Effects of Bivalent Cations on Post-Rest Adaptation in Guinea-Pig Heart Muscle

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Abstract. In isolated papillary muscles of guinea-pig hearts, the inotropic effects of bivalent cations, Ca^{2+}, Ba^{2+}, Sr^{2+}, and Ni^{2+}, were investigated during post-rest adaptation in order to study their individual action on excitation-contraction coupling. Upon exposure to each cation studied, the force of contraction was transiently enhanced, whereas the steady state force was influenced differently: it increased with Ca^{2+}, Ba^{2+} and Sr^{2+} and was depressed by Ni^{2+}. The transmembrane action potentials (measured at 90 % repolarization) were slightly prolonged by Sr^{2+} and even more by Ba^{2+}, and were shortened by Ca^{2+} and Ni^{2+}. After 10 min rest, the post-rest contractions consisted of a late peak (P_{II}) that was enhanced in high Ca^{2+}-solution an by Sr^{2+}. Ni^{2+} and Ba^{2+} depressed P_{II} and during adaptation to pre-rest controls an early peak of contraction (P_I) prevailed. There was no simple relation between post-rest adaptation of force and the duration of action potential in the presence of the bivalent cations tested. During post-rest adaptation the two components of contraction can be separated. The results are interpreted in terms of a model of excitation-contraction coupling which derives Ca ions for contractile activation from two sources: transmembrane calcium influx and calcium release from cellular stores. From the different effects on post-rest adaptation it is concluded that the individual cations influence excitation-contraction coupling more specifically and not merely by "screening-off" the negative surface charges.

Key words: Post-rest adaptation — Action potentials — Two components of contraction — Calcium — Barium — Strontium — Nickel

Introduction

The contractile activation of the heart muscle depends on extracellular Ca^{2+}. During regular stimulation, there is also a substantial release of Ca^{2+} from cellular stores in addition to transmembrane Ca^{2+} entry (Fabiato 1985). The contribution of either source of Ca^{2+} for contraction is influenced by the rate of stimulation (Wohlfart and Noble 1982).

According to Lewartowski et al. (1978) the adaptation of both the electrical and the mechanical activity after a prolonged period of rest can be used to study the dynamics of cardiac excitation-contraction coupling because the first beat after a prolonged pause becomes independent of the preceding contraction and is mainly related to transmembrane Ca^{2+}-influx (Beresewicz and Reuter 1977; Reiter et al. 1984). The first contraction is characterized by a delay in onset and a late peak of force coinciding roughly with the rapid final repolarization phase of the action potential (AP). In the course of adaptation to steady state stimulation an early component of contraction occurs which transiently leads to biphasic contractions. The early component is characterized by a rapid onset following depolarization and has been related to Ca^{2+} release from cellular stores.

In studying inotropic compounds, some knowledge about which component of contraction is predominantly influenced appears useful. Unfortunately the two components of contraction are not readily distinguishable upon regular stimulation at frequencies around 1 Hz; however, they become apparent after rest. We have therefore developed an analysis scheme to separate the post-rest adaptation of the early component from that of the late one. This approach was tested by studying the effects of various bivalent cations that are known to affect contraction force, i.e. Ca^{2+}, Sr^{2+}, Ba^{2+} and Ni^{2+}, on the post-rest adaptation of electrical and mechanical activity in guinea-pig papillary muscles.

Materials and Methods

Freely running papillary muscles, less than 1 mm in diameter, were excised from the right ventricles of guinea-pig hearts and mounted horizontally in a 5 ml chamber that was continuously perfused with oxygenated Tyrode solution at 35 ± 0.5 °C. The papillary muscles were placed with their base under a spring clamp, the tendinous portion was attached through a small glass hook to a strain gauge (Statham UC-2 cell) for isometric force recording. The resting length of the muscle was adjusted by applying a stretching force of 3—5 mN to yield an optimal contraction force. The force was continuously recorded with a pen writer (Helco-scriptor HE 16, Hellige). The preparations were stimulated via platinum point electrodes. The stimulation parameters were: duration 2 ms, intensity 1.5 times the threshold, frequency 1 Hz.

Transmembrane action potentials were recorded with conventional glass micropipettes of 10—20 MΩ resistance when filled with KCl, 3 mol/l. The action potentials (APs) were displayed together with the contraction force on the screen of a dual beam oscilloscope (Tektronix 502 A) and photographed at regular intervals. The electrical and mechanical parameters were measured directly from the photographs by means of a digitizing device (9874A Digitizer, Hewlett Packard). The digital data were stored and processed using a desk-top computer (9845B, Hewlett Packard) a) for accurate estimates of P_I and P_{II} according to the definitions (see page 333), and b) for the calculation of mean ± S.E.M. in the course of post-rest adaptation.

The perfusion medium was modified Tyrode solution with the following composition (in mmol/l): NaCl 137; KCl 5.4; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 12; NaH_2PO_4 0.21; glucose 5.5. The pH of the solution was maintained at 7.4 by gassing with a mixture of 97 % O_2 and 3 % CO_2. The chloride salts of the cations Ba^{2+}, Sr^{2+}, Ni^{2+} and Ca^{2+} were dissolved in distilled water and appropriate amounts of these stock solutions were added to the perfusion medium to yield the final concentration of the respective cation required. The solution containing 0.9 mmol/l Ca_2 was prepared by adding $CaCl_2$ stock solution to nominally Ca^{2+}-free Tyrode solution.

