Ca$_{2+}$ current-mediated regulation of action potential by pacing rate in rat ventricular myocytes

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Abstract

Objective: Pacing rate regulates the duration of the cardiac action potential (AP). It also regulates the decay kinetics of the L-type Ca$_{2+}$ current ($I_{\text{Ca-L}}$) which occurs via modulation of Ca$_{2+}$-dependent inactivation. We investigated whether and how this latter process contributes to frequency-dependent (FD) changes in the AP waveform in rat ventricular cells. Methods: We recorded APs using a microelectrode technique in rat papillary muscles, and using the whole-cell current patch-clamp technique in single rat ventricular cells. Results: The AP duration (APD) was increased by high rates encompassing the physiological range (0.1–5.7 Hz) in both papillary muscles and single cells. This prolongation was accompanied by concomitant depolarisation ($\Delta V_m$ at 5.7 Hz) of the membrane potential (MP) in papillary muscles. Equivalent artificial depolarisation of the MP enhanced the FD prolongation in single cells. The FD prolongation was enhanced in presence of the K current blocker 4-aminopyridine (5 mmol/l), and decreased in absence of extracellular Ca. It was antagonised by Ca channel blockers (Co, nifedipine, nitrendipine) and decreased by use of high EGTA (10 vs. 0.5 mmol/l EGTA) or BAPTA (20 mmol/l) in the patch-pipette. It was prevented by ryanodine or thapsigargin, two drugs that reduce or abolish SR-Ca$_{2+}$ function. Conclusion: $I_{\text{Ca-L}}$ contributes to the FD modulation of the AP, which occurs following a sudden change in cardiac frequency in rat ventricular cells. This highly dynamic physiological process is related to SR-Ca$_{2+}$ release and occurs through beat-to-beat adaptation of Ca$_{2+}$-dependent inactivation of $I_{\text{Ca-L}}$.

Keywords: Ca channel; Calcium (cellular); Heart rate (variability); Membrane potential; SR (function)

1. Introduction

In most mammalian species, including human, elevation of the cardiac beating rate increases steady-state twitch force of the ventricle, which is referred to as the Bowditch phenomenon or the positive force-frequency relationship [1,2]. This short-term regulation of the excitation–contraction (E–C) coupling appears to be an important determinant of cardiac contractility [1–3]. Contractile force is governed by the amount of Ca$_{2+}$ released from the sarcoplasmic reticulum (SR) [4]. This release is triggered predominantly by transmembrane Ca$_{2+}$ entry through L-type Ca$_{2+}$ channels ($I_{\text{Ca-L}}$) during the action potential (AP) [4]. $I_{\text{Ca-L}}$ is also electrogenic and contributes to maintain the AP plateau [3,4]. Amplitude or kinetic variations in the net Ca$_{2+}$ influx via $I_{\text{Ca-L}}$ have potential effects on the shape and duration of the AP, and also on contraction [5,6].

High pacing rates abbreviate the AP duration (APD) in various mammalian species. This is often associated with increased Ca$_{2+}$ transient and contraction, suggesting that Ca$_{2+}$-dependent mechanisms play a role [3,5,7–9]. However, a quite different effect is observed in rat ventricular...
cells. The APD is increased, whereas contraction is usually decreased [10–12]. In parallel, \( I_{\text{Ca,L}} \) undergoes frequency-dependent (FD) facilitation that occurs through changes in current amplitude and kinetics, and results into a slowing of current inactivation promoting increased \( \text{Ca}^{2+} \) entry. This phenomenon, which exists in several mammalian species including human, is particularly robust and consistent in rat ventricular cells [13–17]. The present study was designed to test the controversial hypothesis that, at rates of stimulation encompassing the physiological range, facilitation of \( I_{\text{Ca,L}} \) plays a significant role in the FD prolongation of the AP in rat ventricular cells [11,18,19]. Experiments were performed both on single cells and on papillary muscles in order to achieve more physiological conditions.

2. Methods

2.1. Animals

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH, No. 85-23, revised 1996) and European directives (96/609/EEC). Briefly, 6–10-week-old Wistar Kyoto rats (Janvier, Le Genest, St. Isle, France) were heparinized (0.2 ml, Heparin Gibco* 1000 UI/ml) and anaesthetized with sodium pentobarbital (100 mg/kg, Sanofi Santé, France) by intraperitoneal injection.

