

## Research Article

# Effect of $\text{Ca}^{2+}$ Efflux Pathway Distribution and Exogenous $\text{Ca}^{2+}$ Buffers on Intracellular $\text{Ca}^{2+}$ Dynamics in the Rat Ventricular Myocyte: A Simulation Study

Michal Pásek,<sup>1,2</sup> Jiří Šimurda,<sup>2</sup> and Clive H. Orchard<sup>3</sup>

<sup>1</sup> Institute of Thermomechanics, Branch Brno, Academy of Science of the Czech Republic, Technická 2, 61669 Brno, Czech Republic

<sup>2</sup> Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic

<sup>3</sup> School of Physiology and Pharmacology, University of Bristol, Bristol BS8 1TD, UK

Correspondence should be addressed to Michal Pásek; mpasek@med.muni.cz

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We have used a previously published computer model of the rat cardiac ventricular myocyte to investigate the effect of changing the distribution of  $\text{Ca}^{2+}$  efflux pathways (SERCA,  $\text{Na}^+/\text{Ca}^{2+}$  exchange, and sarcolemmal  $\text{Ca}^{2+}$  ATPase) between the dyad and bulk cytoplasm and the effect of adding exogenous  $\text{Ca}^{2+}$  buffers (BAPTA or EGTA), which are used experimentally to differentially buffer  $\text{Ca}^{2+}$  in the dyad and bulk cytoplasm, on cellular  $\text{Ca}^{2+}$  cycling. Increasing the dyadic fraction of a particular  $\text{Ca}^{2+}$  efflux pathway increases the amount of  $\text{Ca}^{2+}$  removed by that pathway, with corresponding changes in  $\text{Ca}^{2+}$  efflux from the bulk cytoplasm. The magnitude of these effects varies with the proportion of the total  $\text{Ca}^{2+}$  removed from the cytoplasm by that pathway. Differences in the response to EGTA and BAPTA, including changes in  $\text{Ca}^{2+}$ -dependent inactivation of the L-type  $\text{Ca}^{2+}$  current, resulted from the buffers acting as slow and fast “shuttles,” respectively, removing  $\text{Ca}^{2+}$  from the dyadic space. The data suggest that complex changes in dyadic  $\text{Ca}^{2+}$  and cellular  $\text{Ca}^{2+}$  cycling occur as a result of changes in the location of  $\text{Ca}^{2+}$  removal pathways or the presence of exogenous  $\text{Ca}^{2+}$  buffers, although changing the distribution of  $\text{Ca}^{2+}$  efflux pathways has relatively small effects on the systolic  $\text{Ca}^{2+}$  transient.

## 1. Introduction

During the last few years, it has become apparent that the ultrastructure of cardiac ventricular myocytes is critical to their function, with localised ion handling and signalling microdomains playing a key role in cell function. For example,  $\text{Ca}^{2+}$  influx via L-type  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) causes local  $\text{Ca}^{2+}$  release from adjacent sarcoplasmic reticulum (SR) at the dyad [1, 2]; and  $\text{Ca}^{2+}$  released from SR appears to have “privileged” access to the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger (NCX, [3]), presumably because of the proximity of NCX to SR  $\text{Ca}^{2+}$  release channels.

$\text{Ca}^{2+}$  within the dyad—the site of  $\text{Ca}^{2+}$  entry via  $I_{\text{Ca}}$  and  $\text{Ca}^{2+}$  release from the SR—is critical, because it controls  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (CICR) from the SR [1, 2] and  $\text{Ca}^{2+}$ -dependent inactivation (CDI) of  $I_{\text{Ca}}$  [4]. Similarly, bulk cytoplasmic  $\text{Ca}^{2+}$  is critical since it determines contraction

and relaxation. Colocation of different  $\text{Ca}^{2+}$  flux pathways is also likely to be important in  $\text{Ca}^{2+}$  “autoregulation” [5], whereby an increase in intracellular  $\text{Ca}^{2+}$  increases efflux via NCX and decreases influx via  $I_{\text{Ca}}$  [6], and in the genesis of some types of arrhythmia (e.g., delayed afterdepolarizations), which are caused by activation of inward NCX current by spontaneous SR  $\text{Ca}^{2+}$  release [7, 8]. Such localisation may also change in pathological conditions, thereby altering cell function.

