Short communication

Fast Calcium Currents in Cut Skeletal Muscle Fibres of the Frogs *Rana temporaria* and *Xenopus laevis*

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Multiple types of voltage-gated Ca channels have been described in a variety of excitable cells (for a review see Tsien et al. 1987). Electrophysiological and biochemical properties of the slow Ca channels in skeletal muscle have been intensively studied during the last decade (for a review see Beaty et al. 1987; Fosset and Lazdunski 1987). However, recently a fast low threshold Ca^{2+} current was reported in rat skeletal muscle cells in culture (Cognard et al. 1986) and in twitch skeletal muscles of *Rana montezume* (Cola and Stefani 1986) and *Rana pipiens* (Garcia and Stefani 1987; Areola et al. 1987).

The present communication describes fast Ca^{2+} currents in phasic and tonic muscle fibres of the frogs *Rana temporaria* and *Xenopus laevis* and persistence of these currents after denervation.

Similarly as in our previous studies of slow Ca channels in normal and denervated phasic and tonic muscle fibres (Henček et al. 1985; Zacharová et al. 1985) the vaseline gap method of Hille and Campbell (1976) was used to record the fast Ca^{2+} ionic currents. Muscle segments isolated from m. ileofibularis of *R. temporaria* or *X. laevis* were perfused with an internal solution containing (in mmol/l): Cs glutamate 108, $MgCl_2$ 6.5, $CaCl_2$ 0.069, ATP 5, cAMP 0.2, EGTA 5—10, Cs Hepes 20, pH 7.4. The external solution contained (in mmol/l): Na^+ glutamate 110 or the same concentrations of Cs^+ or TMA glutamate respectively, TEA bromide 110, Ca^{2+} glutamate 1.8—10 or Sr^{2+} 5—10, Cs Hepes 10, and TTX 0.001.

Fig. 1 shows two types of inward ionic currents at two voltage pulses lasting several seconds, recorded from 4 different muscle fibre segments of *Rana temporaria*. The ionic currents in *A* were recorded from phasic muscle fibres, those in *B*, *C* and *D* from phasic denervated, tonic, and tonic denervated muscle fibres respectively. At low depolarization a fast activating and non-inactivating current appears as a sole component of the ionic current recorded. At higher depolarizations (−20 mV in A, B, C; −46 mV in *D*) a distinct slowly activating and inactivating current component (slow Ca current) becomes apparent.

At shorter pulses (Fig. 2*A*, *B*) the fast current component appears at wider

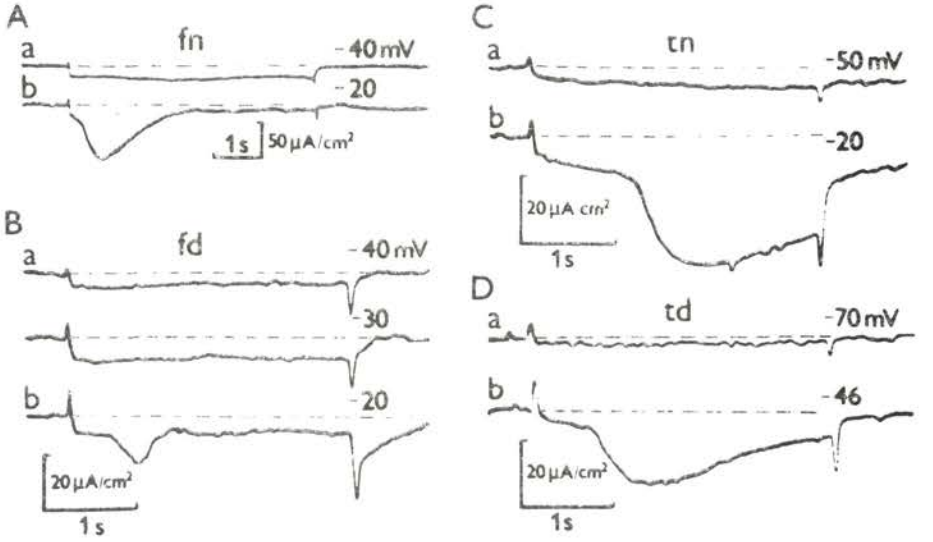

Fig. 1. Fast and slow Ca^{2+} currents recorded in phasic (*A*), phasic denervated (*B*), tonic (*C*) and tonic denervated (*D*) muscle fibres. *Rana temporaria*, m. ileofibularis. *Vh* = −90 mV (*A*, *B*) and −80 mV (*C*, *D*). External solution (in mmol/l): Ca^{2+} 1.8 (10 in *C*, *D*), Na glutamate 110, TTX 0.001.

range of depolarizations. Fig. 2 also reveals that the ionic currents are present both in Ca^{2+} and Sr^{2+} containing salines (*A*, *B*) and in absence of Na ions (*B*, *C*). In *B* and *C*, TMA and Cs ions were substituted for Na ions respectively. The fast calcium currents in denervated tonic muscle fibres (Fig. 2*C*) could be recorded over a wider range of depolarizations as a sole component C; this enabled to construct the current-voltage relationship. The slow Ca current appeared in this case only when the pulse duration was prolonged to more than 1000 ms. The current-voltage relationship reveals that at the given conditions (pulse duration: 100 ms; holding potential: −80 mV) the maximum current is obtained at both −30 mV and −20 mV. The threshold (−60 mV) and maximum values of the fast Ca (Ca_f) current in tonic fibres correlate well with those obtained in phasic fibres of *R. temporaria* and *X. laevis* (Fig. 4) as well as with data from other excitable structures under patch-clamp conditions (Bossu et al. 1985; Mitra et Morad 1986; Carbone et Lux 1987; Narahashi et al. 1987).