2.2. Single cells

Ventricular myocytes were isolated as described [20]. The heart was perfused at 35 °C with a \( \text{Ca}^{2+} \)-free Heps-buffered solution (5 min) containing (in mmol/l): NaCl (117), KCl (5.7), NaHCO\(_3\) (4.4), KH\(_2\)PO\(_4\) (1.5), MgCl\(_2\) (1.7), Heps (21), glucose (11.7), taurine (20); pH adjusted at 7.2 with NaOH. Then, collagenase (1.35 mg/ml; Worthington type IV, 247 UI/mg) was used for 35 min. Successive steps were made to increase \( \text{Ca}^{2+} \) progressively and to eliminate dead cells before storage in a solution containing 1 mmol/l CaCl\(_2\) and 1% BSA, and maintained at 36 °C.

2.3. APs and \( I_{\text{Ca,L}} \) in single cells

APs were recorded in rod-shaped \( \text{Ca}^{2+} \)-tolerant myocytes at 22–24 °C by use of the whole-cell patch-clamp technique (Axopatch 200A, Axon instrument, Burlington, CA, USA). Pipettes had resistance of 2–3 MΩ when filled with the recording solution containing (mmol/l): KCl (130), Heps (25), ATP(Mg) (3), GTP(Na) (0.4), EGTA (10 or 0.5) or BAPTA (20) as specified in the legends of figures; pH adjusted to 7.2 with KOH; 290–310 mOsm. The myocytes were superfused with a Tyrode’s solution containing (in mmol/l): NaCl (135), MgCl\(_2\) (1), KCl (4), glucose (11), Heps (2), CaCl\(_2\) (1.8); pH adjusted to 7.4 with NaOH. In \( \text{Ca}^{2+} \)-free solutions, Mg\(^{2+}\) replaced \( \text{Ca}^{2+} \). APs were elicited by 0.2 ms current injection of supra-threshold intensity. Acquisition rate was 10 kHz. Signals were filtered at 5 kHz (lowpass Bessel Filter). Cells were stimulated routinely at 0.1 Hz until stabilisation of APs (3–5 min). This frequency was taken as the reference since no change occurred at rates lower than 0.2 Hz. For testing at a given frequency, stimulation was started after a rest period of at least 10 s. Between each trial, the frequency was returned to 0.1 Hz. In one set of experiments, \( I_{\text{Ca,L}} \) was recorded in voltage clamp conditions optimised to eliminate contaminating inward \( \text{Na}^+ \) and outward \( \text{K}^+ \) currents [13–17]. Bath solutions contained (mmol/l): TEACl (140), CaCl\(_2\) (1.8), MgCl\(_2\) (2), Heps (10), glucose (10); pH adjusted to 7.4 with TEAOH; 290–310 mOsm. The pipette solution contained (mmol/l): CsCl (140), Heps (10), ATP(Mg) (3), GTP(Mg) (0.4), EGTA (10); pH adjusted to 7.2 with CsOH; 290–310 mOsm.

2.4. Measurements of contraction in single cells

Only myocytes showing clear sarcomeric patterning were selected. Cells were field-stimulated by platinum electrodes at 0.2 and 1.0 Hz. Sarcomere length (SL) was measured accurately SL at 3.3 Hz. The sampling frequency of the video image acquisition was 50 Hz.

2.5. APs in papillary muscles

APs were recorded from right ventricular papillary muscles using a standard microelectrode recording technique (WPI M707, Sarasota, FA, USA) at 37 °C [12]. Muscles were mounted in a bath superfused with Tyrode’s solution containing (mmol/l): NaCl (138.6), KCl (5.4), glucose (11), Hepes (2), CaCl\(_2\) (1.7), Hepes (21), glucose (11.7), taurine (20); pH adjusted to 7.4 with NaOH; and gassed with air. In \( \text{Ca}^{2+} \)-free solutions, Mg\(^{2+}\) replaced \( \text{Ca}^{2+} \). Electrodes resistances ranged between 20 and 40 MΩ. They were filled with a 3-mol/l KCl solution. Unstretched preparations were driven at 1.0 Hz for 3–5 min in order to stabilise electrical activity prior to increasing the rates to 2.5, 3.3 and 5.7 Hz. The AP at each rate was recorded after 30 s, corresponding to steady state effect. The zero-potential, adjusted to the ground potential, was calibrated before and after each period. One or more sites were studied in each papillary muscle. The results presented originated from a single impalement.

