Short communication

Calcium Currents Recorded from Segments of Normal and Denervated Frog Tonic Muscle Fibres

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Recently we have analysed the properties of new sodium channels which appear in tonic muscle fibres after denervation (Zachar et al. 1982). Both the conductance parameters and the selectivity of new sodium channels were in quantitative agreement to those in twitch fibres from the same muscle (Zacharová et al. 1983), except the activation and inactivation rate constants, which were by about one order of magnitude slower in denervated tonic than in denervated twitch muscle fibres. In agreement with the previous evidence (Miledi et al. 1971) we ascribed these differences in rate constants to the effect of a different membrane matrix composition in tonic muscle fibres, on the sodium channel components incorporated after the expression into the membrane. An alternative explanation of both the appearance of the sodium conductance and the rate constant differences is offered on the assumption that the properties of a particular membrane channel were changed after denervation to the extent that it resembles a genuine sodium channel. There is namely recent evidence that the calcium channel can be made permeable for sodium ions under specific experimental conditions. This applies to both the fast calcium channels in nerve (Kostyuk et al. 1973) as well as to slow calcium channels in phasic muscle cells (Almers et al. 1984). Fast calcium channels are absent in both innervated and denervated tonic muscle fibres (Zachar et al. 1982). First, we were therefore aimed at finding out if the tonic muscle fibres posessed slow calcium channels, which are known to exist in twitch muscle fibres (Stanfield 1977; Sanchez and Stefani 1978), and in positive case how they changed after denervation. When this work was in progress, Huerta and Stefani (1983) reported in abstract form on the existence of slow calcium currents in intact tonic muscle fibres of *R. pipiens*. These authors used hypertonic external solution containing (in mmol/l): TEA methane-sulphonate 120; Ca methane sulphonate 10; 3,4 diaminopyridine 5; and sucrose 350. We obtained similar results with muscle fibre segments from tonic muscle fibres of *R. temporaria* (m. ileofibularis), using the vaseline gap method of Hille and Campbell (1976) which enabled the

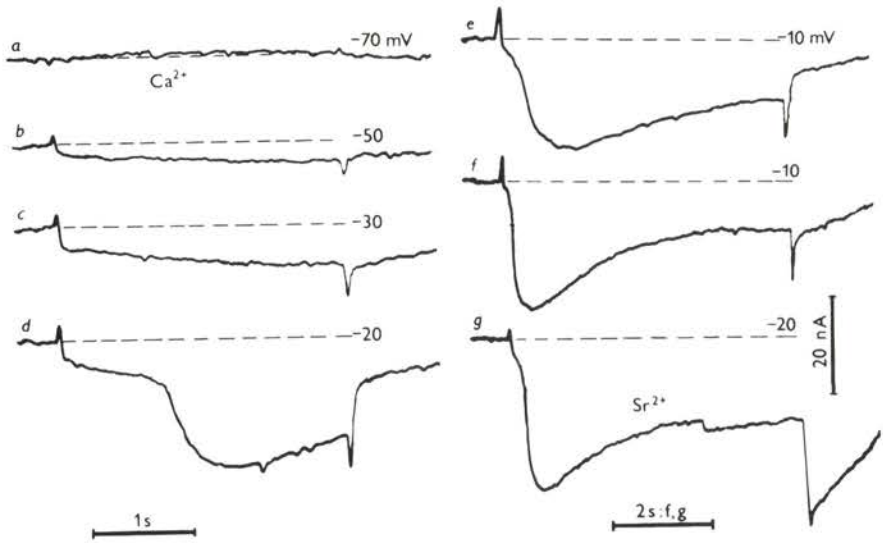

Fig. 1. Slow membrane currents from a perfused segment of tonic muscle fibre (m. ileofibularis of the frog). External solution contained (in mmol/l): 110-Na glutamate, 10-Ca glutamate; in g-Sr was substituted for Ca. Holding potential −80 mV; step depolarization is indicated in mV. Soaked fibre NO 40217.

analysis of slow calcium currents in K free extra- and intracellular environment. In addition we analysed the effect of denervation on the slow calcium currents. Cut tonic fibres were perfused with an internal solution containing (in mmol/l): Cs glutamate 108; $MgCl_2$ 6.5; $CaCl_2$0.069, EGTA 10—20; ATP 5, Cs Hepes 20, pH 7.4 The external solution contained Na or Cs glutamate 110; Ca glutamate 2.0—10; Cs Hepes 10.

