Generation of Twitch Tension in Frog Atrial Fibers by Na/Ca Exchange

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Abstract. Electrical and mechanical responses of frog atrial trabeculae were studied simultaneously using the double-sucrose gap method. Action potentials and twitch tension could be successively generated in fibers in which the slow inward calcium channel current was not observed. As a rule, this could be obtained in the course of a long experiment (3 to 4 hours). Peak tension was shown to increase monotonically with membrane potential in these preparations. In preparations with the slow inward current the total peak tension could be separated into two components. The first component (tonic) monotonically increased with the membrane potential and was probably related to Na/Ca exchange (Horackova 1984). The potential dependency of the second (phasic) component correlated with that of the slow inward calcium current. Only the tonic but not the phasic component could be observed in preparations without the presence of the slow inward calcium current. The tonic component prevailed when both the slow inward current and phasic tension were greatly reduced by nifedipine. Long experiments, long depolarizing clamp pulses, a metabolic inhibitor 2,4-dinitrophenol, inhibitors of Na/K pump ouabain and AR-L57, toxins promoting intracellular sodium accumulation (aconitine, scorpion toxin) were all shown to increase the tonic tension, but not the slow inward current; they induced a transition from biphasic tension-voltage curve into a monotonically increasing one. We concluded that these procedures and agents greatly stimulate Ca influx via Na/Ca exchange. These results show that Na/Ca exchange can function as a reserve system of Ca^{2+} used for contraction, thus supporting the heart function, especially under unfavourable metabolic conditions.

Key words: Frog atrial fibers — Slow inward Ca current — Phasic and tonic tension — Na/Ca exchange

Introduction

Data accumulated during the last decade strongly suggest that extracellular space is the direct source of Ca^{2+} used for contraction in the frog heart (Morad and Orkand 1971; Morad and Goldman 1973; Morad et al. 1983; Klitzner and Morad 1983). Ca^{2+} which activates the myofilaments, can enter the cell through both the slow Ca-channels and the voltage-dependent Na/Ca exchange during excitation. Morad et al. (1983) suggested on the basis of experiments with fast photochemical inactivation of Ca antagonists that the slow calcium channel is the main pathway of Ca^{2+} used for contraction in frog ventricle. However, the absence of any correlation between potential dependency of slow inward calcium current and that of peak tension on frog ventricular fibers (Morad and Goldman 1973; Klitzner and Morad 1983) contradicts this suggestion. On the other hand, both phasic tension related to Ca entry through the slow calcium channels and tonic tension related to Ca entry through Na/Ca exchange have been observed in frog atrial trabeculae (Horackova and Vassort 1976; Horackova and Vassort 1979; reviewed by Horackova 1984).

Presently much attention is being paid to Na/Ca exchange. Some investigators suggest that Na/Ca exchange play a significant role in activating cardiac contraction, especially under the action of cardiac glycosides or toxins, such as veratrine, aconitine, scorpion toxin, sea anemone toxin (Lewartowski et al. 1982; for reviews see Langer 1982; Reuter 1982; Horackova 1984; Honerjäger 1983). It is generally accepted that these compounds exert their positive inotropic effect by increasing intracellular sodium concentration Na_i^+ and subsequently the transmembrane exchange of Na_i^+ for Ca_o^{2+}. Na_i^+ accumulation is due to Na/K pump inhibition by cardiac glycosides and to a prolongation of open state of the fast Na channels by the toxins. A direct quantitative relationship between twitch tension and intracellular sodium activity has been demonstrated in sheep cardiac Purkinje fibers (Eisner et al. 1984).

The question arises whether twitch tension can be generated in frog heart in absence of slow inward calcium current when the main source of Ca^{2+} entering during excitation is Na/Ca exchange.

Materials and Methods

Frog (*Rana ridibunda*) atrial trabeculae, 0.1 to 0.15 mm in diameter, were clamped in a test node, 0.2 mm wide using the double sucrose gap voltage-clamp or current-clamp technique as described previously (Filippov and Porotikov 1983). Electrical and mechanical responses were recorded simultaneously at 18 ± 2°C. Tension was recorded by the optical method (Kass 1981; Filippov and Porotikov 1985). Control resting potential was taken for zero. Membrane potential was measured as the deviation from the resting potential. Holding potential was zero (equal to the control resting

potential) in all experiments. Slow inward current was recorded during the test clamp pulses immediately after a 100 ms conditioning prepulse to +40 mV from the resting potential. The amplitude and duration of the conditioning prepulse were sufficient to inactivate fast sodium channels (Kohlhardt et al. 1972; Reuter 1973, 1979; Filippov and Porotikov 1983). Slow inward current was determined as the difference between the peak of this slow current and the current at the end of the clamp pulse (Horackova and Vassort 1976).