2.6. Analyses and statistics

Data acquisition and analyses were performed using the
Plasm (version 8.1, Axon instruments) and/or Acquis software (G. Sadoc, CNRS URA 1121 Orsay). The MP, the AP amplitude and the APD at 20% and 50% (APD_{50}) and 90% of repolarisation were measured. For brevity, only measurements of the APD_{50}, which best reflects the contribution of I_{Ca-L}, are presented. Only maximal variations (either steady-state or transient) have been included in analyses. All averaged or normalised data are presented as mean±S.E.M. The significance between groups of data was assessed using Student’s t-test (for paired and unpaired samples as appropriate), and one-way analysis of variance (ANOVA test) when three or more groups were compared, with a Newman–Keuls post-hoc test comparing all pairs of group means. Results were considered significant with \( P \) less than 0.05 (*\( P \), 0.05, **\( P \), 0.01, ***\( P \), 0.001).

2.7. Solutions

Ryanodine and thapsigargin (Sigma, St. Quentin Fallavier, France) were prepared as 10-mmol/l stock solution in distilled H \(_2\)O and DMSO, respectively. Nifedipine (Sigma) and nitrendipine (Sigma) were prepared as 10 mmol/l stock solutions in ethanol. Stock solutions were diluted to the desired concentration in the Tyrode solution. 4-Aminopyridine (4-AP; 5 mmol/l; Sigma) was added to block the transient outward K’ current (I_{to}) in some experiments. Co\(^{2+}\) was used to block I_{Ca-L}. The control and test solutions were applied as described before [12,17].

3. Results

3.1. Effect of the rate of stimulation

Fig. 1Aa shows the effect of a stepped increase in the rate of stimulation, from 1.0 to 3.3 Hz and to 5.7 Hz, on the duration of APs recorded in a right papillary muscle at 37 °C. The APD_{50} increased gradually (Fig. 1Ab). A concomitant depolarisation of the MP occurred (Fig. 1Aa,c). Qualitatively similar results were observed in single ventricular myocytes recorded at 22–24 °C with the patch-clamp technique (Fig. 1Ba), though the range of rates where changes occurred was lower than for the papillary muscles recorded at 37 °C. The APD_{50} also increased gradually at higher rates (Fig. 1Ba,b). The FD prolongation reached steady state within four to eight stimulations after the beginning of a train (data not shown). Vice versa, the APD_{50} decreased when the rate of stimulation was lowered (Fig. 1Bc). A small, but significant, depolarisation of the MP occurred (−82.8±0.4 mV at 3.3 Hz vs. −84.2±0.3 mV at 0.1 Hz; \( n = 34, *\( P \), <0.05). Then, we investigated whether a moderate depolarisation (−10 mV), induced by artificially changing the MP of single

![Fig. 1. FD modulation of the AP and of the MP. (A) Steady state APs recorded at 1.0, 3.3 and 5.7 Hz in papillary muscles: (a) original traces in the same preparation; (b) average percentage increases (±S.E.M.) in the APD_{50} at 3.3 and 5.7 Hz, with 1.0 Hz taken as the reference; (c) average percentage FD changes in the MP. (B) Steady state APs recorded at 0.1, 1.0 and 3.3 Hz in single ventricular cells (10 mmol/l EGTA in the pipette): (a) original traces in the same myocyte; (b) average percentage increases (±S.E.M.) in the APD_{50} at 0.1, 1.0 and 3.3 Hz, with 0.1 Hz taken as the reference; (c) steady state effect after a decrease from 3.3 to 0.1 Hz on an AP recorded in a same myocyte (0.5 mmol/l EGTA). Statistical significance between pairs of data is indicated by an asterisk (ANOVA, ***\( P \), <0.001; see Section 2).](https://academic.oup.com/cardiovascres/article-abstract/57/3/670/477683/736747783)
cells, could participate in the FD modulation of the AP in papillary muscles. This manoeuvre induced per se a prolongation of the AP. The APD was longer at the more depolarised MP for a given frequency (0.1 and 3.3 Hz, respectively; Fig. 2A). Moreover, MP depolarisation enhanced significantly the FD prolongation of the APD_{50} (Fig. 2A,B).

