Short communication

DHP-sensitive Ca^{2+} Channels from Crayfish Skeletal Muscle T-tubules Incorporated into Planar Lipid Bilayers

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The voltage-dependent calcium channels play an important role in the mechanism of excitation-contraction coupling. Different types of calcium channels are known to exist, and they differ in the mechanisms controlling their opening and closing. Patch-clamp studies and planar lipid bilayer measurements have provided insight into mechanisms of calcium channel function (Coronado 1987; Pelzer et al. 1988; Rosenberg et al. 1988; Valdivia and Coronado 1988). In view of the presence of calcium currents in single muscle fibres of crayfish *Astacus fluviatilis* (Henček and Zachar 1977; Zahradník and Zachar 1982) and the evidence for dihydropyridine binding to membrane fractions from crayfish (Križanová et al. 1990b) we investigated the properties of the calcium channel from T-tubule membranes after its incorporation into planar lipid bilayers.

T-tubule membrane fractions were prepared as described by Rosemblatt et al. (1981). Planar lipid bilayer measurements were performed as described by Križanová et al. (1990a). The cis side where the vesicles were added was connected to a voltage command signal, and the trans side was connected to a current-voltage converter circuit.

The measurements have confirmed the identity of Ca^{2+} channel sensitive to BAY K 8644. Fig. 1 shows the single channel activity at the holding potential $+50$ mV and $+100$ mV. The channel conductance was 16 pS ($n = 9$) within the range measured (-60 mV to $+130$ mV), and the reversal potential was ≈ -30 mV (Fig. 2). The channel sensitivity to BAY K 8644 was tested in some experiments as follows. A preformed membrane was allowed to incubate for 30 min in solution without BAY K 8644, and then the drug was added to both sides of the membrane to a final concentration of 10 μmol/l. This resulted in a sudden occurrence of channel activity in the membrane at steady holding potentials. No activity was observed in the absence of BAY K 8644 in the bath solution. Based on our results, the channel studied had properties closer to B-type Ca channel as reported by Rosenberg et al. (1988) than to the L-type channel. All the characteristics obtained in our experiments have been very close

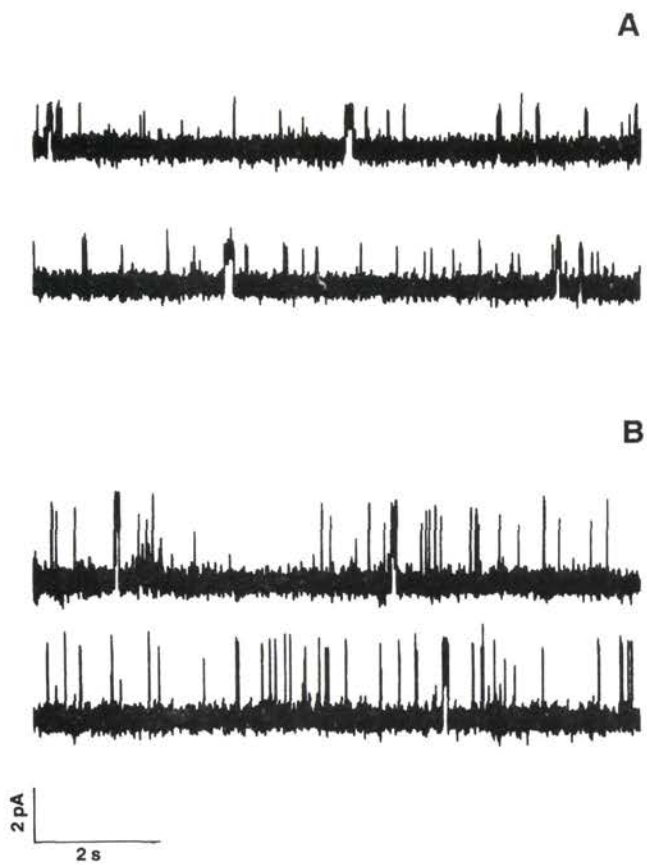

Fig. 1. Recording of single channel activity in 50 mmol/l NaCl, 1 mmol/l EDTA, 10 mmol/l HEPES-NaOH, 10 μmol/l BAY K 8644 (*cis*//*trans*), 100 mmol/l $BaCl_2$ (*cis*), pH = 7.4; at holding potential +50 mV (*A*) and +100 mV (*B*). The openings can be seen as upward deflections.

to those of the Rosenberg et al. (1988), and the selectivity ratio ($P_{Ba}/P_{Na} \approx 4$) was calculated by equation (1) described by Lee and Tsien (1984)

$$E_{rev} = \frac{RT}{F} \cdot \ln \frac{4 \cdot P'_{Ba} \cdot \gamma^{o}_{Ba} \cdot Ba_o + P_{Na} \cdot \gamma^{o}_{Na} \cdot Na_o}{P_{Na} \cdot \gamma^{i}_{Na} \cdot Na_i} \quad (1)$$

where $\gamma^{o}_{Ba} = 0.25$, $Ba_o = 100$ mmol/l, $\gamma^{o}_{Na} = 0.70$, $Na_o = 50$ mmol/l, $\gamma^{i}_{Na} = 0.80$, $Na_i = 50$ mmol/l, $P'_{Ba} = P_{Ba} = [1 + \exp(E_{rev} \cdot F/(R \cdot T))]^{-1}$, with assumption of zero surface potential difference.

The only difference between our channel and that described by Rosenberg

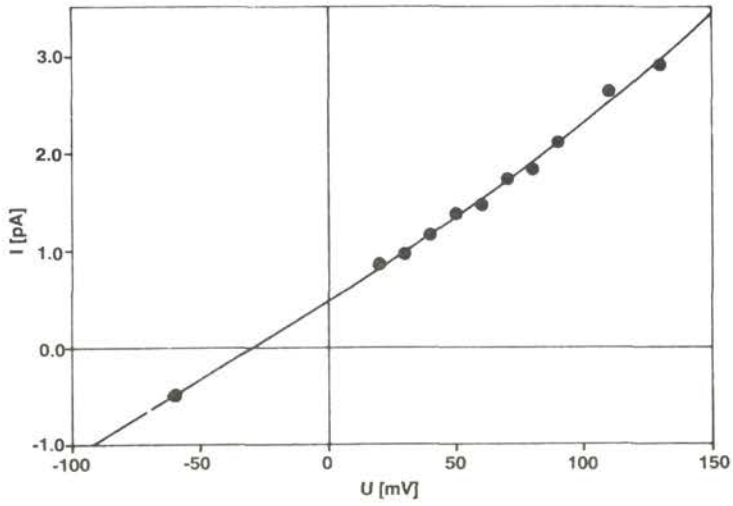

Fig. 2. Current-voltage relationship for DHP-sensitive Ca^{2+} channel. Same conditions as in Fig. 1.

Table 1. Comparison of B-type channel properties

	Calf ventricular muscle*	Crayfish skeletal muscle
Reversal potential (ionic conditions, *cis*/*trans*)	40 mV (100 Ba, 50 Ma// 50 Na)	−30 mV (100 Ba, 50 Na// 50 Na)
Selectivity ratio (P_{Ba}/P_{Na})	10	4
Conductance (100 mmol/l Ba^{2+})	7 10 pS	16 pS
Sensitivity to BAY K 8644	No	Yes

* Rosenberg et al. (1988)

et al. (1988) concerned the sensitivity to BAY K 8644 at the concentration 10 μmol/l. The characteristics of the channel studied in the present experiments are summarized in Table I. The different signs of the reversal potential reflect different voltage definitions.

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