Effects of Chloride Replacement and Chloride Transport Blockade on the Tonic Tension of Frog Atrial Trabeculae

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Abstract. The effects of a chloride-poor medium (methanesulfonate substituted) and a chloride transport inhibitor (SITS) on the outward delayed current and the tonic tension were studied on frog atrial trabeculae under voltage-clamp conditions. The outward delayed current decreased in low-chloride medium (10.5 mmol/l) or in the presence of SITS (2 mmol/l). The tonic tension increased in chloride-poor solution and decreased following SITS. The replacement of chloride by methanesulfonate enhanced the transient increase of tonic tension induced by low external sodium concentration while SITS reduced it. In the same conditions, the effect of the chloride-poor medium was abolished in the presence of SITS. The results showing an increase in Na—Ca exchange in low-chloride medium and a decrease by SITS are discussed assuming that changes in the inner negative charge density influenced the Na—Ca exchange mechanism; the influence of pH_i variation are also considered.

Key words: Chloride ions — SITS — Cardiac contractility — Tonic tension — Na-Ca exchange

Introduction

Detailed information has been obtained concerning the role of chloride ions in electrical properties of cardiac tissue. Substitution of chloride by organic anions has shown that chloride ions make little contribution to the resting conductance of cardiac membrane but become important charge carriers during repolarization (Carmeliet 1961; Hutter and Noble 1961; Peper and Trautwein 1968; Lenfant and Goupil 1977). The replacement of chloride ions by impermeant anions induced in frog myocardium, an increase in the duration of the action potential resulting from a decrease of the outward delayed current and the background current (Bennett and Ware 1966; Anderson and Foulks 1973; Goldman and Morad 1977;

Lenfant and Goupil 1977; Brommundt et al. 1978). Less attention has been paid to the effects of anions on myocardial contractility.

In frog heart, the mechanical activity has been shown to have two components: a phasic one, related to the Ca inward current (Einwächter et al. 1972; Léoty and Raymond 1972; Vassort and Rougier 1972; Horackova and Vassort 1976), and a tonic one, which seems to depend mainly on the Na—Ca exchange mechanism (Goto et al. 1971; Vassort 1973; Chapman 1974; Benninger et al. 1976; Miller and Moisescu 1976; Horackova and Vassort 1979). Such an exchange, affected by changes in extra- and intracellular concentrations of Na and Ca ions, is electrogenic and thus voltage-dependent (Horackova and Vassort 1979; Chapman and Tunstall 1980; Reeves and Sutko 1980; Mentrard et al. 1984).

In frog heart, the substitution of chloride ions by various anions has been shown to induce a positive inotropic action largely dependent on the prolongation of the action potential (Anderson and Foulks 1973). More recently, the positive inotropy of a chloride-poor solution was accounted for by an increase in the two components of contraction: the increase in the phasic tension was related to an increase in the Ca inward current, but no effort was undertaken to explain the mechanism underlying the increase in tonic tension (Horackova and Vassort 1982). We carried out experiments to clarify the influence of chloride ions on the development of tonic tension via the Na—Ca exchange; the effects of a chloride-poor solution (methanesulfonate substituted) and a chloride transport inhibitor (SITS) were investigated in frog atrial trabeculae using a procedure where the Na—Ca mechanism was mainly implicated.

Materials and Methods

Preparation and apparatus

The experiments were carried out at room temperature (18 to 20 °C) on trabeculae (100 to 150 μm in diameter and 3 to 4 mm long), isolated from frog (*Rana esculenta*) auricle. The preparations were mounted in a double mannitol-gap chamber; the experimental apparatus has been described by Léoty and Alix (1976). The tension generated by the portion of the trabeculae in the test gap was measured with a variable resistance transducer (AE 801, Akers Electrotonics). The sensing beam of the transducer was extended by a tungsten needle 5 cm long; the tip of the needle was applied tangentially to the fiber in the central test gap. The fiber was held on each side of the test node by vaseline seals and there was no contraction in the mannitol chambers. The tension was therefore recorded under approximately isometric conditions. However for a large contraction, a small displacement of vaseline seals might have occurred without any variation of the fiber surface in the test compartment. In this case, the mechanical response was intermediate between isometric and isotonic contraction. A similar technique has already been used by Vassort and Rougier (1972), Horackova an Vassort (1976), Potreau and Raymond (1980), Soustre and Rakotonirina (1981).

