Effects of Strontium Ions on Contraction and Action Potential in Rabbit Papillary Muscles. A Comparison with Effects of Tetraethylammonium Ions

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Abstract. The effects of Sr^{2+} on contraction and action potential were studied in rabbit papillary muscles and compared with effects of tetraethylammonium (TEA^+) The membrane potential was measured with KCl-filled microelectrodes and the contraction was simultaneously recorded using a mechanoelectrical transducer A partial (90 %) substitution of extracellular Ca^{2+} (Ca_e^{2+}) by Sr^{2+} produced stimulation frequency-dependent prolongation of the action potential (AP) with a dominant phase "plateau" as well as prolongation of the contraction At low frequencies where the AP prolongation was well pronounced, the contraction became biphasic The effect of Sr^{2+} on both AP and contraction was blocked by nifedipine (10 μmol/l) or by increasing Ca_e^{2+} Ryanodine suppressed the early contraction component only AP was prolonged to a similar extent and in the same frequency-dependent manner by TEA^+ (20 mmol/l) Despite similar AP configuration, no biphasic contraction developed in the presence of TEA^+ High Ca_e^{2+} (10 mmol/l) or low Na_e^+ (70 mmol/l) suppressed the TEA^+ effect on AP The data indicate that the two components of the biphasic contraction are of different origin, the early one is activated by activator cation released from the sarcoplasmic reticulum while the late one results from the Sr^{2+} entry across the sarcolemma via L-type Ca^{2+} channels

Key words: Heart — Strontium — Biphasic contraction — Tetraethylammonium

Introduction

Sr^{2+} can substitute for Ca^{2+} in some electrophysiological processes as well as in generating tension at myofibrillar level it was reported both to permeate calcium channel as a charge carrier of the L-type calcium current (I_{CaL}, Kohlhardt et

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al 1973, Hess et al 1986) and to actıvate the contractıle apparatus (Kerrıck et al 1980) However, there ıs a dıfference observed ın cardıac muscle Sr^{2+} prolongs, usually quıte markedly, the durations of AP and of contractıon (Kondo 1987) These effects, however, were studıed at low stımulatıon frequencıes (Braveny and Šumbera 1972, Kıng and Bose 1983, Kondo 1987) and theır frequency-dependence was not descrıbed At low stımulatıon frequencıes, partıal (90%) substıtutıon of Ca^{2+} wıth Sr^{2+} ın dog trabeculae (Kıng and Bose 1983) or ın dıfferent specıes (Bravený and Šumbera 1972) was reported to ınduce development of bıphasıc contractıons where the early component was attrıbuted to ıntracellular release of actıvator catıon and the late one to Sr^{2+} entry across sarcolemma The way of Sr^{2+} entry, however remaıned obscure The L-type Ca^{2+} channels were suggested but Sr^{2+} could enter the cell also vıa the Na^+/Ca^{2+} exchanger workıng ın the reverse mode

In thıs study, the frequency-dependence of Sr^{2+} effects on both AP and contractıon wıll be descrıbed Furthermore, the Sr^{2+}effects wıll be compared wıth the effects of a classıcal potassıum conductance blocker, TEA^+ (for revıew, see Stanfield 1983), another ıntervention whıch prolongs the duratıon of the actıon potentıal (APD) Applıcatıon of TEA^+ prolongs APD ın neurons (Tasakı and Hagıwara 1957), cardıac Purkınje fibers (Ito and Surawıcz 1981), and workıng ventrıcular myocardıum (Ochı and Nıshıye 1974) The effect was attrıbuted to a block of out ward potassıum current (Armstrong 1971) It was also shown, ın canıne Purkınje fibers that the effect on APD was strongly frequency-dependent showıng a reverse use dependence (Ito and Surawıcz 1981) The compaı ıson of Sr^{2+} and TEA^+ ef fects allows (under certaın assumptıons) to dıscuss the ımportance of I_{CaL} and/or Na^+/Ca^{2+} exchange for development of the late component In addıtıon, ıt wıll be shown that the TEA^+ effect ıs antagonızed by ınterventıons ıncreasıng ıntracellulaı Ca^{2+} whıch could play a role ın the reverse use dependence of the TEA^+ effect

Materials and Methods

Adult rabbıts (1 5 3 kg) of eıther sex were anaesthesızed wıth pentobarbıtal (60 mg/kg body weıght and 1000 IU/kg heparın, ı v) Hearts were quıckly excısed and placed ın Tyrode solutıon (see the compositıon below) Thın papıllary muscles (dıameter 0 4 0 8 mm, length $\sim$ 4 mm) were dıssected from the rıght ventrıcle and placed ın the experımental chamber, where they were attached to an ısometrıc foıce transducer (RCA 5734, for more detaıl, see Pučelık et al 1983) After an ınıtıal stabılızatıon perıod, the restıng tensıon was set so that the developed twıtch tensıon reached 90 95% of maxımum at 1 Hz stımulatıon frequency The bath was peıfused wıth Tyrode solutıon at 36 °C at a constant flow rate (6 10 ml/mın)

Muscle preparatıons were stımulated by punctate electrodes (square-wave voltage pulses 0 1 ms duratıon, amplıtude $\geq$50% above threshold) at a chosen stımulatıon frequency (3 33, 2, 1 43, 1, 0 2, 0 1, or 0 03 Hz) Membrane potentıal was

measured with glass microelectrodes (filled with 3 mol/l KCl, resistance ~ 20 MΩ), simultaneously with the isometric tension viewed on an oscilloscope and recorded using an analogue tape recorder (Racal Thermionic, GB) and/or a pen recorder (RFT NEK polygraph, FRG).