In all experiments muscles were allowed to equilibrate until all the parameters measured became constant (60 min). Cumulative concentration-response curves were obtained by perfusing the bath with solutions containing increasing concentrations of the respective cation and allowing 15 min for equilibration at each concentration.

The influence of Ba^{2+}, Sr^{2+}, Ni^{2+} or Ca^{2+} on post-rest adaptation was studied at concentrations that produced half maximum effect on contraction force as determined from the concentration-response curves. In these experiments, stimulation was interrupted for 10 min at the end of the equilibration period, and after resuming stimulation, APs and contraction force were observed until a new steady state was reached (usually within 5 min). The preparations were then exposed to the respective cation and post-rest adaptation was measured once more after 30 min exposure. The reversibility of the effects was tested after 30 min washing in normal Tyrode solution.

Results

1. *EC_{50} for the inotropic effects of bivalent cations*

The effects of bivalent cations on post-rest adaptation were investigated at concentrations inducing half maximum inotropic responses as determined by the EC_{50} values from the concentration-response curves. Fig. 1 illustrates the effects of cumulative concentrations of Ca^{2+}, Sr^{2+}, Ba^{2+} and Ni^{2+} on contraction force and the duration of action potential at 20 % and 90 % repolarization (APD_{20} and APD_{90}, respectively). Under our experimental conditions the EC_{50} of contraction force strengthening induced by Ca^{2+} and Sr^{2+} was 3.6 and 5 mmol/l, respectively. In the upper concentration range, the determination of the concentration-response curve for Ca^{2+} was limited by solubility, that for Sr^{2+} by the saturation of the effect. Both cations shortened APD_{20}, APD_{90} was shortened by Ca^{2+} and prolonged by Sr^{2+} (see Discussion). Ba^{2+} was very effective in prolonging the duration of the action potential, and moderately effective in strengthening contraction force. EC_{50} was 0.2 mmol/l. The occurrence of arrhythmias (Antoni and Oberdisse 1965; Reid and Hecht 1967) and contractures limited the concentration-response curve to 3 mmol/l. The negative inotropic effect of Ni^{2+} (EC_{50} 0.5 mmol/l) was accompanied by shortening of the AP plateau phase (APD_{20}) and by a slight prolongation of APD_{90}.