### 3.2. Effect in the presence of 4-AP

Two kinetically distinct voltage-dependent K⁺ currents have been identified in rat ventricular cells: (i) a 4-AP-sensitive transient current, I_{to}, activating and inactivating rapidly, and (ii) a delayed rectifier, I_{K}, which activates too slowly to be involved in the process described here [22]. To study the participation of I_{to} in the FD modulation of the APD, we recorded APs in presence of 5 mmol/l 4-AP to totally block I_{to}. The 4-AP slowed the fast repolarizing phase of the AP and prolonged the plateau markedly, which could be inhibited by the inorganic Ca²⁺ channel blocker Co²⁺ (2 mmol/l) (Fig. 3A). In the presence of 4-AP, a graded increase in frequency between 0.1 and 3.3 Hz produced a graded and large prolongation of the AP (Fig. 3B,C). This effect reached a maximum within two to five stimuli and was followed by a partial recovery after 10–30 stimuli, which occurred mainly at rates higher than 1 Hz (data not shown). Examination of I_{to} in voltage-clamp conditions revealed the existence of a use-dependent unblock of the effect of 4-AP on I_{to}, but this occurred only for frequencies higher than 1.0 Hz (data not shown) [23]. Furthermore, FD prolongation was observed following changes from 0.1 Hz to rates as low as 0.3 Hz or even 0.2 Hz that had no effect in control conditions (data not shown), whereas no difference was observed between 1.0 and 3.3 Hz (Fig. 3C). The FD prolongation was abolished by Co²⁺ suggesting involvement of Ca²⁺ influx (Fig. 3D).

### 3.3. Involvement of Ca²⁺ channels

Fig. 4A illustrates the typical FD facilitation of I_{Ca-L} observed in absence of other currents in rat ventricular cells (for details, see Refs. [13–17]). This regulation is highly consistent among cells. Since AP FD-prolongation is also observed in nearly all cells, the two regulations were expected to occur in association in same cells. Indeed, when AP type of recording solutions and voltage-clamp conditions (HP of −60 mV) designed to minimise contamination by K⁺ and Na⁺ currents were used, both FD prolongation of AP and FD change in the inward current occurred (Fig. 4B) in all of three cells tested. Although this change was reminiscent of I_{Ca-L} facilitation, we could not exclude the participation of residual K⁺/Na⁺

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**Fig. 2.** Influence of the MP on the FD prolongation of APs in single cells (10 mmol/l EGTA). (A) Steady-state APs recorded at 0.1 Hz and 3.3 Hz from three different diastolic MPs, artificially controlled using current injection, in the same cell. (B) Average increases (±S.E.M.) in the APD_{50} (Δt, ms) when increasing the rate from 0.1 Hz (reference) to 3.3 Hz. Statistical significance between pairs is indicated by an asterisk (ANOVA, *P<0.05).
Fig. 3. Effect of 4-AP in single cells (0.5 mmol/l EGTA). (A) Effect of 4-AP (5 mmol/l) and 4-AP+Co2+ (2 mmol/l) at low rate (0.1 Hz). (B) Original traces of steady state APs recorded at 0.1, 0.33, 1.0, and 3.3 Hz in the presence of 5 mmol/l 4-AP in the same cell. (C) Average percentage increases (±S.E.M.) in the APD at various rates, with 0.1 Hz taken as the reference. Statistical significance between pairs is indicated by an asterisk (ANOVA, *P<0.05). (D) Suppression of the FD prolongation by 2 mmol/l Co2+ in the presence of 5 mmol/l 4-AP. The cell was the same as in (B).