The Tyrode solution had the following composition (in mmol/l): NaCl 137, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.5, $NaHCO_3$ 11, glucose 10. The solution was aerated with 95% O_2 and 5% CO_2 (pH 7.4). Tetraethylammonium bromide (TEA^+; 20 mmol/l) was added to the normal Tyrode solution to make the TEA^+-medium. In Sr^{2+}-medium, 90% of Ca^{2+} was substituted by Sr^{2+} (the solution thus contained 1.8 mmol/l Sr^{2+} + 0.2 mmol/l Ca^{2+}). In some experiments, Sr^{2+} and Ca^{2+} concentrations were different: 5.8 mmol/l + 0.2 mmol/l or 4 mmol/l + 2 mmol/l, respectively. Solutions containing nifedipine (Sigma, St. Louis, USA) were protected from light. Ryanodine and other chemicals were from Sigma.

Only steady state AP and contractions were used for analysis. Since both Sr^{2+} and TEA^+ slowed down the terminal repolarization, and measurement of APD at more positive levels would therefore underestimate their effect, APD was measured at a level corresponding to 90% repolarization. For mechanical data, peak values of active tension are reported and expressed in arbitrary units (*a.u.*). The resting tension level was always taken as zero. When means were calculated the values were standardized to contraction at 1 Hz under control conditions, and are given in relative units (*r.u.*). The contraction duration and the time to peak of contraction were measured from the onset of AP. Data are presented as mean ± S.E.M. When appropriate, Student's *t*-test was used for statistical analysis. Differences at $p \leq 0.05$ were considered to be significant.

Results

A partial (90%) substitution of extracellular Ca^{2+} by Sr^{2+} (Sr^{2+} 1.8 mmol/l + Ca^{2+} 0.2 mmol/l) caused considerable changes of both AP and contraction. AP was prolonged and the effect was stimulation frequency-dependent (Fig. 1). The effect was more pronounced at low stimulation frequencies, e.g. at 1 Hz frequency APD increased from 235.0 ± 15.0 ms (n = 6) in control to 508.8 ± 69.8 ms in Sr^{2+}-containing medium, and at 0.1 Hz from 181.7 ± 12.2 ms to 2400.0 ± 239.3 ms. The shape of the AP was quite characteristic: dominant phase 2 (plateau) terminated by a relatively fast terminal repolarization (Fig. 1, insets). There was a tendency for mild depolarization of the resting membrane potential (RMP), the changes, however, were not significant (e.g., −80.2 ± 2.6 mV (n = 6) in control and −77.0 ± 3.0 mV in Sr^{2+}-medium at 0.1 Hz).

In the presence of Sr^{2+}, the contraction was prolonged correspondingly with the AP prolongation in a frequency-dependent manner (Fig. 2*A*): e.g., at 1 Hz, the contraction duration increased from 311.7 ± 11.9 ms (n = 6) in control to