Time-to-peak tension (TtPT) was shortened upon increasing Ca^{2+} con-

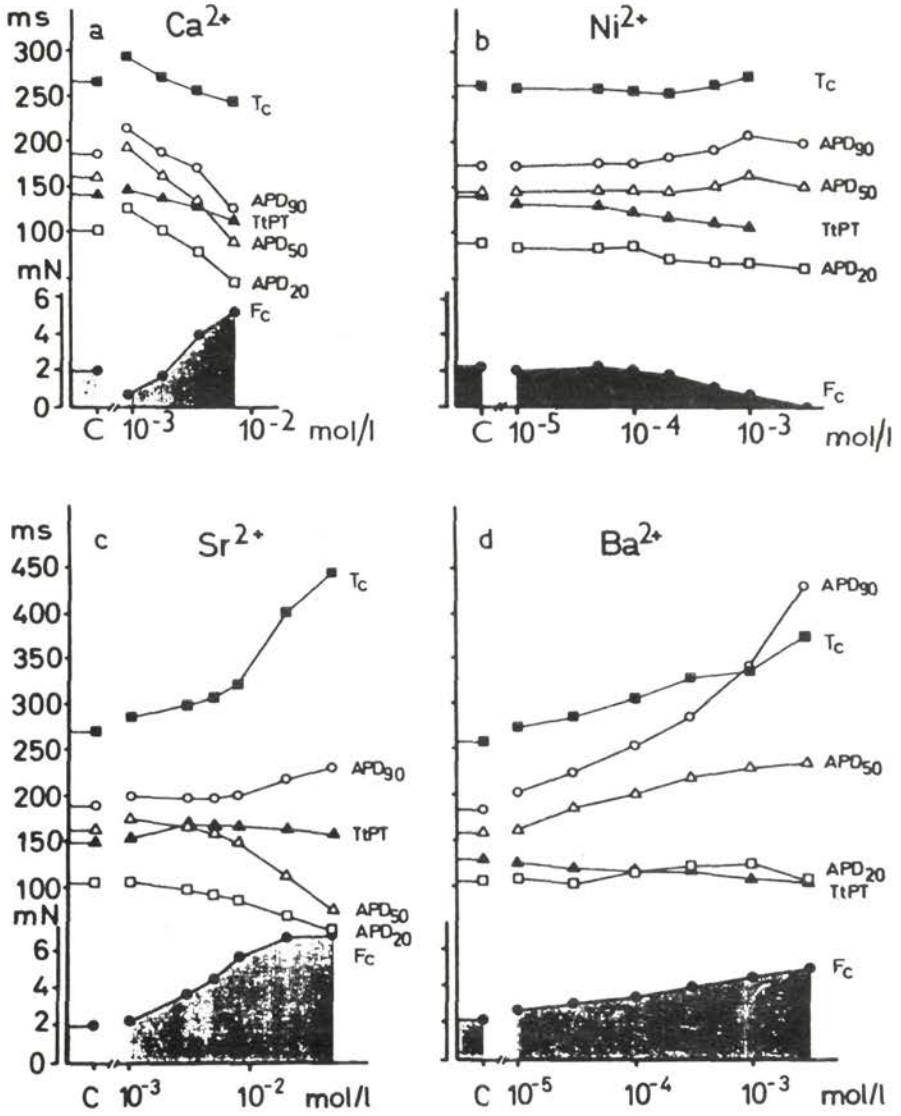

Fig. 1. Cumulative concentration-response curves for the effects of calcium, nickel, strontium and barium on electrical and mechanical parameters in guinea-pig papillary muscles stimulated at 1 Hz. Ordinates: Duration of the action potential at 90 %, 50 % and 20 % repolarization (APD_{90}, APD_{50} and APD_{20}), time-to-peak tension (TtPT) and duration of contraction (T_c) in ms; force of contraction in mN. Abscissae: concentration (in mol/l) of the respective cation. The values at the left of each curve represent the individual controls (Ca^{2+} concentration in the perfusion medium: 1.8 mmol/l) before the addition of the cations. Note that at the beginning of the concentration-response curve for calcium the first concentration was lower (0.9 mmol/l) than that during the equilibration period (1.8 mmol/l). Mean values from 5—8 experiments, S.E.M. not shown (< 10 % of the mean).

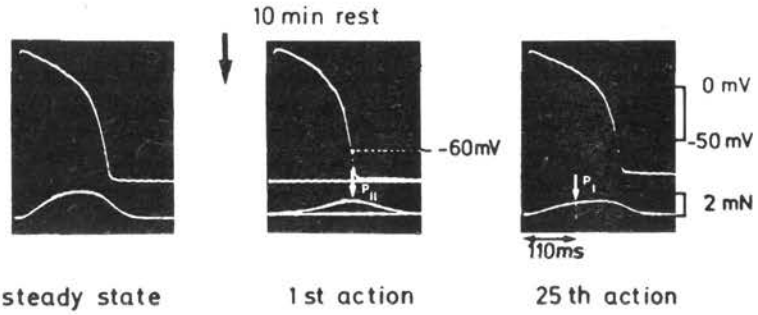

Fig. 2. Original records of action potentials and contraction force of guinea-pig papillary muscle during regular stimulation at 1 Hz (steady state) and after a 10 min period of rest. The first post-rest contraction is characterized by a late component of force development (P_{II}). With the following actions, an early component (P_I) can be distinguished (25th action potential after the rest).

centrations ($P < 0.05$ for 7.2 mmol/l). The small decrease observed with Ba^{2+} and Ni^{2+} and the increase observed with Sr^{2+} were statistically insignificant. The overall duration of contraction strongly increased with Sr^{2+} and Ba^{2+}, was shortened upon increasing Ca^{2+} concentration and remained unaffected by Ni^{2+}.

2. *The two components of contraction*

The characteristic changes in contraction force and in APs after a transient interruption of stimulation for 10 min, are illustrated in Fig. 2. The first contraction after rest consisted of the typical late component of contraction only (downward arrow, P_{II} in Fig. 2) but the 25th action also had an early component (downward arrow, P_I in Fig. 2). The following criteria were used for the differentiation of P_I from P_{II} in the course of post-rest adaptation (though a substantial overlapping of both components occurred within a constant interval in the contraction cycle, i. e. between 100—120 ms (mean 110 ± 4.1 ms, $n = 50$) after AP upstroke. Therefore P_I was defined as the force amplitude measured 110 ms after the initiation of the contraction cycle. P_{II} varied with APD. Its peak occurred roughly at the moment when membrane potential had returned to -60 mV. This relationship was used to define P_{II} for the measurements.

After 10 min of rest also the electrical parameters changed characteristically. The duration of the first AP was prolonged and APD increased up to the 10th action. Thereafter the APD slowly returned to the pre-rest values. Fig. 3 shows mean values of the transient prolongation of APD_{90}, of the overall contraction amplitude, and of the two peaks during post-rest adaptation. Two groups of results are presented since although APD showed similar time cour-

