculated from reversal potential shows the following sequence $pK^+:pNa^+:pCs^+:pRb^+:pNH_{4}^{+} = 1.00:0.16:0.16:0.09:0.06$. TEA-insensitive outward currents, carried mainly by Cs⁺, may be recorded in the presence of different external solutions. Voltage-dependence and equilibrium potential of this channel in physiological conditions allows to postulate its contribution to maintain the cell depolarized during repetitive firing.

Key words: Crab axon — TEA-insensitive channels — Ionic channels — Voltage clamp

Introduction

Membrane currents of crab giant axons under voltage clamp may be analyzed into several components (Quinta-Ferreira et al. 1982a, b, 1985: Soria et al. 1985): 1) An asymmetric capacitative transient, 2) An inward sodium current (blocked by TTX), 3) Two outward potassium current components, a fast one with activation-inactivation kinetics, and delayed one, with kinetics similar to those recorded in other nerves, with both the fast and delayed components blocked by TEA, either internally (Quinta-Ferreira et al. 1985) or externally applied (Soria et al. 1985), and 4) A "leakage" current not affected either by TTX or TEA.

Leakage currents in the crab giant axon

In their classic work, Hodgkin and Huxley (1952) approximated the leakage current in the squid giant axon by an equivalent conductance constant with time and membrane potential. Despite its importance in maintaining the resting membrane potential and in the repolarization of the action potentials the leakage

conductance has received little attention. In most reports leakage current is substracted by analogic or digital procedures and no more comments are given. Leakage conductance of the Ranvier node, which is as high as 40 mS/cm², contributes to repolarization of action potential in myelinated fibres (Hille 1973). Its early appearance in cultures of the avian neural crest has been reported (Bader et al. 1983). Before the introduction of potassium channels blockers and sodium channel blockers. Adelman and Taylor (1961) made an elegant description of leakage current rectification in the squid axon. They concluded that leakage channel rectifies, its time constant is shorter than 100 μ s and is mainly due to the outward movement of so far unidentified internal ions.

Leakage current in the crab giant axon was studied after block of sodium currents with 300 nmol/l external TTX and of potassium currents with 40 mmol l TEA. The nerve chamber, electrode arrangement and feed-back amplifier used have been described before (Quinta-Ferreira et al. 1982a). The current injected by the feed-back amplifier was measured as a voltage drop across a 1000 Ω resistor in series with the current injecting electrode or as the voltage in this electrode divided by the resistance of the axoplasm. In general, there was agreement between this two measurements. Of the four pools of the nerve chamber, pool A (external membrane side) was perfused with K-free artificial sea water (K-free ASW, composition in mmol/1:: NaCl 470, CaCl₂ 12, MgCl₂ 14, Tris-HCl 5), TTX 300 nmol/l was added to block currents through the sodium channel. To test ion permeability Na⁺ was substituted by the corresponding monovalent cation. The axoplasm was equilibrated with internal solution (composition in mmol/1: CsF 470, TEA 40, EGTA 1, Tris-HCl 5) by diffusion from pools C and E. Holding potential was estimated from the position of Na-conductance inactivation curve.

Membrane currents recorded after blocking sodium and potassium currents are composed of two components: an asymmetric capacitive transient and a leakage current. In the presence of K-free artificial sea water, steady state I-Vm curves show two apparent components: a linear and non-linear rectifying component (Fig. 1). The linear component has a very low slope conductance at the beginning of the experiment and may increase after 1—2h to values above 80 mS/cm² (this component probably reflects the state of damage of the membrane). The non-linear component remains constant all over the experiment. After substraction of the linear component from the total leakage current the resultant was analyzed in terms of a membrane channel exploring which ions are permeant through it.

Monovalent cation permeability of the TTX and TEA insensitive component

The permeability ratio for a given pair of monovalent cations was calculated using the Goldman-Hodgkin-Katz equation and measured values of the reversal potenTEA-Insensitive K-Channels

tial. A detailed description of the method is given in Quinta-Ferreira et al. (1985). The reversal potential, Er(zero current potential) for current in the non-linear component of leakage currents is measured in K-free artificial sea water and then in solutions containing the test cation. The permeability ratio pX/pY is calculated from the change in Er using the relation:

$$Er_{Y} - Er_{x} = (RT/F) \ln \{(pY[Y]/(pX[X]))\}$$

where R, T and F have their usual meaning, pX and pY are the permeabilities of the cations X and Y, and [X] and [Y] represent thermodynamic activities of the cations. The thermodynamic activities , sed were calculated from the activity coefficient (Robinson and Stokes 1959). This method avoids the requirement of knowing the absolute value of the membrane potential and the internal concentration of permeant cations at the time when the reversal potential is measured (Hille 1975). This is rather important because of Cs⁺ and TEA⁺ permeability through these channels.



Fig. 1. Current-voltage curves at 25 ms with the axon in Na-ASW. Dashed line: linear component. External solution: NaCl 470 mmol/l, CaCl₂ 12 mmol/l, MgCl₂ 14 mmol/l, Tris-HCl 5 mmol/l, TTX 300 nmol/l. Internal solution: CsF 500 nmol/l, EGTA 1 mmol/l, Tris-HCl 5 mmol/l. Holding potential: - 100 mV. Temperature: 16°C.