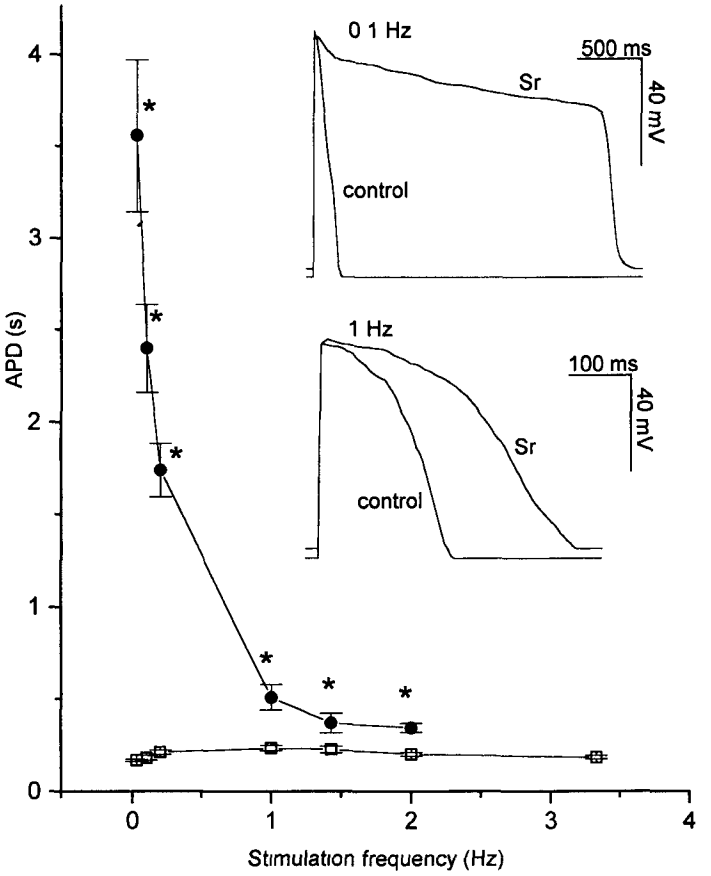

Figure 1. Effect of Sr^{2+} on APD at various stimulation frequencies Sr^{2+} replacing 90% of extracellular Ca^{2+} (Sr^{2+} 1 8 mmol/l + Ca^{2+} 0 2 mmol/l) $n = 6$, *, significantly different from control, $p \leq 0\ 05$ Open squares control Filled circles Sr^{2+} Data point reflecting APD in Sr^{2+}-medium at the highest frequency (3 33 Hz) was omitted since APD was longer than the cycle length (300 ms), hence the real frequency was lower The effect of Sr^{2+} increases with the decreasing frequency Top inset Effect of Sr^{2+} on AP at the stimulation frequency of 0 1 Hz Bottom inset Effect of Sr^{2+} on AP at the stimulation frequency of 1 Hz

582 5 ± 73 8 ms in Sr^{2+}-containing solution, and at 0.1 Hz from 331 7 ± 19.9 ms to 2628 ± 268 ms. Moreover, at low stimulation frequencies (0 2 Hz and less) where the AP prolongation was well pronounced, the contraction became clearly biphasic with the first fast and the second slow components. The relative amplitude of the second component was frequency-dependent, being 31.1 ± 4.8% ($n = 6$) of the

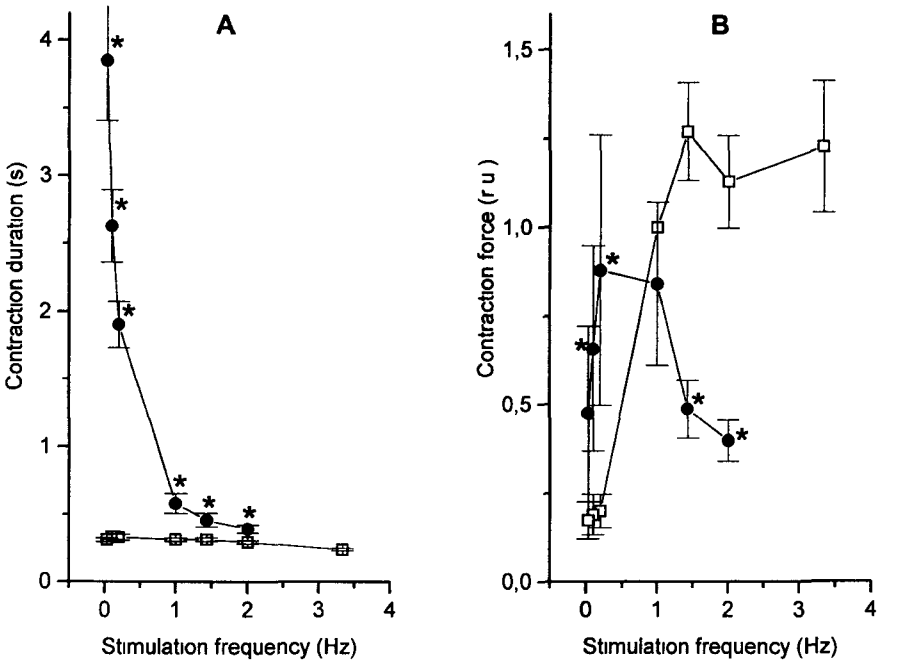

Figure 2. Effect of Sr^{2+} on contraction Sr^{2+} replacing 90% of extracellular Ca^{2+} (Sr^{2+} 1 8 mmol/l + Ca^{2+} 0 2 mmol/l) $n = 6$, *, significantly different from control, $p \leq 0\ 05$ Open squares control Filled circles Sr^{2+} Data point reflecting contraction duration (A) or contraction force (B) in Sr^{2+}-medium at the highest frequency (3 33 Hz) was omitted since APD was longer than the cycle length (300 ms), hence the real frequency was lower A Effect of Sr^{2+} on contraction duration at various stimulation frequencies (in the case of biphasic contractions, the values represent total duration) B Effect of Sr^{2+} on contraction force at various stimulation frequencies (in the case of biphasic contractions, the values represent peak force of the contraction i e , peak force of the first component) Contraction force was standardized to contraction at 1 Hz in control

first component at 0.2 Hz, $39.9 \pm 4.9\%$ at 0.1 Hz and $48.8 \pm 6\ 8\%$ at 0.03 Hz. It is possible that both components were also present at higher frequencies but they were not clearly distinguishable. The time to peak of contraction was prolonged at any frequency tested, e.g. 103.3 ± 7.8 ms ($n = 6$) in control and 165.8 ± 12.9 ms in Sr^{2+}-containing solution at 0.1 Hz. The contraction force was reduced at high stimulation frequencies (2 and 1 43 Hz), while an increase was observed at low frequencies (0.2 and 0 1 Hz; Fig 2*B*)