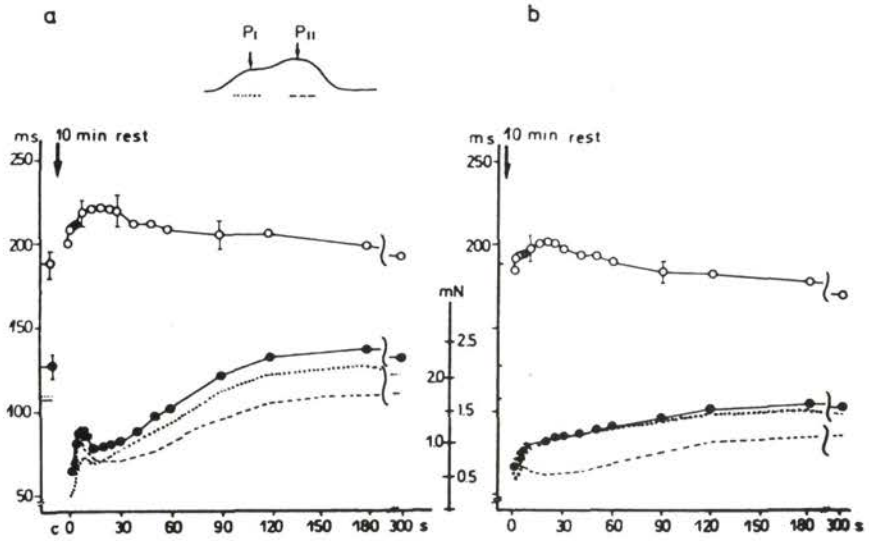

Fig. 3. Contraction force (closed circles) and action potential duration at 90 % repolarization (open circles) after the interruption of regular stimulation for 10 min (downward arrows). The dotted and hatched lines represent the time courses of post-rest adaptation of the early and late component of contraction, respectively (see the inset: contraction amplitude composed of an early (P_I) and a late (P_{II}) peak). The papillary muscles were divided in two groups according to their adaptation patterns of contraction force during the first 20 beats after rest: (*a*) muscles that showed a transient hypercontractility in the early phase of post-rest adaptation; (*b*) muscles with an essentially monophasic time course of post-rest adaptation. Ordinates: action potential duration in ms; contraction force in mN (middle). Abscissae: time in s after resuming regular stimulation (1 Hz). Mean values ± S.E.M. from 8 (*a*) and 17 (*b*) experiments.

ses, two different patterns of contraction force were observed. As no age-, weight- or sex-relations could be established, we assumed that the particular pattern of adaptation presents an intrinsic characteristic of the individual papillary muscle. In the first group (Fig. 3*a*), peak force was increasing up to the 8th beat, decreased until about the 25th beat and then slowly returned to the pre-rest values. The rested-state contraction consisted of the late component only (a small value was obtained for P_I as P_{II} had already started after 110 ms). It is interesting to note that the early transient increase in contractility was mostly due to P_{II} it peaked around 8 s after the rest and then declined. P_I rose almost monotonously after rest.

In the majority of preparation (Fig. 3*b*), post-rest adaptation consisted of an initial phase of rapid force increase lasting about 10 s, followed by a second slow phase reaching pre-rest values within 4—5 min (compare Seibel 1986). In these muscles the first contraction after 10 min of rest still contained a distinct

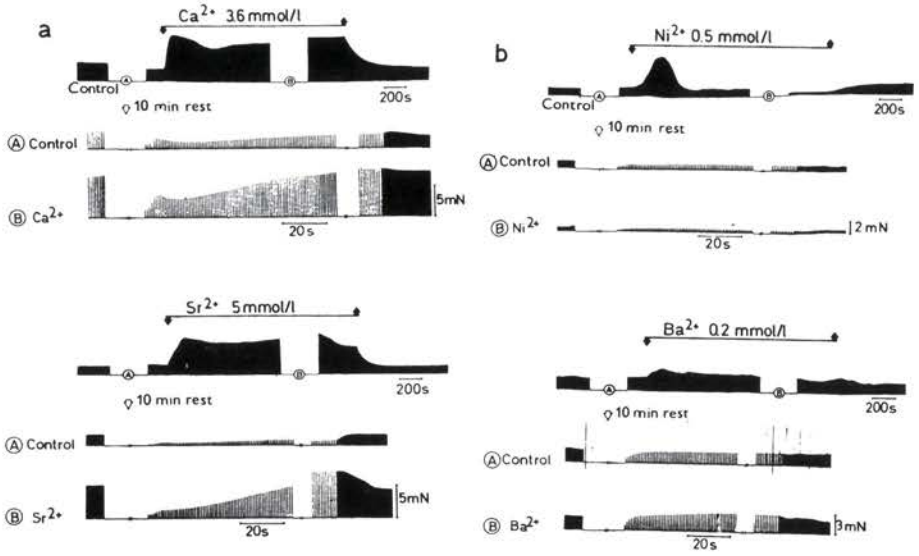

Fig. 4. Effects of the four cations of contraction force of guinea-pig papillary muscles during regular stimulation at 1 Hz (*upper traces*) and after 10 min rest under control conditions (*middle traces*) and in the presence of the respective cation (*lower traces*). (*A*) and (*B*) indicate the original position of the respective mechanograms of post-rest adaptation. Note the different time scales. (*a*) Calcium 3.6 mmol/l and strontium 5 mmol/l (upper and lower set of records, respectively); (*b*) nickel 0.5 mmol/l and barium 0.2 mmol/l (upper and lower set of records, respectively).

early component P_1 as defined by the criteria given above (see p. 333). Even after prolonged periods of rest (up to 30 min), each muscle maintained its typical pattern of post-rest contractions.