Fast activating and inactivating Ca^{2+} inward currents were frequently observed upon applying 20 - 100 ms pulses to phasic muscle fibre segments from *Xenopus laevis* (Fig. 3*A*, *B*, *C*). The records in the left column were obtained at physiological concentrations of Ca and Na ions (1.8 and 110 mmol/l respectively). The amplitudes of these Ca^{2+} currents were considerably increased after blocking the K channels with TEA^+ substituted for Na ions. The

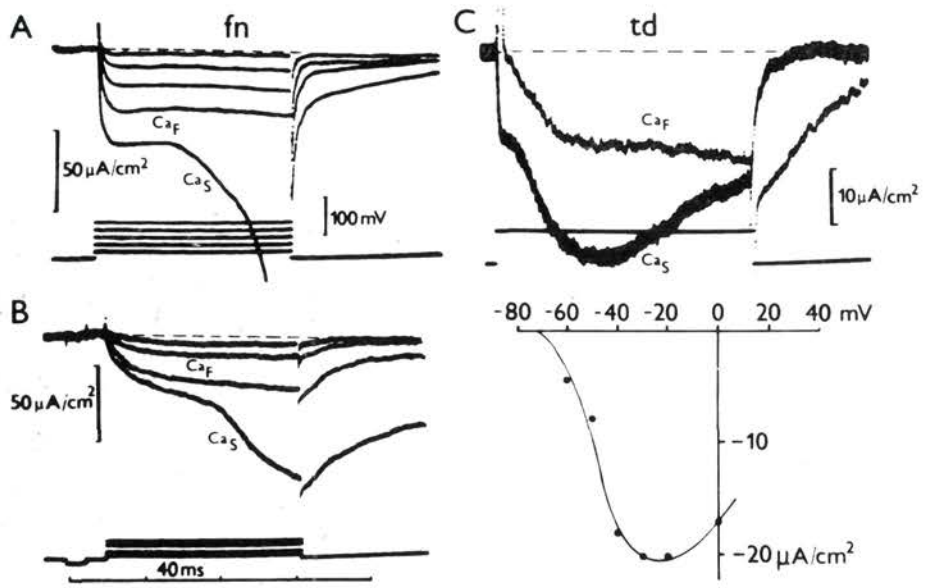

Fig. 2. Activation of fast (Ca_f) and slow (Ca_s) calcium and/or strontium currents. *A*, *B*: Phasic fibres. External solution: *A*: Sr^{2+} 10, Na glutamate 110 (+TTX 0.001), *B*: Sr^{2+} 5, TMA glutamate 110 (+TTX); $Vh = -90$ mV, *C*: Tonic denervated fibre: Ca^{2+} 10, Cs glutamate 110. Two superimposed records with different time bases: Ca_f (100 ms), Ca_s (1000 ms). Inset: Current-voltage relation for a tonic denervated fibre, $Vh = -80$ mV.

tail current in TEA^+ solution reversed its sign in comparison with that in control solution, where an inward current, most probably the slow Ca^{2+} current, started developing. The time constants of activation (τ_m) and inactivation (τ_h) changed from 1.1 to 1.05 ms and from 17 to 30 ms respectively, and conductance (G) increased from 1.56 to 2.65 mS/cm^2. Qualitatively the same changes occurred in solution with Cs ions (Fig. 3*B*; *C* increased from 0.6 to 1.4 mS/cm^2). The calcium nature of this fast calcium current is supported by the effect of calcium blockers, i.e. Ni ions (Fig. 3*C*). When the inward current was blocked, an outward current appeared, as also observed by Cota and Stefani (1986) who used Mg ions as blockers.

On replacing external Na and Cl ions with Cs glutamate, transient fast Ca^{2+} currents have been observed on several occasions also in twitch muscle fibres of Rana temporaria. Fig. 4 shows that the transient fast Ca^{2+} current activated at -60 mV and reached a peak value at -20 mV. The current-voltage relationships of these currents from twitch muscle fibres of *R. temporaria* correlate very well with those in X. laevis fibre. Table 1 gives selected parameters of these currents.