Upon the application of long (2—10 s) square pulses slow ionic currents could be recorded, as shown on Fig. 1. Ca current in this experiment developed after a delay during depolarization to −20 mV from the holding potential of −80 mV. The peak amplitude increased and the delay shortened when depolarization was increased to −10 mV. The Ca currents slowly increased (the time to peak was 0.7 s at this depolarization) and then declined to a steady value (Fig. 1*e*). As shown in Fig. 1*g*, Sr ions can carry currents through the calcium channel as well; the kinetics is, however, accelerated and the inward current is increased (compare *d* and *g*). The rate of repolarization was variable. Both the current occurence and its amplitude were dependent on the concentration of Ca ions in external solution (Fig. 2*A*). Step depolarization to the membrane potential of −10 or 0 mV did not evoke the typical slow current with 2 mmol/l Ca_o, and the current appeared when 10 mmol/l Ca_o were added. Fig. 2*B* illustrates that the sodium-free environment

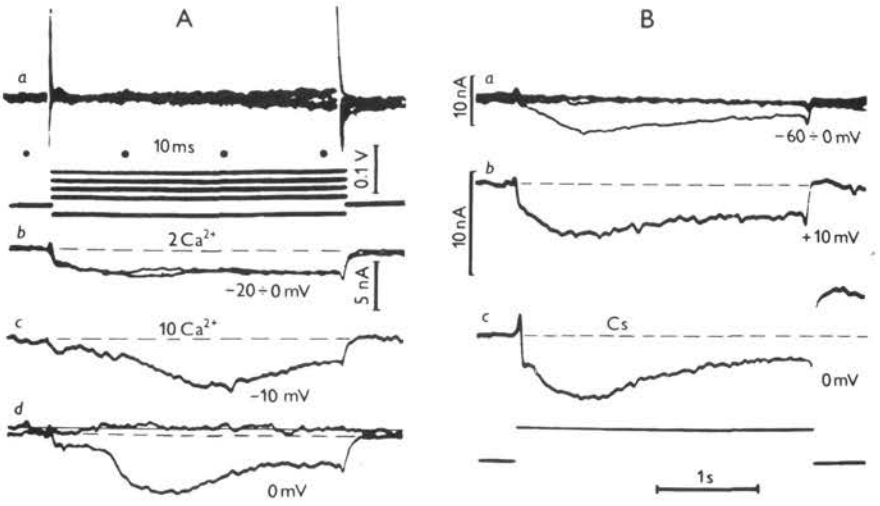

Fig. 2. Effect of Ca (A) and Na-free external solutions (B) on slow membrane currents. Note 2 mmol/l Ca in Ab and substitution of Cs for Na in Bc. Soaked fibres No 40222 (A) and No 40214 (B).

(Cs ions substituted for Na ions) had no substantial effect on these currents.

The ionic blockers Ni^{2+} and Co^{2+} decreased or entirely blocked the slow currents; this blockade was reversible. The effect of Ni (2 mmol/l) on Ca currents recorded from denervated tonic muscle fibre is shown in Fig. 3 (*a*—*f*). Ca currents in denervated tonic muscle membrane were insensitive to TTX (see Fig. 3, *g* and *h*). The denervated fibre lacked the non-inactivating component of the Ca current, as follows from a comparison of inactivation time course of Ca currents in normal (Figs. 1 and 2) and denervated (Fig. 3) tonic muscle fibres.

It follows from the results that the slow calcium currents recorded from tonic muscle fibres of the frog (m. ileofibularis) resemble by both the activation and inactivation characteristics Ca currents recorded under similar conditions in phasic muscle fibres of the same muscle. Table 1 shows selected electrical parameters of slow calcium currents in tonic muscle fibres and reference data from phasic muscle fibres obtained under identical conditions. The amplitude of the calcium current presents the highest difference. The maximum inward current in tonic fibres makes up in average only 1/3 of the I_{Ca} amplitude obtained in phasic fibres. The amplitude is very variable. No calcium currents at all could be recorded from several segments. This difference may be explained on the assumption that slow Ca channels are sited in the tubular system membranes of muscle fibres; this has also been suggested for phasic fibres (Nicola Siri et al. 190; Almers et al. 1981). It is known that surface area and volume of the T system network relative to the