Before adding the respective cation, each muscle served as its own control (Fig. 4). We prefered to add the bivalent cations rather than substitute them for calcium in order to simplify the concentration changes in the extracellular space, i.e. filling with the tested cation only instead of simultaneous depletion of calcium. The positive inotropic effect of Ca^{2+}, Sr^{2+} and Ba^{2+} passed through a transient maximum ("hypercontractility"). This initial phase of force increase was observed even with Ni^{2+}, the negative inotropic acting cation, and hypercontractility was in fact most prominent with Ni^{2+}. With respect to post-rest adaptation, the papillary muscles exposed to high Ca^{2+} or to Sr^{2+} (Fig. 4*a*), showed the early phase of transient increase in post-rest contraction whereas those exposed to Ba^{2+} did not. With elevated Ca^{2+} and with Sr^{2+}, the amplitudes of post-rest contractions were enhanced and the force increase was stimulated. Nickel depressed contraction force throughout the adaptation period. With Ba^{2+} the first post-rest contraction could not be distinguished from

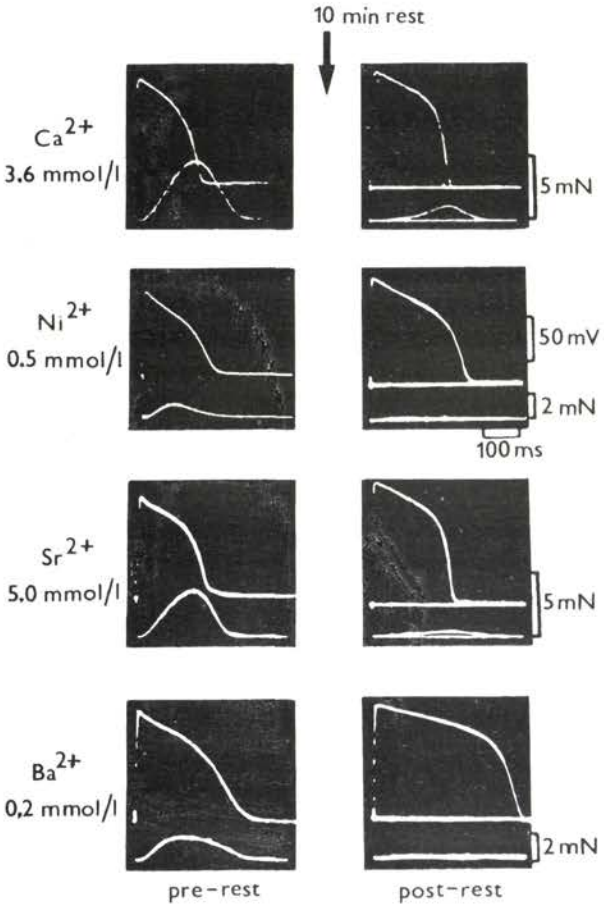

Fig. 5. Action potentials and contraction amplitudes during regular stimulation at 1 Hz (pre-rest) and after 10 min rest (post-rest) in the presence of high Ca^{2+} (3.6 mmol/l), Ni^{2+} (0.5 mmol/l), Sr^{2+} (5 mmol/l) or Ba^{2+} (0.2 mmol/l). Guinea-pig papillary muscles.

the control, but the contraction force was enhanced during further adaptation. None of the cations did alter the adaptation pattern characteristic for the individual preparations.

3. *Does the shape of the action potential determine the contribution of P_I and P_{II} to overall contraction amplitude during post-rest adaptation?*

The effects of the bivalent cations on the 1st AP after 10 min of rest are illustrated in Fig. 5 (original recordings). Irrespective of the change induced at regular pacing, the 1st AP was prolonged in all papillary muscles. Although