The bundles were stimulated every 20s under current-clamp conditions and every 5s under voltage-clamp conditions. Solutions used (in mmol/l): Ringer's solution (pH = 7.5): NaCl 110; KCl 2.5; $CaCl_2$ 1.8; $MgCl_2$ 1; $NaHCO_3$ 2.4; glucose 5.5; isotonic sucrose solution (sucrose dissolved in redistilled water): sucrose 230; glucose 5.5. The solutions were not gassed.

Results

In some preparations no slow inward calcium current was present (Fig. 1*A*). As a rule this was the case in the course of a long experiment (3 to 4 hours). However, these preparations could generate action potentials with a relatively short plateau and successive twitch tension (Fig. 1*B*). In voltage-clamp experiments, the peak tension was shown to increase monotonically with the potential (Fig. 1*C*). This was not the case with preparations in which the slow inward current was present (Fig. 2). In these preparations the total tension can be separated into two components using the procedure described by Horackova

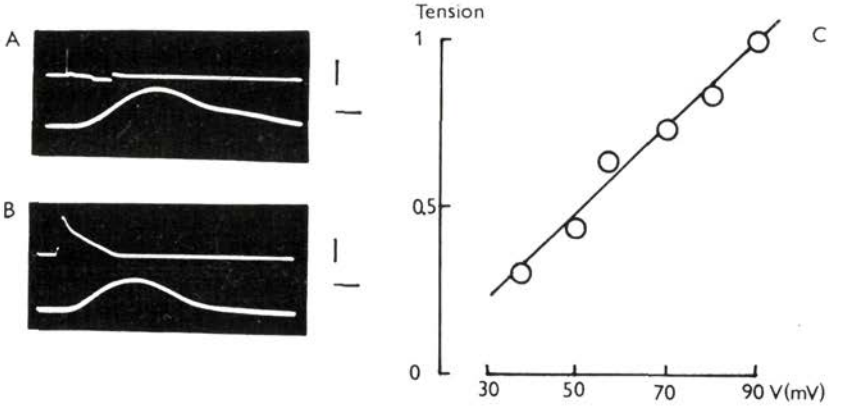

Fig. 1. A preparation with no slow inward current. *A* — Oscilloscope traces of membrane current (*top*) and tension (*bottom*) of frog atrial trabeculae. The membrane was clamped for 80 ms at +70 mV (from the resting potential) immediately after a conditioning prepulse for 100 ms to +40 mV. Upward deflection corresponds to inward current. Only fast inward current is observed on the first pulse. No slow inward current is generated on the test pulse; however, succesive tension develops. Vertical bar = 0.4 μA, horizontal bar = 100 ms. *B* — Traces of action potential (*top*) and the corresponding twitch tension (*bottom*). Vertical bar = 50 mV, horizontal bar = 100 ms. *C* — Voltage dependence of normalized peak tension.

and Vassort (1979). The tonic component monotonically increases with the potential and is related to the Na/Ca exchange (Horackova and Vassort 1979; Horackova 1984). The potential dependency of the phasic component correlates with that of the slow inward current (Fig. 2). It can be seen than no phasic component exists in preparations in which the slow inward current is absent. Only the tonic component related to Na/Ca exchange occurs (Fig. 1*C*).

Is there any procedure or intervention that can induce a transition from biphasic tension-voltage curve (Fig. 2, upper panel) into a monotonically increasing one (Fig. 1*C*)?