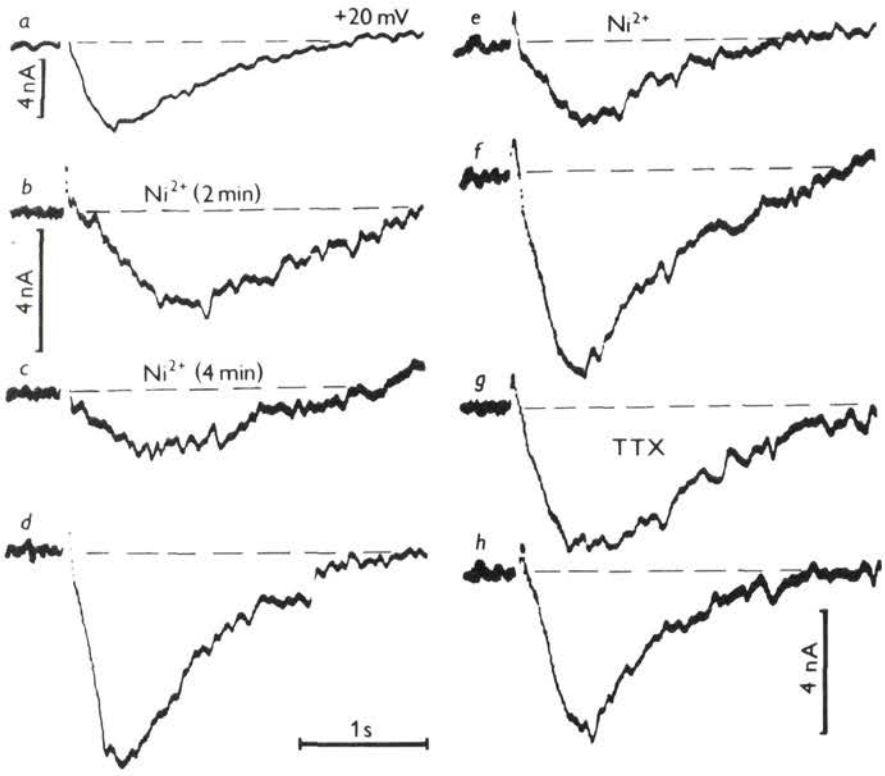

Fig. 3. The effect of Ni^{2+} (2 mmol/l) and TTX (10^{-3} mmol/l) on slow inward currents in a denervated tonic muscle fibre. Soaked fibre No 40124.

external surface area and total volume of the fibre are less than in twitch fibres (Flitney 1971; Franzini-Armstrong 1984). Further differences in the above parameters (time to peak and half-time of inactivation) may also be due to the tubular system geometry of the tonic muscle fibre. The most conspicuous change after denervation concerns the time course of inactivation. The disappearance of the steady-state component of the inactivation phase of calcium currents following denervation of tonic fibres will require, however, further quantitative analysis; this mainly applies to the comparison of activation and inactivation rate changes observed in phasic (twitch) muscle fibres following denervation (Henček et al. 1985).

Table 1. Electrical parameters of Ca currents in normal tonic muscle fibres of the frog

Muscle type	Fibre code	I_{max} (nA)	V_t (mV)	V_{Ca} (mV)	t_a (s)	t_i (s)
Tonic	40118	8	−50	—	—	—
	40119	2.3	−50	52	0.5	1.5
	40214	7	−40	60	0.75	0.83
	40217	26	−40	—	0.7	1.02
	40222	2.5	−50	46	1.17	0.58
	40224	5	−50	40	0.4	0.85
	40321	3	—	20	—	—
	40323	10	—	—	0.37	0.63
	40328	9	−50	—	—	—
	40330	14	−60	—	2	3
	Average	8.7	−49	44	0.84	1.20
Twitch	Average	33	−59.4	43	1.31	1.94
Ratio	Tonic / Twitch	0.3	0.82	1.02	0.64	0.62

I_{max} — maximum Ca inward current, V_t — threshold potential, V_{Ca} — reversal potential, t_a — time to peak, t_i — half-time of inactivation

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Received June 16, 1985/Accepted July 18, 1985