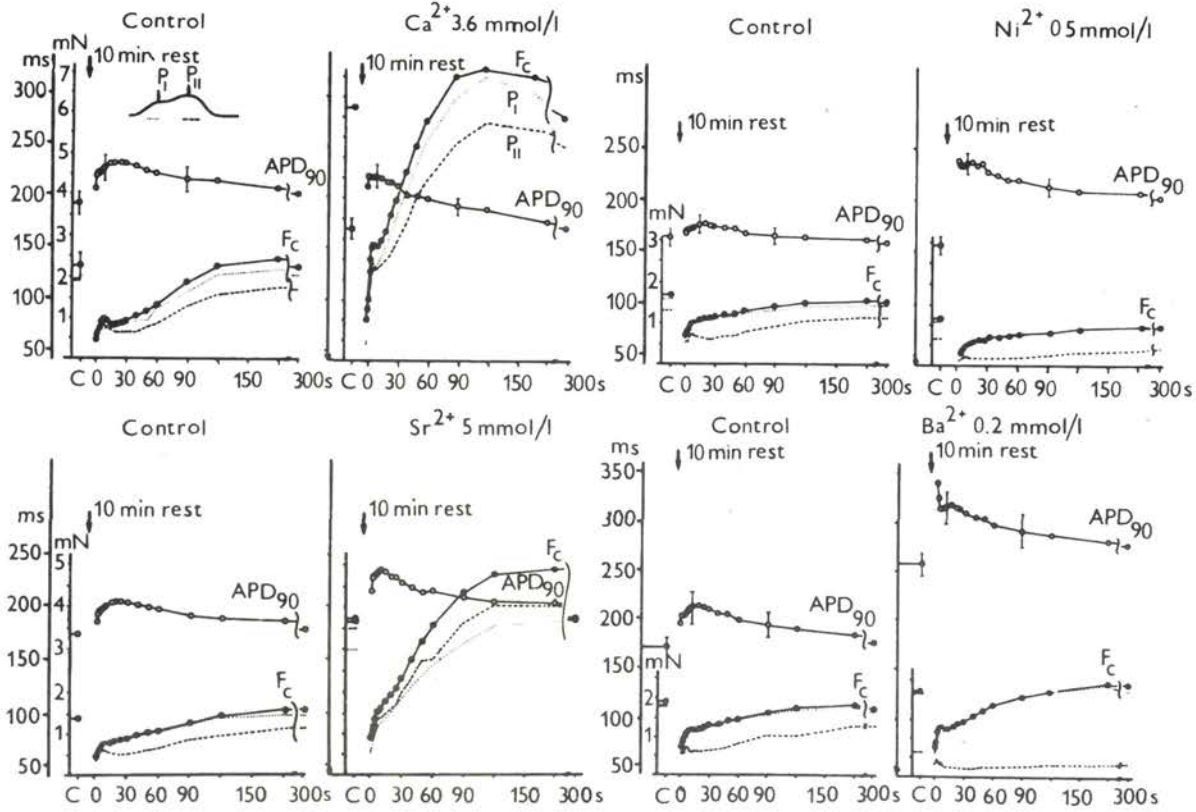

Fig. 6. Post-rest adaptation of action potential duration at 90% repolarization (APD_{90}) and contraction force (F_c) in guinea-pig papillary muscles under control conditions and in the presence of cations: (*a*) calcium 3.6 mmol/l; (*b*) nickel 0.5 mmol/l; strontium 5 mmol/l; and (*d*) barium 0.2 mmol/l. The contractions were analysed to separate P_I and P_{II} (see the inset). Ordinates: APD_{90} in ms (*left*) and contraction force in mN (*right*); abscissae: time in s after resumption of regular stimulation at 1 Hz following 10 min rest. Mean values ± S.E.M. of 5—7 experiments.

Ni^{2+} and Ba^{2+} were the strongest in affecting APD after a pause, the amplitude of the post-rest contraction could barely be distinguished from zero, whereas with Ca^{2+} and Sr^{2+} prominent P_{II} components of contraction could be observed.

The effects of the bivalent cations on post-rest adaptation time courses of contraction force and APD are summarized in Fig. 6. In the respective control runs, the post-rest increase in P_I generally determined the overall amplitude of contraction, while P_{II} was leading only during the first few beats. This pattern of contribution of P_I and P_{II} during post-rest force adaptation was maintained with elevated Ca^{2+}-concentrations. With Ni^{2+} and Ba^{2+}, also P_I was the leading component since P_{II} was actually depressed. With Sr^{2+} the late peak P_{II} clearly prevailed and was the dominant contraction component throughout the adaptation.

The time couse of post-rest adaptation of APD_{90} remained biphasic in the presence of high Ca^{2+} and Sr^{2+}. However, with Ba^{2+} and Ni^{2+}, APD_{90} shortened from the 1st through the 4th post-rest action, then it was prolonged again until the 15th action before it declined monophasically to pre-rest values.

We did not observe any consistent relationship between contraction force and APD after a pause in the presence of any of the bivalent cations studied.

Discussion

The effect of bivalent cations during regular stimulation

The effect of bivalent cations on excitation-contraction coupling will depend on their ability to substitute for or compete with Ca^{2+} at the various cellular levels. Sr^{2+} and Ba^{2+} are known to serve as charge carriers for the slow inward current, i_{si} (Kohlhardt et al. 1973; Mascher 1973), whereas Ni^{2+} competes with Ca^{2+} in this respect (Kohlhardt et al. 1979). Sr^{2+} and Ba^{2+} may activate contractile proteins directly (Kerrick et al. 1980; Mascher 1973), both cations are taken up by the sarcoplasmic reticulum of cardiac muscle (Winegrad 1973 — Sr^{2+}; Weber et al. 1966 — Ba^{2+}, in skeletal muscle); however, Sr^{2+} seems to be released from the sarcoplasmic reticulum less easily than Ba^{2+} (King and Bose 1983; Mascher 1973). These findings taken together readily explain the positive inotropic effect observed both with Sr^{2+} and Ba^{2+}. The transient inotropic effect observed immediately after the exposure to Ni^{2+}-containing solution was surprising although a similar finding had been documented previously (see Kaufmann and Fleckenstein 1965). If nickel competes with calcium in the slow inward channel resulting in a block of the calcium current as suggested by Kohlhardt et al. (1979), a negative inotropic effect would be expected to occur. Rubányi et al. (1980) have shown that nickel concentrations far below the range necessary for