To investigate the mechanisms underlying the effect of Sr^{2+}, the following experiments were performed When the Sr^{2+} concentration in the experimental solution was increased from 1.8 to 5.8 mmol/l (0 2 mmol/l Ca^{2+} present in both

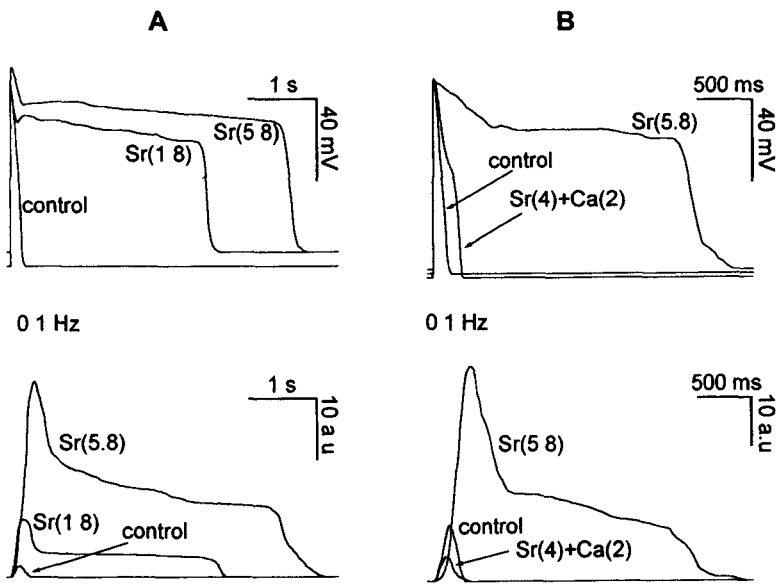

Figure 3. Modulation of the Sr^{2+} effect by Ca^{2+} and by Sr^{2+} Top panels APs Bottom panels Contractions Stimulation frequency 0 1 Hz *A* Increasing the Sr^{2+} concentration (from 1 8 to 5 8 mmol/l with 0 2 mmol/l Ca^{2+} present all the time) further enhanced the effect *B* Increasing the Ca^{2+} concentration from 0 2 to 2 mmol/l (simultaneously decreasing the Sr^{2+} concentration from 5 8 to 4 mmol/l to keep the total concentration constant) suppressed the effect

solutions) the above described changes of both AP and contraction became more obvious (Fig. 3*A*). Interestingly, these effects were blocked by the application of a solution containing 2 mmol/l Ca^{2+} and 4 mmol/l Sr^{2+} (Fig. 3*B*). The application of solution with Ca^{2+} totally omitted and substituted by Sr^{2+} (2 or 6 mmol/l) led to permanent depolarization and refracterity. The application of nifedipine (10 μmol/l) blocked the Sr^{2+}-induced prolongation of AP and totally suppressed the contraction (Fig. 4*A*). Ryanodine (1 μmol/l), a selective blocker of sarcoplasmic reticulum release channels, eliminated the first contraction component and induced a further prolongation of AP and of the second contraction component (Fig. 4*B*).

TEA^+ (20 mmol/l) induced a prolongation of AP as expected. The effect was stimulation frequency-dependent (Fig. 5), small at high frequencies (1 Hz and higher) and very pronounced at low ones (0.2 Hz and less). While APD at the stimulation frequency of 1 Hz increased from 228.0 ± 11.6 ms ($n = 6$) to only 435.0 ± 14.8 ms, at 0.1 Hz APD changed from 180.0 ± 8.9 ms to 2190.0 ± 533.0 ms. Also, the shape of AP in the presence of TEA^+ was strikingly similar to that in

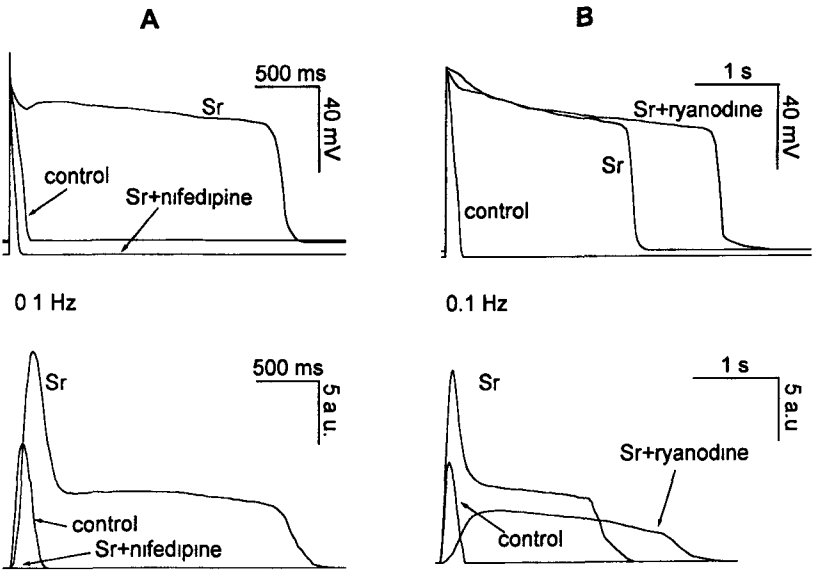

Figure 4. Pharmacological modulation of the Sr^{2+} effect Top panels APs Bottom panels Contractions Stimulation frequency 0 1 Hz *A* Nifedipine (10 μmol/l) eliminated Sr^{2+}-dependent prolongation of AP and totally suppressed the contraction *B* Ryanodine (1 μmol/l) eliminated the first contraction component only, while AP and the second contraction component became more prolonged

the presence of Sr^{2+}; the dominant phase 2 (plateau) terminated by a relatively fast terminal repolarization (Fig. 5, insets). RMP did not change significantly (e.g., -73.0 ± 4.1 mV in the absence and -76.8 ± 2.5 ms in the presence of TEA^{+} at 0.1 Hz; $n = 6$).