Fig. 4. FD modulation of \( I_{\text{Ca-L}} \) and AP in single cells. (A) Effect of an increase from 0.1 to 3.3 Hz on \( I_{\text{Ca-L}} \) recorded in absence of intra- and extracellular Na+ and K+ (see Section 2). \( I_{\text{Ca-L}} \) was evoked at −10 mV from a holding potential of −80 mV (EGTA, 10 mmol/l). Note the slowing of decay kinetics of \( I_{\text{Ca-L}} \). (B) Effect of an increase from 0.1 to 3.3 Hz on both \( I_{\text{Ca-L}} \) and AP recorded in a same cell using AP type solutions (intracellular K+, extracellular Na+; see Section 2). \( I_{\text{Ca-L}} \) was recorded at −10 mV from −60 mV in order to minimise the contribution of contaminating inward \( I_{\text{Na}} \) and outward \( I_{\text{K}} \) (EGTA, 0.5 mmol/l). Facilitation of \( I_{\text{Ca-L}} \) can be observed in these conditions [13].
currents. It was impossible to depolarise the HP further to eliminate these currents completely because this manipulation also abolishes facilitation of $I_{Ca-t}$. Thus, we used pharmacological approaches. First, we found that FD prolongation of the AP was decreased in Ca$^{2+}$-free solutions both in papillary muscles (Fig. 5A) and single cells (Fig. 5B). Although a residual prolongation remained, the decrease was highly significant and consistent (Fig. 5Ab,Bb), indicating that Ca$^{2+}$ entry is important. We next assessed the effect of the Ca$^{2+}$ channel antagonists nifedipine and nitrendipine. These dihydropyridines (DHPs) were chosen because they are devoid of use-dependent block, compared to other Ca$^{2+}$ blockers [24]. In single cells, we applied the DHPs at 2 µmol/l because this concentration blocks approximately 50% of $I_{Ca-t}$ evoked from a HP of −80 mV and has moderate non-specific effects [25]. We used a higher concentration of DHPs (10 µmol/l) for papillary muscles in order to compensate for the less efficient diffusion of the drug. The FD prolongation of the AP was significantly decreased by DHPs both in papillary muscles (Fig. 6Aa,b) and in single cells (Fig. 6Ba,b), suggesting a contribution of $I_{Ca-t}$. In addition, the DHPs prevented the effect of MP depolarisation on the FD modulation of the APD in single cells. Not only was the FD prolongation at a given MP (−90 and −80 mV, respectively) attenuated, but also the enhancement of the FD prolongation by MP depolarisation (Fig. 7A,B), indicating that $I_{Ca-t}$ is also involved.

3.4. Effects of intracellular Ca$^{2+}$ buffering

To assess the role of intracellular Ca$^{2+}$, we performed patch-clamp recordings using two different concentrations of EGTA in the pipette. At 0.1 Hz, high EGTA increased the APD (Fig. 8Aa,b). On average, the APD$_{50}$ increased from 10.6±0.8 ms (EGTA 0.5 mmol/l; $n=24$) to 16.2±1.5 ms (EGTA 10 mmol/l; $n=60$; ***$P<0.001$). For comparison, the APD$_{50}$ was 11.2±0.3 (n=52) in papillary muscles driven at low rate (1.0 Hz). We found that an increase in the stimulation frequency from 0.1 to 3.3 Hz induced a larger prolongation of the APD$_{50}$ in presence of low EGTA (42±5%; $n=7$) as compared to high EGTA (20±5%; $n=12$) (Fig. 8Abc,B). Moreover, FD prolongation was abolished when high BAPTA (20 mM), a faster and more efficient Ca$^{2+}$ chelator than EGTA at the sub-cellular level, was used (Fig. 8Ac,B). These results suggested that the intracellular Ca$^{2+}$ concentration not only influences the APD at low pacing rates but also determines the lengthening of AP at high rates. It should also be noted that high rates induced a transient effect at frequencies >1 Hz when low EGTA (0.5 mmol/l) was used (Fig. 8C). First, the FD prolongation reached a maximum within four to 10 stimuli after starting a train. Then, a partial recovery developed within 10 to 20 stimuli before the AP reached its final waveform. This recovery was absent when external Na$^{+}$ was replaced by Li$^{+}$ (data not shown). The overall FD prolongation was also less in these conditions, suggesting a secondary role of the $I_{Na-Ca}$ exchanger.

3.5. Effect of ryanodine and thapsigargin

Ryanodine binds to SR-Ca$^{2+}$ release channels (RyRs) and is known to reduce SR Ca$^{2+}$ release and contraction,
Fig. 6. Effect of dihydropyridines. (A) Steady state APs recorded at 1.0 and 5.7 Hz in the absence and presence of nifedipine (10 μmol/l) in papillary muscles: (a) original traces in the same preparation; (b) average percentage increases (±S.E.M.) in the APD at 5.7 Hz. (B) Steady state APs recorded at 0.1 and 3.3 Hz in the absence and presence of nitrendipine (2 μmol/l) in single myocytes (10 mmol/l EGTA): (a) original traces in the same cell; (b) average percentage increases (±S.E.M.) in the APD at 3.3 Hz.