Solutions

The physiological solution (normal Ringer's solution) contained (mmol/l): NaCl 100; KCl 2.5; $CaCl_2$ 2; $MgCl_2$; Hepes buffer 10. The pH was adjusted with NaOH to 7.8.

Chloride-poor solution was prepared by replacing NaCl by an equimolar amount of Na methanesulfonate prepared by NaOH neutralization of methanesulfonic acid; the concentration of chloride ions was 10.5 mmol/l. Substitution of methanesulfonate for chloride has been shown to have no significant effect on the calcium activity of the physiological solution (Kenyon and Gibbons 1977; Horackova and Vassort 1982). The calcium activity of the chloride-poor solution measured using a Ca^{2+} specific electrode (Orion 93. 20.00) was 96% of that of the normal Ringer; this value was probably underestimated because of negative interference of methanesulfonate with the Ca^{2+} electrode (Dani et al. 1983). Consequently, methanesulfonate was used without calcium adjustement.

In some solutions, the sodium concentration was lowered to 35 or 70 mmol/l by replacing NaCl or Na methanesulfonate by LiCl or Li methanesulfonate prepared by LiOH neutralization of methanesulfonic acid.

SITS (4-acetamino-4'-isothiocyano 2-2'stilbene disulfonic acid) (2 mmol/l) was used to inhibit the transmembrane chloride exchange (Vaughan—Jones 1979). In some experiments (Fig. 2 and Fig. 3), tetrodotoxin (1.5×10^{-3} mmol/l) and $MnCl_2$ (3 mmol/l) were added in all solutions as fast and slow inward current inhibitors respectively.

Procedure

All experiments were carried out under voltage clamp conditions; depolarizing pulses were applied from the holding potential defined as zero reference. The holding potential was adjusted until the amplitude of the fast inward current elicited by a 40 mV step depolarization reached its maximum value ($h\infty \simeq 1$). The preparations were continuously stimulated at a frequency of 0.1 Hz.

In order to specify the effects of Cl^- ions on the Na—Ca exchange mechanism, atrial trabeculae were depolarized every 10 s by about 500 ms long pulses of 140 mV amplitude; at this potential above the apparent reversal potential for the slow inward current, the membrane current was entirely outward and the contractile force consisted of tonic tension which, at this duration of the depolarizing pulse, did not reach a plateau level. Membrane current, tonic tension and the imposed voltage were displayed on an oscilloscope and photographed; tonic tension was continuously recorded by a pen recorder (Gould Brush 220). Fig. *1B* shows a curve obtained by plotting the peak amplitude of tonic tension measured every 10 s. A reduction of the external sodium concentration to 35 mmol/l induced initially an increase in the peak amplitude of tonic tension and then a decrease below the control level. According to Horackova and Vassort (1979), the transient increase results from an activation of Na—Ca exchange: Ca-influx linked to Na-efflux is enhanced while Ca-efflux linked to Na-influx is inhibited. The decrease of tonic tension is due to an inhibition of the Na—Ca exchange resulting from a decrease in Na_i secondary to a change in Na_o (Horackova and Vassort 1979; Chapman and Tunstall 1984): the Na-efflux dependent Ca-influx is decreased. On return to normal Ringer's solution the peak amplitude of tonic tension further decreased before returning to the control level. These changes in tension were accompanied by insignificant changes in membrane outward current (Fig. 1).

In experiments illustrated in Fig. 4 to 7 the transient increase in peak tonic tension was taken as an index of the Na—Ca exchange activity. To this end, a low-sodium solution was applied for a short period (about 1 min), so that the transient increase in peak tonic tension was indeed maximal. In this condition, the transient increase in tonic tension may be related only to the decrease in Na_o generating a more important Ca influx because it may be assumed that the changes in Na_i were limited (Ellis et al. 1981; Chapman et al. 1984), that they were reversible when Na_o was raised back to 100 mmol/l (Chapman et al. 1984) and that no pH_i variation was induced (Deitmer and Ellis 1980). It should be