In contrast to the considerable effect of TEA^{+} on APD, the contraction time course was not influenced markedly. Although a mild prolongation (15–20%) was observed at stimulation frequencies of 1 Hz and 0.5 Hz, it was by far not comparable with the Sr^{2+} effect (Figs. 6*A*, 2 *A*). The late contraction component did not appear at any stimulation frequency. The time to peak of contraction remained also unchanged in the presence of TEA^{+} at any frequency tested (e.g., 102.5 ± 7.0 ms and 104.2 ± 7.2 ms at 0.1 Hz; $n = 6$). The contrast between the effects of TEA^{+} on APD and contraction duration is well documented in Fig. 7. It shows that the contraction duration is independent on the TEA^{+}-induced prolongation of AP On the other hand, the Sr^{2+}-dependent prolongation of AP correlates well with the contraction prolongation. At low frequencies (1 Hz and less), the application of TEA^{+} elicited a pronounced positive inotropic effect (Fig. 6*B*) e.g., at 0.1 Hz

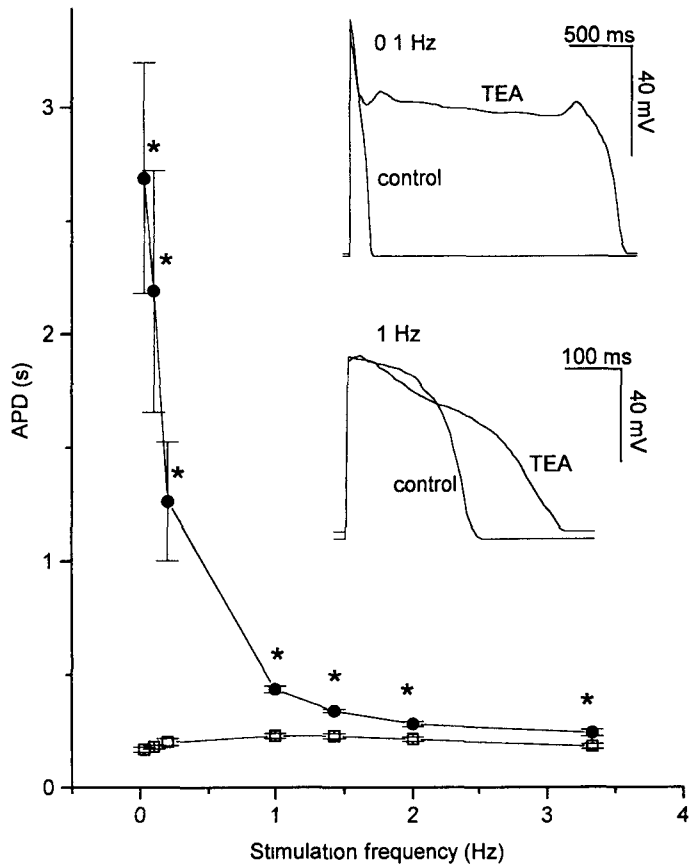

Figure 5. Effect of TEA^+ on APD at various stimulation frequencies $n = 6$, *, significantly different from control, $p \leq 0\ 05$ Open squares control Filled circles TEA^+ The effect of TEA^+ increases with the decreasing frequency Note the similarity with the effect of Sr^{2+} Top inset Effect of TEA^+ on AP at the stimulation frequency of 0 1 Hz Bottom inset Effect of TEA^+ on AP at the stimulation frequency of 1 Hz

TEA^+ increased the contraction force from 0.3 ± 0.1 *r.u.* ($n = 6$; standardized to the contraction force at 1 Hz in control) to 1.8 ± 0.3 *r.u.* On the other hand, at high frequencies (3.3 and 2 Hz), there was a tendency to reduced contraction force.