at micromolar concentration [26,27]. A secondary effect is to prevent the SR-Ca\(^{2+}\) release-induced fast inactivation of \(I_{\text{Ca-L}}\) [17,27]. We found that ryanodine (1–10 μmol/l) markedly prolonged the AP both in papillary muscles (Fig. 9Aa) and in single cells at low rates (Fig. 9Ba). In addition, ryanodine attenuated the FD prolongation at 5.7 Hz in papillary muscles (Fig. 9Aa,b) and abolished it at 3.3 Hz in single cells (Fig. 9Ba,b). Thapsigargin, known to inhibit SR-Ca\(^{2+}\) uptake and to slow the decay kinetics of \(I_{\text{Ca-L}}\) [28], had essentially similar effects as ryanodine in single cells. Thapsigargin (1 μmol/l) prolonged dramatically the AP 5 min of extracellular superfusion, and abolished the FD prolongation (n=3; data not shown).

3.6. FD decrease of contraction in single cells

Fig. 10 shows that an increase in the stimulation rate from 0.2 to 1.0 Hz induced a decrease of the contraction in single cells. This decrease occurred immediately at the first beat following the change and contraction remained stable. Consistent results were obtained in eight cells.

4. Discussion

An increase in the stimulus frequency prolongs the AP at physiological heart rate in rat single ventricular cells and papillary muscles [10–12,18,19]. Several ionic currents, including \(I_{\text{na}}\), are potentially involved [18,19]. The present study demonstrates that \(I_{\text{Ca-L}}\) plays a role. This contribution may occur via changes in its decay kinetics in relation with SR-Ca\(^{2+}\) release and Ca\(^{2+}\)-dependent inactivation of Ca\(^{2+}\) channels. This regulation probably plays a highly dynamic role in the regulation of APD following sudden changes in cardiac frequency. We also show that FD depolarisation of the MP has a part in the FD prolongation of the AP in papillary muscles. The overall prolongation is
Fig. 8. Effect of intracellular Ca\(^{2+}\) buffer. (A) Steady state APs recorded at 0.1 and 3.3 Hz in single myocytes loaded with 10 mmol/l EGTA (a: high), 0.5 mmol/l EGTA (b: low) or 20 mmol/l BAPTA (c), respectively, in the patch-pipette. (B) Average percentage increases (±S.E.M.) in the APD\(_{50}\) at 3.3 Hz. Statistical significance between pairs is indicated by an asterisk (ANOVA, ** P < 0.01). (C) Partial recovery of the FD prolongation of the AP observed over time with 0.5 mmol/l EGTA in the patch pipette.

Fig. 9. Effect of ryanodine. (A) Steady state APs recorded at 1.0 and 5.7 Hz in absence and presence of 10 \(\mu\)mol/l ryanodine in papillary muscles: (a) original traces in the same preparation; (b) averaged percentage increases (±S.E.M.) in the APD at 5.7 Hz. (B) steady state APs recorded at 0.1 and 3.3 Hz in absence and presence of 1 \(\mu\)mol/l ryanodine in single myocytes (10 mmol/l EGTA): (a) original traces in the same cell; (b) averaged percentage increases (±S.E.M.) in the APD at 3.3 Hz. Similar effect was observed at 10 \(\mu\)mol/l (data not shown).
likely to reflect the combination of two distinct effects on Ca\(^{2+}\) channel activity.

4.1. Modulation of APD by frequency and MP

Adaptation of APD to heart rate varies among species. The APD shortens with stepped increases in frequency in guinea pig and in diseased human cardiomyocytes [5,7–9], whereas a prolongation occurs in rat ventricular cells [10–12,18,19]. Here, we found similar FD prolongation both in rat papillary muscles and rat single ventricular myocytes despite some quantitative differences that may be partly related to differences in intracellular Ca\(^{2+}\) buffering. A FD depolarisation also occurred in the papillary muscles, in agreement with previous observation [11]. It is known that increased stimulation frequency causes depolarisation of the MP in thin multicellular preparations, which has been previously related to an increase of the cleft K\(^+\) concentration [3,7,8]. This depolarisation was, however, very small (<2 mV) in single cells, reflecting possibly better washout or oxygenation of the cells by the bathing solution or, alternatively, temperature difference. Interestingly, we showed here that an artificial moderate depolarisation of the MP (<10 mV) enhances the FD prolongation of the AP in single cells, suggesting that this process contributes to the FD adaptation occurring in papillary muscles.