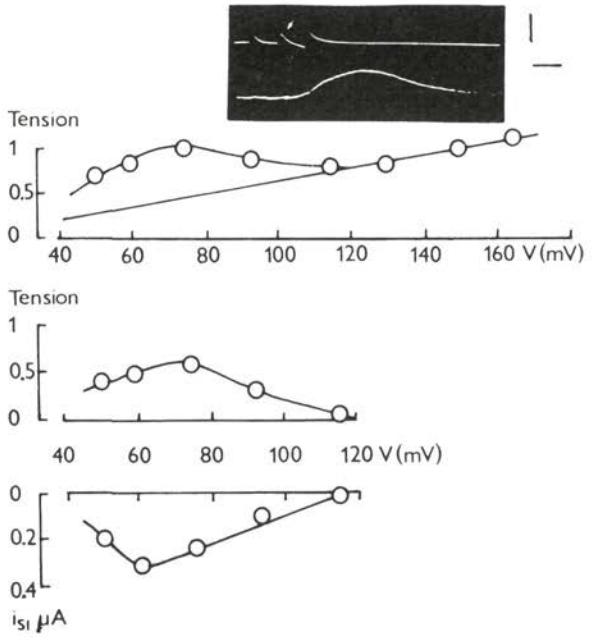

Fig. 2. A preparation with the slow inward current. Upper panel: Normalized peak tension-membrane potential relationship. The phasic tension was subtracted from total peak tension by extrapolating the tonic component (straight line) to membrane potentials below 120 mV. The resulting peak phasic tension (middle panel) and the peak slow inward current (lower panel) plotted against membrane potential. Inset: traces of membrane current (*top*) and tension (*bottom*). The same clamp pulses as in Fig. 1*A*. Arrow point to the slow inward current measured on the test pulse. Vertical bar = 0.4 μA, horizontal bar = 50 ms. Upward deflection corresponds to inward current.

The tendency to transition was observed when the slow inward current and phasic tension had been reduced by a Ca-channel blocker nifedipine (Fig. 3).

It should be noted that such a transition could routinely be obtained in the course of a long experiment. Accordingly, the slow inward current decreased or entirely disappeared. This allowed us to suggest that energy depletion in cardiac

cells can induce a transition. Indeed, some rectification of the tension-voltage curve was observed when a metabolic inhibitor 2,4-dinitrophenol was added to the Ringer solution (Fig. 4). This rectification was due to an increase in the tonic component of tension and to a decrease of the phasic component of tension. Also, the slow inward calcium current decreased.

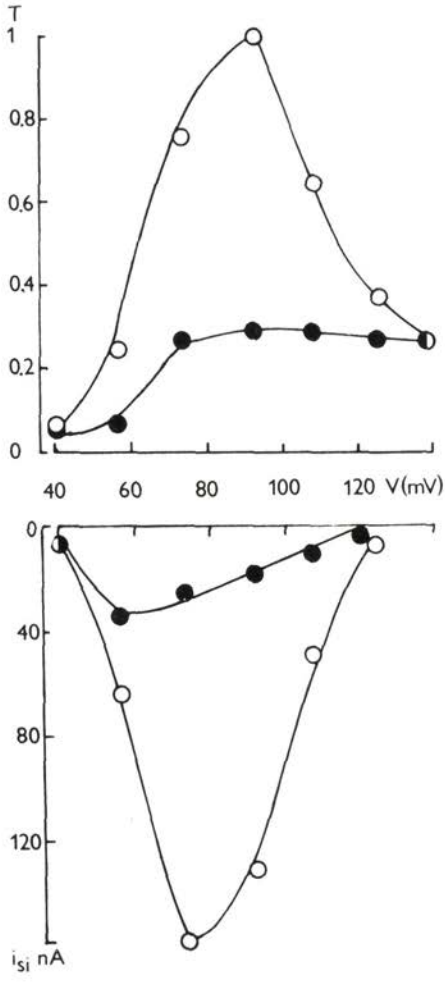

Fig. 3. Effect of Ca-channel blocker nifedipine on the normalized peak tension (*top*) and peak slow inward calcium current (*bottom*) plotted against test potential. Open circles-control, filled circles — nifedipine 10^{-5} mol/l 3 min.

A tendency to transition could be observed in absence of any drug when the duration of the test clamp pulse was increased from 70 ms to 300 ms or more (Fig. 5). This has been reported earlier, and interpreted as being a consequence of a stimulation of tonic component of tension related to Na/Ca exchange by a long depolarizing pulse (Horackova 1984). It can thus be suggested that

compounds which stimulate Ca transport into the cell by Na/Ca exchange may induce transition. Indeed, cardiac glycoside ouabain and another sodium pump inhibitor AR-L57 (Honerjäger et al. 1980) or Na channel modifiers aconitine and scorpion toxin induced in our experiments transition and greatly increased the tonic component of tension (Figs. 6,7). Twitch tension associated with action potential also increased, although the slow inward current decreased or remained unaffected. Similar effects have been reported earlier for alkaloid veratrine (Horackova and Vassort 1974).