To obtain some additional information about the possible role of the reverse mode Na^+/Ca^{2+} exchange in the development of the late contraction component, we performed experiments with the reverse mode enhanced by increasing Ca_e^{2+} (up to 10 mmol/l) or decreasing Na_e^+ (70 mmol/l, completed by sucrose). Interestingly, these interventions completely suppressed the TEA^+-dependent AP prolongation

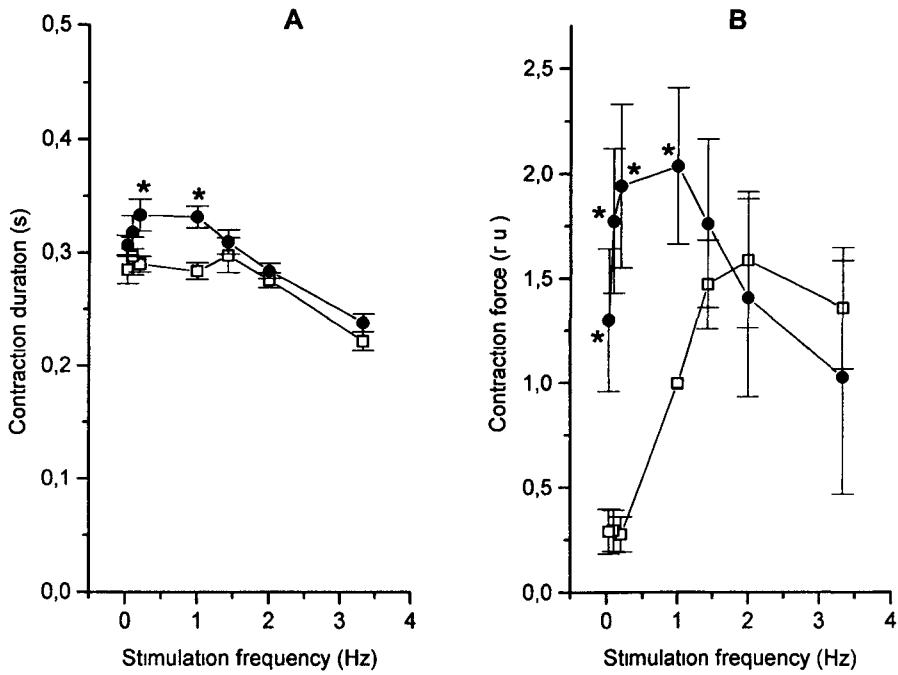

Figure 6. Effect of TEA^+ on contraction $n = 6$, *, significantly different from control, $p \leq 0\,05$ Open squares control Filled circles TEA^+ *A* Effect of TEA^+ on contraction duration at various stimulation frequencies *B* Effect of TEA^+ on contraction force at various stimulation frequencies Contraction force was standardized to contraction at 1 Hz in control

(Fig. 8*A*, *B*). Both interventions would be expected to increase Ca_i^{2+} and subsequently the contraction force. Regarding this issue, the results were somewhat controversial. We observed reduction, increase as well as no change of the contraction force. It may be related to the fact that tension was not measured continuously in our experiments, and the resting tension level was reset to zero before each record. An increase of the resting tension could then be responsible for the apparent reduction or no change of active contraction.

Discussion

The present results confirm the findings of Bravený and Šumbera (1972) and King and Bose (1983) that at low stimulation frequencies, partial substitution of Ca_e^{2+} by Sr^{2+} induces an AP prolongation as well as the development of biphasic contraction Furthermore, we observed a frequency-dependence of these effects in the wide range

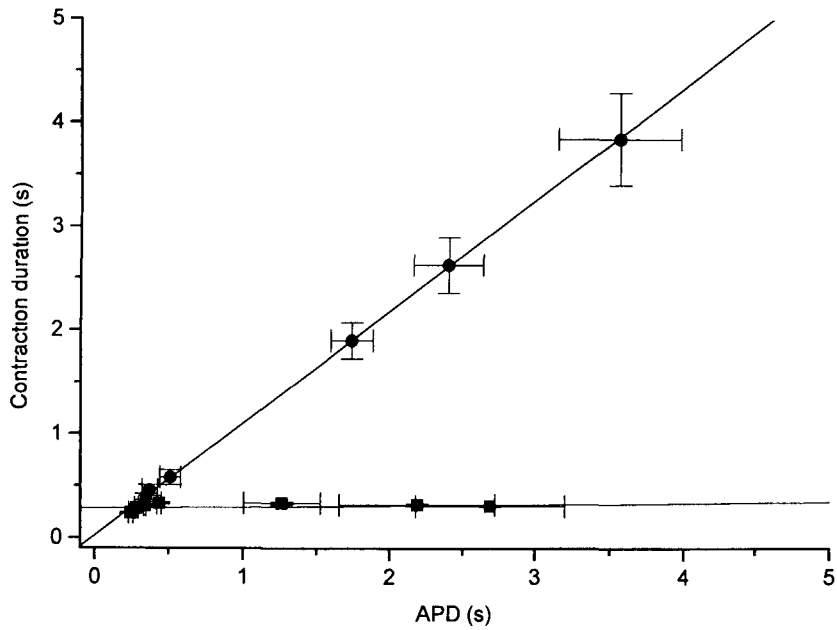

Figure 7. Dependence of contraction duration on APD in the presence of TEA^+ or Sr^{2+} $n = 6$ Squares TEA^+ Circles Sr^{2+} In the presence of Sr^{2+}, APD and contraction duration were prolonged correspondingly and correlated well On the other hand, TEA^+ prolonged AP but not contraction, hence contraction duration was independent on APD

of stimulation frequencies (from 0 03 Hz to 3 33 Hz) and suggested that the reverse mode Na^+/Ca^{2+} exchange plays only a negligible role in the development of the late component of the biphasic contraction which is therefore mainly due to Sr^{2+} influx through I_{CaL} channels Another new finding is that the effect of TEA^+, a potassium conductance blocker, is antagonized by interventions increasing Ca_i^{2+} which could play a role in the reverse use dependence of the TEA^+ effect