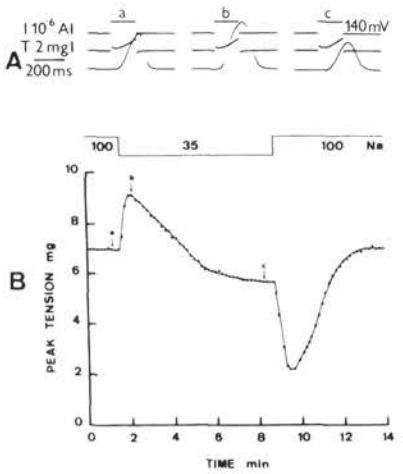

Fig. 1. Effects of a low-sodium solution (35 mmol/l) on peak tonic tension recorded under voltage clamp. *A*: recording of the membrane current (middle trace) and tonic tension (lower trace) in normal solution (*a*) and after 45s (*b*) and 6 min 45 (*c*) in low-sodium solution. *B*: the curve represents the envelope of the peak amplitude of tonic tension measured every 10 s; arrows indicate times of current and tension recordings, illustrated in *A*.

noted that only preparations showing no development of contracture in low-sodium solution were considered. Each curve in the Results section is representative of four to six experiments.

— *Symbols*

V variation of membrane potential from holding potential; depolarizations are positive values
I membrane outward current
T peak amplitude of tonic tension

Results

1 — Effects of a chloride-poor solution and SITS on delayed outward current and tonic tension

The effects of a chloride-poor solution (Fig. 2) and SITS (Fig. 3) on the delayed outward current and the tonic tension induced by variable depolarizing clamp pulses were studied. Current and tension, first recorded in control solution, were measured at maximal effects of low-chloride solution (3.30 min) and SITS (4 min). The amplitude of the outward delayed current was determined as the difference between the total outward current measured at the end of the clamp pulse and the leakage current measured at the beginning of the impulse. The current-voltage and

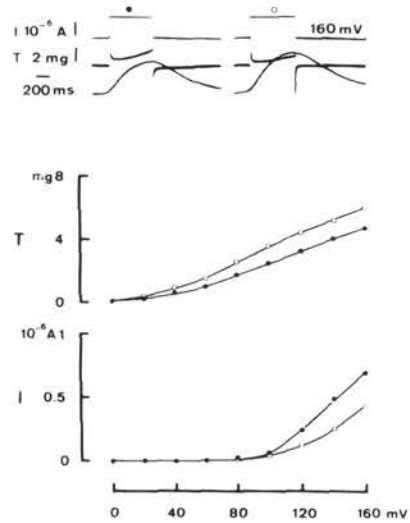

Fig. 2. Effects of a chloride-poor solution on outward delayed current (I) and tonic tension (T). *Top:* current and tension recorded for 160 mV depolarization. *Bottom:* current-voltage and tension-voltage relationships. ● Control solution; ○ chloride-poor solution.

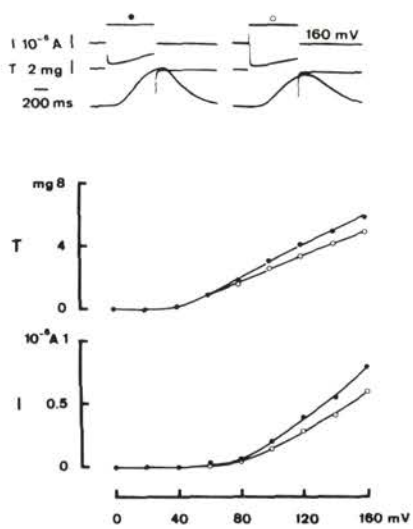

Fig. 3. Effects of SITS (2 mmol/l) on outward delayed current (I) and tonic tension (T) *Top:* current and tension recorded for 160 mV depolarization. *Bottom:* current-voltage and tension-voltage relationships. ● Control solution; ○ SITS.

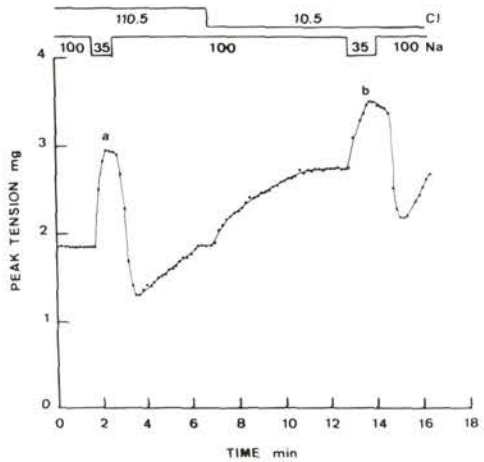

Fig. 4. Effects of a low-sodium solution (35 mmol/l) on peak tonic tension in normal chloride medium (*a*) and in chloride-poor medium (*b*).