The reversal potentials in different external cation are: Na⁺ (470 mmol/l) -60 mV. K⁺ (500 mmol/l) 13 mV, Cs⁺ (500 mmol/l) -59 mV, NH⁺₄ (400 mmol/l) -88 mV and Rb⁺ (500 mmol/l) -74 mV. Relative permeabilities with internal cesium calculated from reversal potentials show the following sequence, pK⁺:pNa⁺:pRb⁺:pNH⁺₄ = 1.00:0.16:0.16:0.09:0.06. This sequence is clearly

different from that of the two types of potassium channels in the same preparation (Quinta-Ferreira et al. 1985), $pK^+:pRb^+:pNH_4^+:pCs^+:pNa^+$ = 1.0:0.9:0.2:0.1:0.1, evidencing that the non-linear component represents a channel different from the described potassium channels. So far, there has only been one report on the ionic conductance of the so-called leakage channels (Hille 1973). The leakage conductance (from Table III of Hille 1973) in myelinated nerve decreases in the sequence $pK^+:pCs^+:pNH_4^+:pRb^+:pTI^+:pNa^+$ = 1.0:1.0::0.93:0.92:0.88:0.84:0.79. In the crab giant axon "leakage" channels are potassium-prefering channels although show a low selectivity for other monovalent cations.

Outward currents through TEA insensitive channels

The data presented in the previous section suggest the presence of a rather large cation selective channel with high permeability for caesium ions. In fibres perfused with internal CsF and in presence of TTX and TEA, large outward currents may be observed, current density reaches values of approximately 2 mA/cm² and the slope conductance from the I-Vm curves is approximately 30 mS/cm². Cs⁺ blocks voltage-gated K-channels (Fukushima 1982) and inward rectifier channels (Bezanilla and Armstrong 1972), both of them are potassium preferring cation channels. The so-called multi-block mechanism supports the idea that cesium blocks potassium channel entering the pore. In a larger pore, cesium will not only enter but act as a current carrier. In internally perfused nerve cell bodies of *Limnea stagnalis* (Byerly and Hagiwara 1982) when K⁺ is removed from both sides, time-dependent, voltage-dependent outward currents are observed at positive potentials. This non-specific outward currents can be carried by Tris⁺ and TEA⁺, as well as Cs⁺, but the Cs⁺ currents are several times larger.

In Rb-ASW the linear component of the so-called leakage is lower than with other "depolarizing" cations. In the membrane currents shown in Fig. 2 leakage was not substracted. Membrane currents in the presence of Rb-ASW (with internal CsF) show inward and outward components. The outward components may be depicted blocking Rb inward current through the potassium channel by externally applied 100 mmol/l TEA. Then, the TEa-insensitive current may be subtracted from each record of the total current (Fig. 2), the resultant is Rb-current through the potassium channel. TEA-insensitive current shows both inward and outward components. The predominant cation inside is Cs⁺ (470 mmol/l), therefore this outward current is carried mainly by Cs⁺, although a small contribution from 5 mmol/l Trist is not excluded. Outside there are Rb⁺ (400 mmol/l) and TEA⁺ (100 mmol/l). Rb⁺ permeates the non-linear components of the "leakage" current and TEA⁺ has been described to permeate non-specific channels in nerve cell bodies from *Limnea stagnalis* (Byerly and Hagiwara 1982) and in voltage clamped

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hair cells (Corey and Hudspeth 1979). It is reasonable to think that both Rb⁺ and TEA⁺ are responsible of the inward current insensitive to TEA. Internally perfused snail neurons also show a TEA-resistant voltage-dependent outward K-current (Kostyuk et al. 1980). The permeability to Tris⁺ is much higher than would be expected from the K-selectivity filter in frog nerve and muscle.



Fig. 2. Membrane currents recorded with the axon in Rb-ASW. External solution (in mmol/l): RbCl 370, Tris-HCl 105, CaCl₂ 12, MgCl₂ 14. TTX (300 nmol/l) was added to external solution. To record TEA-insensitive currents 100 mmol/l Tris-HCl was substituted by 100 mmol/l TEA. Internal solution (in mmol/l): CsF 470, EGTA 1, Tris-HCl 5. Holding potential -102 mV. Temperature 15 °C. *a*) Total current, *b*) TEA sensitive currents obtained after substract *c* from *a*, *c*) 100 mmol/l TEA insensitive currents. (\bullet), TEA-insensitive currents (\triangle) and Rb⁺ currents through the potassium channel (\bigcirc). Calibration : vertical 5 mA/cm², horizontal 50 ms.

In conclusion, in addition to one sodium and two potassium channels, crab axons have another time and voltage dependent channel. This channel activates around -50 mV and its selectivity against potassium compared to other monovalent cations is lower compared to the potassium channels described previously (Hille 1973). Its equilibrium potential in physiological conditions is dominated by its permeability to K⁺ and Na⁺ (pK⁺:pNa⁺ = 1.00:0.16). From the Goldman equation an equilibrium potential between -40 and -30 mV may be postulated. Thus, this TEA-insensitive potassium channel may contribute to maintain the cell depolarized when firing repetitively, the more relevant physiological property of these axons.

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