Replacing 90% of Ca^{2+} in the extracellular solution with Sr^{2+} (1 8 mmo/l Sr^{2+} + 0 2 mmol/l Ca^{2+}) induced a marked prolongation of AP The effect was even more pronounced when the Sr^{2+} concentration was increased (5 8 mmol/l Sr^{2+} + 0 2 mmol/l Ca^{2+}) Since the inactivation of I_{CaL} is not only voltage- but also Ca^{2+}-dependent (for review, see Pelzer et al 1990), the rate of inactivation decreases when cations other than Ca^{2+} carry the current (Hess et al 1986, McDonald et al 1986) A slower inactivation of I_{CaL} and subsequently longer influx of Sr^{2+} could explain the observed AP prolongation Another possibility is a block of repolarizing currents Certainly, the Ca^{2+}-activated currents, in the heart first of all the Ca^{2+}-dependent transient outward current (Sipido et al 1993), will be sup-

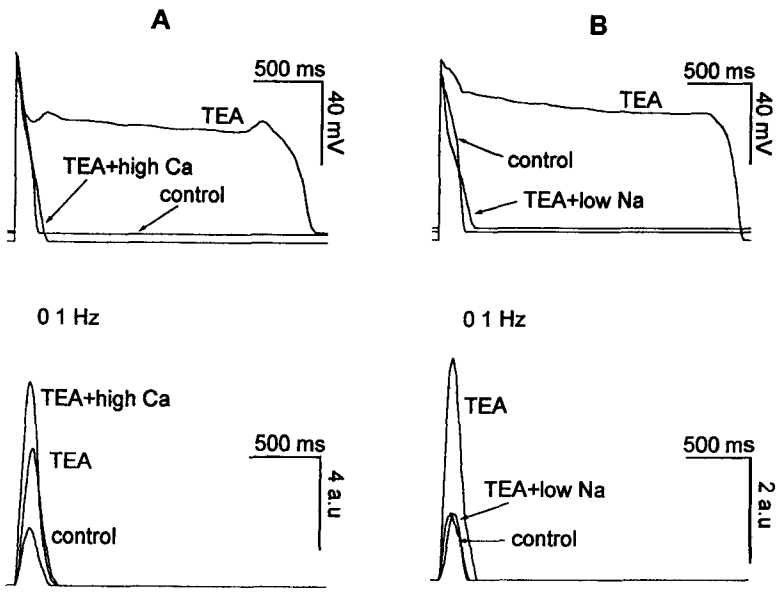

Figure 8. Modulation of TEA^{+} effect by interventions increasing $Ca^{2+}{}_{i}$. Top panels APs Bottom panels Contractions Stimulation frequency 0 1 Hz *A* The effect of TEA^{+} on AP was blocked in the presence of high extracellular Ca^{2+} (10 mmol/l) *B* Application of extracellular solution with low Na^{+} concentration (70 mmol/l) suppressed the effect of TEA^{+} on AP

pressed. Furthermore, in frog skeletal muscle Ba^{2+} and Sr^{2+} were reported to block the inwardly rectifying K^{+} conductance (Standen and Stanfield 1978). Nevertheless, Sr^{2+} was around 400 times less effective than Ba^{2+}, and should not have any major effect at concentrations used in our experiments. The results are in favour of the first hypothesis: the Sr^{2+} effect was blocked by nifedipine, a selective blocker of I_{CaL}, as well as by raising the Ca^{2+} concentration in external solution from 0.2 to 2 mmol/l (while decreasing the Sr^{2+} concentration from 5.8 to 4 mmol/l to keep the total concentrations of both divalent cations constant). Although suppression of the effect by raising Ca_e^{2+} does not allow to distinguish between the above mentioned possibilities (increased Ca^{2+} would both enhance the Ca^{2+}-dependent inactivation of I_{CaL} and activate the Ca^{2+}-dependent repolarizing currents), experiments with nifedipine strongly support the first hypothesis. Since nifedipine is a selective blocker of I_{CaL}, suppression of the Sr^{2+} effect is consistent with the first hypothesis. On the other hand, nifedipine was reported to inhibit transient outward K^{+} current (Gotoh et al. 1991) and therefore, if the Sr^{2+} effect was related to a block of repolarizing currents, nifedipine should promote the effect of Sr^{2+} or have

no effect at all which was not the case. Further support is provided by the fact that, in Sr^{2+}-treated preparation, APD and contraction duration always correlate very well suggesting a substantial influx of divalent cation, activator of contraction, during all AP.