ıension-voltage relationships show that the delayed outward current was decreased for any potential under either condition, and that the peak amplitude of tonic tension increased in the low-chloride solution while it decreased in the presence of SITS. Current and tension recorded for a 160 mV clamp pulse are shown. The effects of the low-chloride solution were reversible, but the effects of SITS were poorly or not reversible, because of covalent binding in cellular membrane (Cabantchik and Rothstein 1972).

2 — Effects of a chloride-poor solution on tonic tension in normal and low-sodium solution

As illustrated in Fig. *4a*, lowering external sodium to 35 mmol/l in the presence of chloride ions induced a transient increase in peak tonic tension to about 59% of the control level. After the return of tonic tension to the control level in normal Ringer's solution, the preparation was perfused by a low-chloride solution; tonic tension increased to a new steady level within about 4 min. The application of a low-Na solution in chloride-poor medium elicited a transient increase in tonic tension of only 28% of the initial value (Fig. *4b*). The result may suggest either a reduction of the low-sodium effects on tonic tension in chloride-poor solution or a saturation of the contractile proteins as a result of too a high concentration of internal free calcium. The latter hypothesis seems to fit with the following experiments.

When the external sodium concentration was lowered to 70 mmol/l instead of

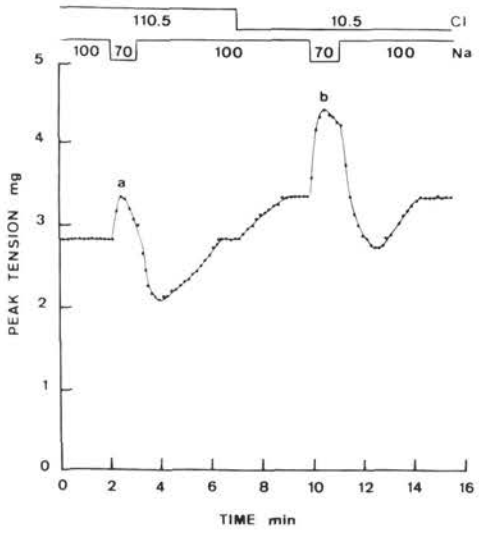

Fig. 5. Effects of a low-sodium solution (70 mmol/l) on peak tonic tension in normal chloride medium (*a*) and in chloride-poor medium (*b*).

35 mmol/l, the transient increase of tonic tension in low-chloride medium reached 32% of the initial value (Fig. *5b*) against 18% in the normal chloride solution (Fig. *5a*). The saturation of the contractile system could also have been avoided in testing the effect of the 35 mmol/l Na solution before the steady state of positive inotropic action of the low-chloride medium was achieved. Fig. *6a* shows that in normal chloride medium, tonic tension increased to about 57% of the control value by lowering external sodium to 35 mmol/l; a simultaneous reduction of external Cl to 10.5 mmol/l and external sodium to 35 mmol/l (Fig. *6b*) induced a more important transient increase in tonic tension (68% of the initial value), while at maximal positive inotropic effect of the low chloride medium (Fig. *6c*) the transient increase in tonic tension in 35 mmol/l Na medium was only 26% of the initial value (see also Fig. *4b*).

3 — Effects of SITS on tonic tension in normal and low-sodium solution

As shown in Fig. *7a*, tonic tension transiently increased to 51% of the control level upon lowering external sodium to 35 mmol/l. In the presence of SITS, tonic tension decreased to a new steady value; the addition of a low-sodium solution (35 mmol-/l) induced a less important increase in tonic tension (37% of the initial value) (Fig. *7b*).