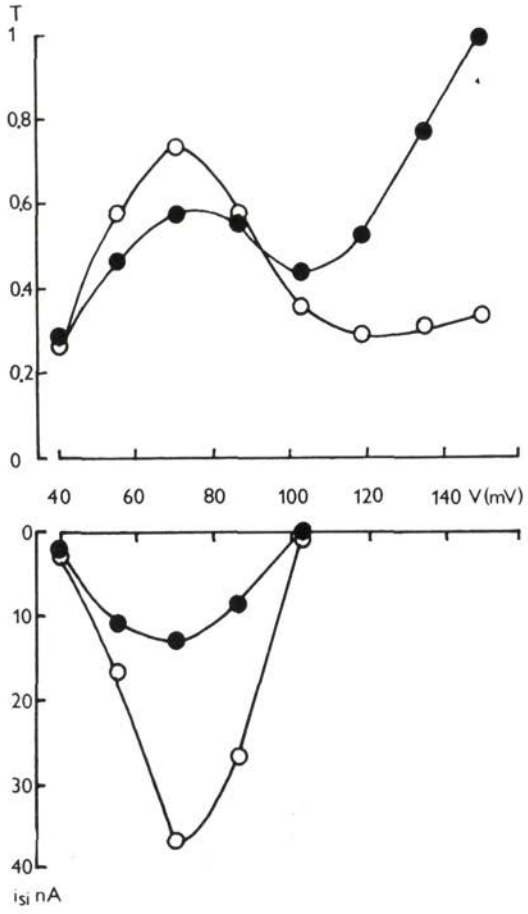

Fig. 4. Effect of an electron transport uncoupler 2,4-dinitrophenol on the normalized peak tension (*top*) and peak slow inward calcium current (*bottom*) plotted against test potential. Open circles-control, filled circles — dinitrophenol 10^{-4} mol/l, 15 min.

Discussion

Our data strongly suggest that Na/Ca exchange can generate twitch tension in frog atrial trabeculae when slow inward calcium current is absent or when this current has been diminished.

Why does the slow inward calcium current disappear in some, especially long, experiments? We do not believe that the inward calcium current overlaps with the outward current generated by Na/Ca exchange during depolarization or with a delayed outward potassium current. Outward exchange current meas-

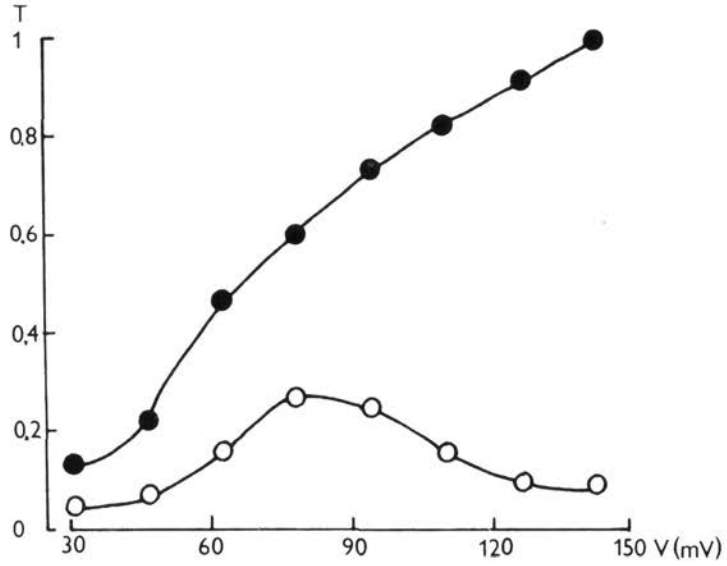

Fig. 5. Effect of the test clamp pulse duration on the normalized peak tension plotted against test potential. Open circles: 70 ms test pulses; closed circles; 300 ms test pulses.