The contraction in Sr^{2+}-containing medium became biphasic, with the first, fast and the second, slow components. Ryanodine, a blocker of sarcoplasmic reticulum release channels, suppressed the first component but did not influence the second one. The results suggest that the first component is caused by release of activator cation from the sarcoplasmic reticulum, which is in good accordance with the data of King and Bose (1983) obtained in dog trabeculae. The time to peak of contraction in Sr^{2+}-treated muscle (i.e., the time to peak of the first component) was always longer than in control suggesting a lower efficiency in activation of sarcoplasmic reticulum release (if we admit that Sr^{2+} and Ca^{2+} are equally efficient in activating cardiac myofibrils as shown by Kerrick et al. 1980). However, we are not able to distinguish whether the activator of the release is Sr^{2+} (then it must be a weaker activator than Ca^{2+}) or Ca^{2+} (a lower efficiency due to lower external Ca^{2+} concentration). In a recent study Spencer and Berlin (1997) demonstrated that Ca^{2+} influx, but not Sr^{2+} influx, via sarcolemmal I_{CaL} channels can induce Sr^{2+} release from the sarcoplasmic reticulum in rat ventricular myocytes; therefore, the second possibility appears to be more probable.

With regard to the late contraction component, its mechanism remained obscure. There was an agreement that external Sr^{2+} entering the cell during the prolonged AP is responsible but the pathway remained unclear. King and Bose (1983) concluded that I_{CaL} channel is a probable candidate but they admit that at least a part of the late component could result from the Na^+/Ca^{2+} exchange. The exchanger is able to transport Sr^{2+} instead of Ca^{2+} (Na^+/Sr^{2+} exchange, Kimura et al. 1987). If, during the plateau phase, the membrane potential is more positive than the reversal potential of the exchange, the exhanger would work in the reverse mode, thus transporting Sr^{2+} into the cell. Experiments with nifedipine, a selective blocker of I_{CaL} channels, seem to confirm the conclusion of King and Bose (1983), that I_{CaL} is the main pathway. One has to keep in mind, however, that nifedipine, by blocking the AP prolongation, would also prevent the reverse mode of the Na^+/Ca^{2+} exchange, and therefore, these experiments do not provide sufficient information. To distinguish between I_{CaL} and Na^+/Ca^{2+} exchange, it would help to depolarize the membrane as in the presence of Sr^{2+}, but without affecting I_{CaL}. This is possible either pharmacologically (by blocking the repolarizing currents) or by artificial depolarizing current as in sucrose gap experiments by Bravený and Šumbera (1970). In their experiments, the artificial AP prolongation was accompanied by sustained contraction, but the membrane potential was held at potentials more positive than the plateau potentials in the presence of Sr^{2+}. In our experiments, we used the pharmacological approach: block of repolarizing cur-

rents by TEA^+ TEA^+ was described to prolong AP in canine and sheep Purkinje fibers (Ito and Surawicz 1981, Kenyon and Gibbons 1979, respectively) or in guinea pig papillary muscles (Ochi and Nishiye 1974) The underlying mechanism is probably a block of time-dependent outward K^+ currents, transient outward current, I_{to}, and delayed rectifier, I_K (Kass et al 1982, Kenyon and Gibbons 1979), while the background K^+ current, I_{K1}, does not seem to be influenced significantly (Ito and Surawicz 1981) We characterized the TEA^+ effect and found that, concerning AP, it is strikingly similar to the effect of Sr^{2+} The late contraction component, however, never developed suggesting that the Ca^{2+} influx via the reverse mode Na^+/Ca^{2+} exchange during prolonged AP plateau is not sufficient to activate the contraction If we then assume that concentration gradients for Ca^{2+} in TEA^+ experiments and for Sr^{2+} in Sr^{2+} experiments (subsequently the reversal potentials of the Na^+/Ca^{2+} and Na^+/Sr^{2+} exchangers) are similar, it is possible to conclude that the role of Na^+/Sr^{2+} exchange in the development of the late contraction component is negligible

To make it more clear, experiments were performed with the reverse mode Na^+/Ca^{2+} exchange enhanced by increasing Ca_e^{2+} or decreasing Na_e^+ Interestingly, these interventions did not produce the late component but suppressed the TEA^+-dependent AP prolongation This effect is probably related to an increase of Ca_i^{2+} which is known to modulate many membrane currents One possibility is that increased intracellular Ca^{2+} would stimulate I_{to}, its Ca^{2+}-sensitive component (Coraboeuf and Carmeliet 1982, Sipido et al 1993) and/or I_K which was also reported to be Ca^{2+}-sensitive (Tohse et al 1987) Other explanation could be the existence of Ca^{2+} dependent K^+ current, $I_{K(Ca)}$ Such a current is well known in arterial smooth muscle (for review, see Nelson and Quayle 1995) but there is no evidence for its existence in the heart ventricle although it was reported in Purkinje fibers (Callewaert et al 1986) and atrial myocytes (Baro and Escande 1989) This phenomenon could also help explain the reverse use dependence of the TEA^+ effect Since Ca_i^{2+} is increased at high frequencies, the effect of TEA^+ is antagonized, while at low frequencies when Ca_i^{2+} is decreased the TEA^+ effect becomes more pronounced Beside the effect on AP, TEA^+ had a positive inotropic effect at low stimulation frequencies It is probably due to Ca^{2+} influx (and subsequently increased sarcoplasmic reticulum Ca^{2+} load) via the Na^+/Ca^{2+} exchange working in reverse mode during long lasting AP This influx, however, has to be quite small, since it is not able to induce the late contraction component

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