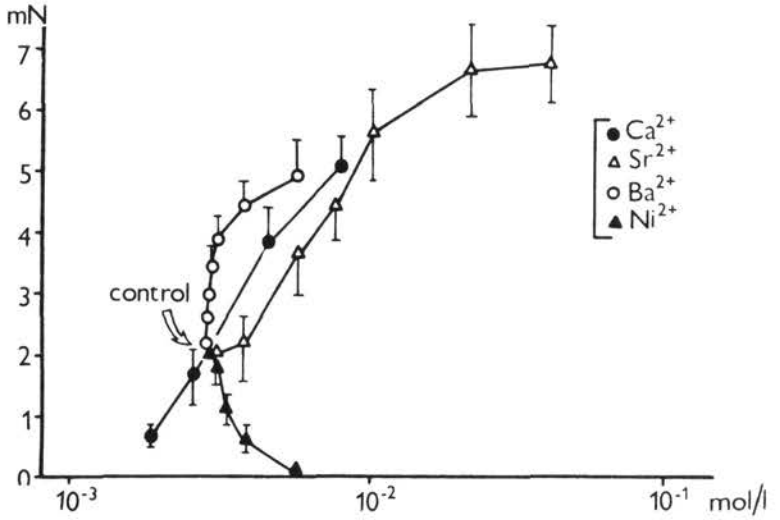

Fig. 7. Concentration-response curves for the effects of Ca^{2+}, Ni^{2+}, Sr^{2+} and Ba^{2+} on contraction force of guinea-pig papillary muscles. Ordinate: contraction force in mN, abscissa: concentrations (mmol/l) of bivalent cations. The data are taken from the same experiments as shown in Fig. 1.

the calcium channel block (1 μmol/l) may cause morphological alterations of intracellular structures. Therefore, nickel must be able enter the intracellular space and probably does so also in the concentrations used in the present study. If nickel competes for calcium also at cellular binding sites (Lüllmann and Peters 1977), the displaced Ca ions may become available for contractile activation before they are sequestered, and thus cause the transient inotropic effect. In the long run, however, a negative inotropic effect develops because Ni^{2+} cannot activate the contractile proteins directly (Ong and Bailey 1973).

The changes in the shape of the transmembrane APs are in line with the reports in the literature, i. e. a shortening of the overall duration in the presence of high extracellular calcium concentrations (Reiter and Stickel 1968), a prolongation of APD_{90} with Sr^{2+} (King and Bose 1983) and even more with Ba^{2+} (Mascher 1973; Potreau 1982), and a shortening of the AP plateau phase (APD_{20}) with Ni^{2+} (Kaufmann and Fleckenstein 1965; Kecskemeti et al. 1985).

Inomata and Kao (1985) discussed that the bivalent cations may be effective by screening off the negative surface charges that are of great importance for channel properties. Such an unspecific general mechanism might be involved but cannot be the only action, since the shapes of the concentration-response curves of each of the bivalent cations studied are distinctly different from one another even when the effects are plotted against the total concentration of all bivalent cations present in the solution (e. g. calcium, magnesium, and the respective

cation under investigation, Fig. 7). Therefore, some additional and more specific action has to be assumed.

Post-rest adaptation of mechanical and electrical activity

A single cardiac contraction cycle strongly depends on previous contractions during regular stimulation but becomes independent after a long period of rest (Koch-Weser and Blinks 1963). In guinea-pig ventricular myocardium the first post-rest beat is characterized by a small amplitude, a delay in the onset of tension development and by a late tension maximum that coincides with the final phase of repolarization (Reiter et al. 1984). The amplitude of the post-rest contraction is closely related to the slow inward current (Beresewicz and Reuter 1977). The early contraction component developing during subsequent beats is related to calcium release from the sarcoplasmic reticulum (Lewartowsky et al. 1978). This hypothesis could be confirmed by recent results concerning subcellular Ca distribution obtained with X-ray microstructure analysis (Wendt-Gallitelli 1985): after a long period of rest, the sarcoplasmic reticulum became depleted of its calcium content.

There are several reports in the literature on methods for the analysis of the two peaks of contraction used to draw conclusions about the source of calcium ions for contractile activation (Braveny and Sumbera 1970; Ochi and Trautwein 1971; Allen et al. 1976; Henderson and Cattell 1976; Beresewicz and Reuter 1977; Bogdanow et al. 1979). In this paper we separated the phasic contraction into two peaks in the course of the first few beats after rest. The criteria defined for obtaining P_I and P_{II} were useful also for the analysis of contractions during regular stimulation when to two peaks cannot be readily differentiated. Certainly, this procedure does not yield sharp separation, because if P_{II} is large, some tension development measured 110 ms after the stimulus artifact is erroneously regarded as P_I and inversely, a fraction of P_I tension still present at the membrane potential of -60 mV will be regarded as P_{II}. However, despite this overlap, the two peaks give some estimate of how the two components contribute to total contraction.