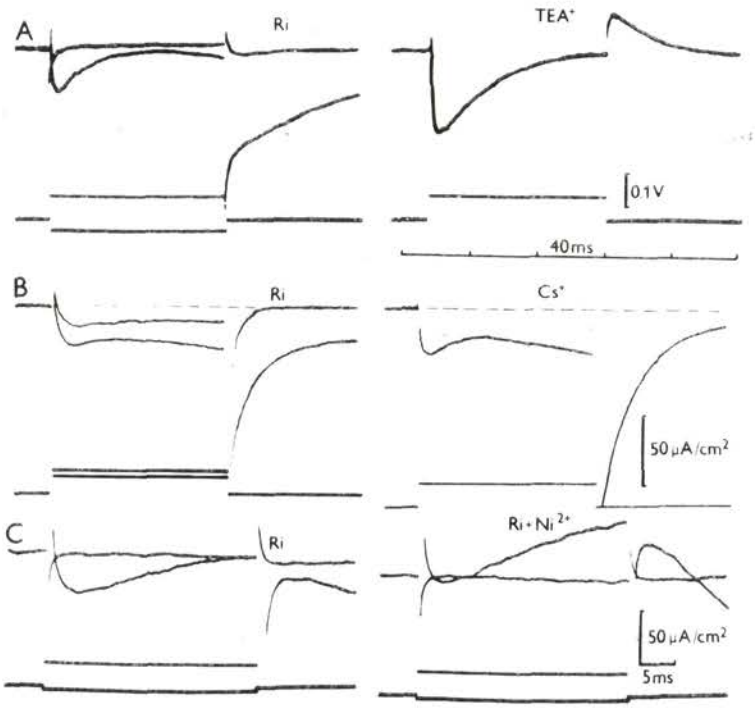

Fig. 3. The effect of TEA^+, Cs^+ and Ni^{2+} on Ca_i in muscle fibres of *Xenopus laevis* (three different fibres). *A, B, C: Left:* in control solution Ri (Ca^{2+} 1.8 mmol/l, Na glutamate 110 + TTX 0.001. *Right:* in TEA^+ bromide 110, Cs ions substituted for Na ions, and after the addition of $NiCl_2$ (2 mmol/l).

Table 1. Conductances and kinetics of transient fast Ca channels in muscle fibres of *Xenopus laevis* and *Rana temporaria*

External solution (mmol/l)	Membrane depolarization (mV)	τ_m (ms)	τ_h (ms)	G mS/cm²	n
Na glutamate 110, Ca glut. 1.8—5	−30	2.00 ± 1.7	21.12 ± 9.22	0.49 ± 0.52	8
Cs glutamate 110 or TEA bromide 110 Ca 1.8	−20	2.08 ± 1.61	20.83 ± 7.77	1.49 ± 0.83	6

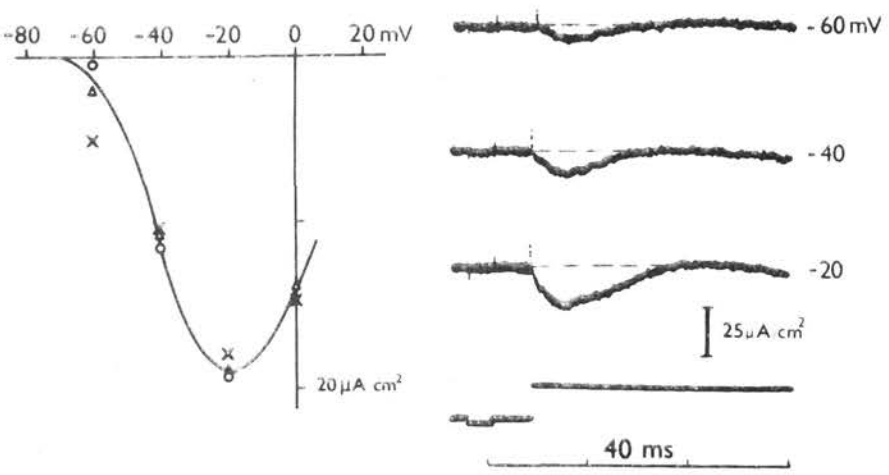

Fig. 4. Transient fast Ca^{2+} currents in muscle fibre segments of *Rana temporaria*. External solution (in mmol): Ca^{2+} 1.8, Cs glutamate 110 + TTX 0.001. Current-voltage relations: *Rana temporaria* (circles, triangles), *Xenopus laevis* (crosses).

In conclusion, our experiments provided evidence for two calcium currents, a fast and slow, present in twitch and tonic muscle of the frog *R. temporaria* and in twitch fiber of *X. laevis*. Both types of Ca channels persist after denervation. The fast Ca^{2+} currents inactivated either very slowly, in particular in response to long lasting stimuli, similarly as in muscle fibres of *Rana montezume* and *R. pipiens* (Cota and Stefani 1986; Arrecola et al. 1987), or rapidly on shorter lasting depolarizations. Both inactivation states could be seen in the same experiments. In denervated twitch and tonic muscle fibers very slow inactivation was observed.

Analysis of the different factors which may play a role in these changes is in progress although it is not sure whether this can be do under voltage clamp conditions.

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