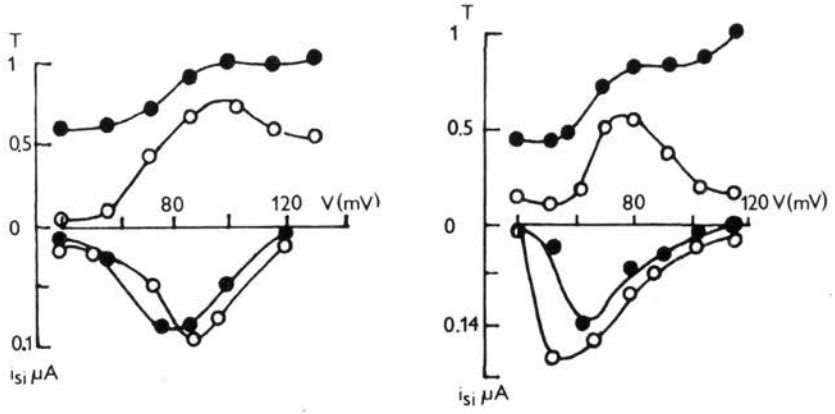

Fig. 6. Effect of sodium pump inhibitors ouabain 10^{-4} mol/l (*left*) and AR-L57 10^{-5} mol/l (*right*) on the normalized peak tension (*top*) and peak slow inward calcium current (*bottom*) plotted against test potential. Open circles — control; filled circles-experimental, 5 min.

ured during depolarization in low Na solution has been shown to be at least 10-times smaller than passive inward calcium channel current (Mentrard et al. 1984). The amplitude of exchange current would be even smaller in normal sodium solution. Moreover, no evidence for a time dependence of exchange current which could mask the time course of slow inward current has been found by these authors. The activation time of delayed outward potassium current is known to be much slower than that of inward calcium current (see, for example Ojeda and Rougier 1974). It would be more reasonable to assume that the decay of the slow inward calcium current is a result of energy depletion in cells of some preparations, especially during long lasting experiments. This suggestion is favoured by the finding that metabolic inhibition decreases the slow inward calcium current in cardiac cells (Nargeot 1976; Nargeot et al. 1978; Kohlhardt et al. 1977; Payet et al. 1978, also see Fig. 4).

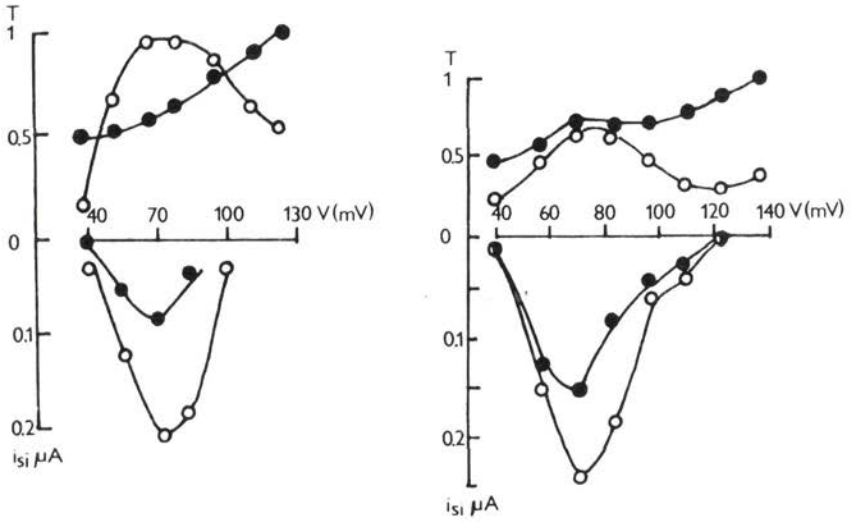

Fig. 7. Effect of agents promoting intracellular sodium accumulation, aconitine 2×10^{-5} mol/l (*left*) and scorpion toxin 10^{-4} mg/ml (*right*) on normalized peak tension (*top*) and peak slow inward calcium current (*bottom*) plotted against test potential. Open circles — control, filled circles — experimental, 5 min.

It is easy to imagine that Na/Ca exchange can operate as a reserve system of Ca^{2+} used for contraction thus supporting the heart function, particularly under unfavourable metabolic conditions when membrane Ca channels, known to be under a strong metabolic control (Nargeot 1976; Nargeot et al. 1978; Reuter 1983) are not operative. Under similar conditions, Ca entry via Na/Ca exchange can be additionally stimulated by an elevation of internal Na concentration, which in turn can be due to ATP deficit and sodium pump inhibition.

This suggestion is favoured by our observation that metabolic inhibitor dinitrophenol, sodium pump inhibitors and toxins promoting intracellular sodium accumulation produce a qualitatively similar increase in tonic tension related to Na/Ca exchange.

Our data also indirectly support the view that Na/Ca exchange is responsible for the positive inotropic effect of sodium pump inhibitors and toxins promoting intracellular Na accumulation. Ca entering the cell via Na/Ca exchange can both, directly activate myofilaments and additionally stimulate Ca release from the sarcoplasmic reticulum, thus promoting a further increase in tension in the mammalian heart (Fabiato 1985).

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