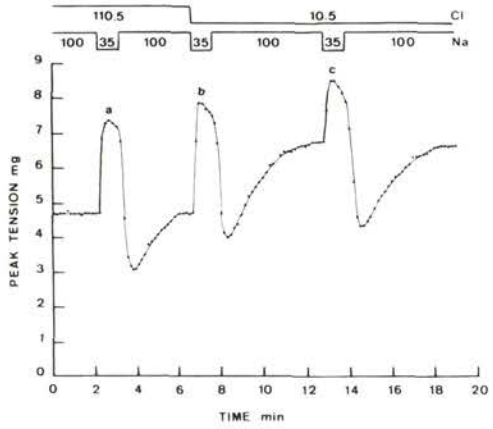

Fig. 6. Effects of a low-sodium solution (35 mmol/l) on peak tonic tension in the presence of chloride ions (*a*), in chloride-poor medium with Na_o and Cl_o simultaneously decreased (*b*), in chloride poor medium (*c*).

4 — Influence of SITS on the effects of a low-chloride, low-sodium solution on tonic tension

In the presence of SITS, after recovery of tonic tension previously decreased by low-sodium solution (Fig. 7*b*), the external chloride and sodium concentrations simultaneously decreased to 10.5 mmol/l and 35 mmol/l, respectively (Fig. 7*c*). The transient increase in tonic tension (35% of the initial value) was not significantly different from that obtained in Fig. 7*b*; thus SITS abolished the enhancement of the transient increase in tonic tension induced by application of a low-chloride, low-sodium solution as illustrated in Fig. 6*b*.

Discussion and Conclusion

The present study shows that the replacement of chloride ions by methanesulfonate ions induces a decrease in the delayed outward current. This finding is in agreement with the studies of Lenfant and Goupil (1977) and Horackova and Vassort (1982) who used various substituted anions on frog atrial preparations. It might be supposed that like methylsulphate (Lenfant and Goupil 1977), methanesulfonate decreases the second component of the delayed outward current. SITS, which had been shown to block chloride influx and chloride efflux in cardiac Purkinje fibres (Vaughan—Jones 1979), decreases the delayed outward current. A similar result was obtained by Taupignon et al. (1982) who described a decrease in the second component of the delayed outward current in the presence of SITS.

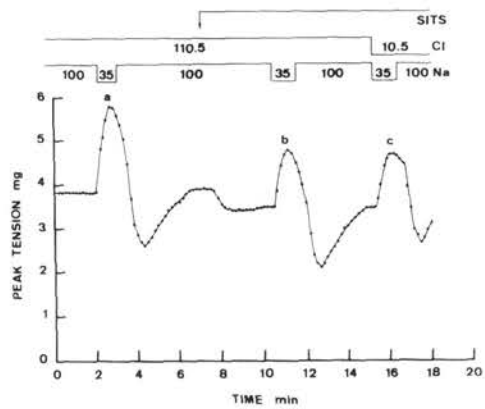

Fig. 7. Effects of a low-sodium solution (35 mmol/l) in the absence of SITS (*a*), in the presence of SITS (*b*), in the presence of SITS with Na_o and Cl_o simultaneously decreased (*c*).

Though chloride-poor solution and SITS have similar effects on the delayed outward current, their inotropic actions are opposite: tonic tension is increased in chloride-poor solution and decreased by SITS. There is no correlation between the variation in delayed outward current and the variation in tonic tension.

The transient increase in tonic tension elicited by a low-sodium solution was used to evaluate the Na—Ca exchange activity involved in the development of tonic tension (Horackova and Vassort 1979). It is noteworthy that the time-course of peak tonic tension changes obtained in low-sodium medium (Fig. 1) was similar to the time-course of the variations of the exchange current (I_{ex}) observed by Mentrard et al. (1984); their results suggested that the Na—Ca exchange may generate an outward current when Ca ions are moving into the cell.

The transient increase in tonic tension in low-sodium solution is more accentuated in chloride-poor medium, suggesting that the Na—Ca exchange is more sensitive to sodium alterations: the sodium-dependent Ca-influx which contributes to the activation of tonic tension may be enhanced in low-chloride medium. The inhibition of chloride transport by SITS leads to a reduction of the transitory increase of tonic tension in low-sodium solution, resulting from a decrease in Na—Ca exchange activity. Accordingly, in normal sodium solution, the increase in tonic tension in chloride-poor medium and its decrease in the presence of SITS can be explained by activation and reduction of the Na—Ca exchange mechanism respectively. It might be expected that in chloride-poor medium and in the presence of SITS the Na—Ca exchange current exhibited the same variations as those of the Na—Ca exchange; this current was not measurable in our experimental conditions.

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Received February 7, 1985/Accepted October 11, 1985