4.2. Role of I\(_{Ca-L}\) and SR Ca\(^{2+}\) release

An increase in the APD is expected to reflect alter-
of the AP in rat ventricular cells. This control occurs via Ca\(^{2+}\)-dependent inactivation of I_{Ca-L} as anticipated from mathematical modelling and experimental approaches [33,34]. This mechanism is probably well developed in rat ventricle where high SR Ca\(^{2+}\)-ATPase activity is known to favour high SR-Ca\(^{2+}\) load, and thus high Ca\(^{2+}\) release [4]. This interpretation is also consistent with the decrease in contraction that was observed immediately after increasing the pacing rate in single cells (see also Ref. [10,11]), though additional mechanisms may also be involved because changes in APD were slower to reach steady state.

### 4.3. Limiting role of MP and I_{\text{to}}

The I_{\text{to}} blocker 4-AP induced large FD prolongation of the AP, suggesting that I_{\text{to}} is a limiting factor, probably because this current shortens the AP and, thereby, limits the recruitment of I_{Ca-L}. This indirect role of I_{\text{to}} may partially explain the larger FD prolongation correlated with decreased I_{\text{to}} in diabetic rats [18]. Our study also showed that the enhancement of the FD AP-prolongation by depolarisation of the MP, physiologically observed in papillary muscles, reflects the contribution of two distinct effects on I_{Ca-L}. One effect reflects changes in gating properties, leading to facilitation (slowing of current inactivation). The other is probably related to changes in the number of Ca\(^{2+}\) channels activated during the AP. Since there is a reduction in the rate upstroke (dV/dt) and a delay in the repolarisation of the AP, resulting from voltage-dependent inactivation of both I_{Na} and I_{\text{to}}, more Ca\(^{2+}\) channels may be recruited and, thereby, available for FD facilitation.

### 4.4. Possible role and relevance for pathophysiology

Adaptation of the APD to heart rate is essential for co-ordinated control of electrical activity and Ca\(^{2+}\) homeostasis during systolic and diastolic periods. The FD adaptation described in this study occurs very rapidly and at physiological rates. It is expected to occur during sudden variations of the heart rate on a beat-to-beat basis. Its role may be to supply the cell with additional Ca\(^{2+}\) ions from the extracellular source in order to refill the SR at high pacing rates. Removal of Ca\(^{2+}\) release-induced inactivation of I_{Ca-L} has indeed been shown to increase Ca\(^{2+}\) entry under extreme conditions of SR-Ca\(^{2+}\) depletion or during heart failure (HF) [30,35].

Prolongation of the AP is a hallmark feature of hypertrophy, and HF, which can disturb electrical heterogeneity and induce arrhythmias leading to sudden death [36–38]. Reduction of I_{\text{to}} is involved, whereas I_{Ca-L} density is probably unchanged [39,40]. Suppression of I_{\text{to}} by 4-AP or decreased K\(^-\) channel expression prolongs the AP in post infarction and increases both Ca\(^{2+}\) influx and Ca\(^{2+}\) transient in rat cardiomyocytes [37,39,41]. Our experiments with 4-AP suggest that one consequence of I_{\text{to}} blunting is to enhance the FD AP-prolongation. This enhancement could be particularly important if the SR-Ca\(^{2+}\) release is unaffected or increased [39]. A marked AP prolongation could result in a large, time-dependent positive inotropic effect [41]. Moreover, any depolarisation of the MP, equivalent to that described 8 weeks following left coronary artery ligation in rat (~10 mV) [39], might also enhance dramatically the effect of high rates on the APD and on intracellular Ca\(^{2+}\) load.

In summary, we have related sudden changes of rat ventricular AP that occur physiologically in a highly dynamic manner during variations in frequency, to changes in Ca\(^{2+}\) channel activity. These effects seem to be related to facilitation of I_{Ca-L} and to SR-Ca\(^{2+}\) dependent modulation of Ca\(^{2+}\) channel inactivation. We emphasise that this modulation is an obligatory, highly regulated, intermediate in the communication between SR-Ca\(^{2+}\) and the APD, which contributes to the electrical control of Ca\(^{2+}\) cycling during the diastolic and systolic phases.

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