In addition to P_I and P_{II} of the individual contraction, two phases of adaptation can be distinguished. The first phase comprises some 6 to 8 beats of rapid force increase. It is separated from a late peak by an intermediate phase of very little or no change; during the late phase the contraction amplitude slowly recovers with variable degrees of hypercontractility before pre-rest control values are reached again. The transient hypercontractility may be caused by intracellular redistribution of Ca ions (see Ziegler and Ziehm 1978). Seibel (1986) postulates that this second phase of adaptation is caused by a stimula-

tion-induced accumulation of intracellular Na ions and that Na ions control the release of Ca ions from cellular stores.

A transient interruption of regular stimulation does not only affect the contraction force but also the shape of APs (Ravens 1983). The first post-rest AP is prolonged at all stages of repolarization (Becher and Ravens 1982; Beyer et al. 1986), but species differences have been described; e. g. in rabbit heart muscle, the plateau phase of the first AP after a prolonged pause is shortened (Pucelik et al. 1983; Ravens 1983). The time course of the subsequent post-rest adaptation is biphasic: APD continues increasing through some 10 to 15 beats and then it slowly shortens to pre-rest values. The duration of the first post-rest AP seems to terminate P_{II}, hence the definition of P_{II} measurement. However, in the course of post-rest adaptation there is no consistent relation between the changes observed in APD and contraction force: during the first phase of rapid force increase, APD is prolonged whereas it is shortened during the second phase of slow force adaptation. The slowly increasing force during this latter phase reflects higher levels of intracellular free Ca^{2+} which in turn activate the K conductance (Bassingthwaighte et al. 1976). Increased K conductance shortens the AP. However, it remains unclear why this mechanism should be effective only in the slow phase of adaptation and not in the initial one. Clearly, voltage clamp studies are required to elucidate the changes in membrane currents underlying the biphasic pattern of post-rest adaptation of APD.

Effects of bivalent cations on post-rest adaptation

In high Ca solution, P_{II} dominates the amplitude of the post-rest contraction which is increased as compared to controls. This finding supports the concept of 2 sources of calcium for contractile activation because an increase in the calcium gradient across the membrane is expected to enhance the inward current i_{si}. Since P_{II} also increases during the subsequent beats, the cellular stores are rapidly refilled so that P_I becomes dominant at a higher contraction level and appears earlier than under control conditions. Pre-rest values are reached already 1 min after resumption of regular stimulation. P_I is the leading force component during the marked hypercontractility. Possible stores of Ca during the redistribution phase underlying the decline in hypercontractility (Ziegler and Ziehm 1978) may include the slowly adapting mitochondria (Carafoli et al. 1977).

In high Ca solution the time course of adaptation of APD remains biphasic, but the turning point is reached already after 3—4 APs (control 10—15). The increased intracellular free Ca^{2+} concentration should enhance i_{si} inactivation (Kass and Sanguinetti 1984) and K conductance (Isenberg 1975; Bassingthwaighte et al. 1976). However, additional effects might also contribute, as the

degree of post-rest shortening of APD during the slow phase of adaptation is similar after exposure to either high Ca^{2+}, Sr^{2+}, Ba^{2+} or Ni^{2+}, yet the respective slow increase in contraction amplitude is highly variable ($Ca^{2+} > Sr^{2+} > > Ba^{2+} > Ni^{2+}$).

The time courses of post-rest adaptation of contraction force are quite similar in high Ca^{2+} and in Sr^{2+}-containing solution with one exception; namely in Sr^{2+} media P_{II} predominates throughout the adaptation process. Although Sr^{2+} may mimick Ca^{2+} in many aspects it seems to be released less easily from the cellular binding sites and activates contraction less effectively because of a lower affinity to troponin.

In preparations exposed to Ba^{2+}, the P_I component of contraction is increased because Ba^{2+} enhances the release of Ca^{2+} from cellular stores (Potreau and Raymond 1982). Also after rest, P_I remains the predominant tension component. Since the Ca-channels conduct Ba^{2+} better than Ca^{2+}, i_{si} is expected to increase. However, this only holds true if each cation is on its own; in fact, Ca^{2+} may even inhibit the Ba^{2+} current when both cations are present (Potreau and Raymond 1982; Inazawa et al. 1984). In our experiments, the P_{II} component depending on i_{si} was depressed suggesting a competition of the two cations also at the level of activation of the contractile proteins. Ba^{2+} causes a triphasic time course of adaptation of action potential duration with particularly long duration of the first post-rest AP. Based on potential measurements only, any explanation has to remain speculative; nevertheless Ba^{2+}-induced decrease in K conductance may be involved.

Similar post-rest adaptation patterns as with Ba^{2+} are observed with Ni^{2+}, although tension development remains at a lower level. P_I dominates and P_{II} is effectively absent as expected from the decrease in i_{si} (Kohlhardt et al. 1979).

In the present work we investigated the two components of contraction in the presence of various bivalent cations by analysing P_I and P_{II} during post-rest adaptation. After rest, P_I and P_{II} recovered with different time courses, and the bivalent cations preferentially influenced one or the other. Based on the assumption that P_I and P_{II} reflect contractile activation by Ca^{2+} derived from the release from cellular stores and from transmembrane influx, respectively (Wohlfart and Noble 1982), it is suggested that the analysis of differential effects on either component of contraction may provide some clue as to the possible site of action of the inotropic agent.

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