Many studies have investigated the location and colocation of  $\text{Ca}^{2+}$  influx and release pathways and their importance for cell function. Although  $\text{Ca}^{2+}$  efflux occurs predominantly in the t-tubules, which are, therefore, likely to play a role in ensuring rapid and uniform relaxation of the cell [9, 10], less is known about the ultrastructural location and colocation of  $\text{Ca}^{2+}$  efflux pathways and how critical such location is to cell function. While it appears likely that the localisation of

NCX is important in cell function (above), little is known about the relevance of the distribution of SR  $\text{Ca}^{2+}$  ATPase (SERCA), which biochemical studies have shown throughout the SR [11], while immunohistochemical studies suggest that it is located predominantly at the Z-line and, thus, close to the t-tubules and the site of SR  $\text{Ca}^{2+}$  release [12]. We have, therefore, used a computer model of the rat ventricular myocyte to explore the sensitivity of intracellular  $\text{Ca}^{2+}$  cycling to changes in dyadic  $\text{Ca}^{2+}$  handling brought about either by altering the distribution of  $\text{Ca}^{2+}$  efflux pathways between the dyad and bulk cytoplasm or by addition of  $\text{Ca}^{2+}$  buffers that are used experimentally to differentially buffer  $\text{Ca}^{2+}$  within the dyad and bulk cytoplasm.

## 2. Methods

The model used in this study (Figure 1) was based on that described by Pásek et al. [13], which was modified to explore the effect of the distribution of  $\text{Ca}^{2+}$  removal pathways on intracellular  $\text{Ca}^{2+}$  dynamics. The distribution of NCX, sarcolemmal  $\text{Ca}^{2+}$  ATPase, and L-type  $\text{Ca}^{2+}$  channels between the t-tubular and surface membranes was as determined experimentally and described in [13]. The fraction of the  $\text{Ca}^{2+}$  extrusion pathways located at the t-tubular and surface membrane dyads ( $f_{\text{NaCa,d}}$  and  $f_{\text{pCa,d}}$ ) was varied independently between 0 (their normal value in the model) and 0.3, with reciprocal variation of the corresponding extradyadic fraction at each membrane. Thus, when  $f_{\text{NaCa,d}}$  or  $f_{\text{pCa,d}}$  was set to 0.3, the fractions of the corresponding ion transporter at t-tubular dyadic space, surface dyadic space, t-tubular subsarcolemmal space, and surface subsarcolemmal space were, respectively,  $0.3 \times$  t-tubular fraction of ion transporter,  $0.3 \times$  surface fraction of ion transporter,  $0.7 \times$  t-tubular fraction of ion transporter, and  $0.7 \times$  surface fraction of ion transporter. The fraction of L-type  $\text{Ca}^{2+}$  channels located at the dyads ( $f_{\text{Ca,d}}$ ) was maintained constant at 1.

The fraction of SR  $\text{Ca}^{2+}$  ATPase (SERCA) at the dyad ( $f_{\text{up,d}}$ ) was also varied between 0 (its normal value in the model) and 0.3, with the remaining fraction  $1 - f_{\text{up,d}}$  in the bulk cytosolic space. The distribution of the dyadic fraction of SERCA between the surface and t-tubular dyads was set proportional to the fraction of dyads at the t-tubular and surface membranes (0.8 and 0.2, resp. [13]). Thus, for a total dyadic fraction of SERCA of 0.3, its fraction at the t-tubular membrane was  $0.3 \times 0.8$  and that at surface membrane was  $0.3 \times 0.2$ . To simplify presentation, unless stated otherwise (e.g., Figure 7), data are shown only for t-tubular dyads, since there are more dyads at the t-tubules than at the surface membrane [14], so that they play a greater role in excitation-contraction coupling, even though the gain of CICR appears to be similar at the two sites [15]. The behaviour of dyads at the two sites in response to changes in the distribution of  $\text{Ca}^{2+}$  efflux pathways was also qualitatively similar in the present study.

Extracellular  $[\text{Ca}^{2+}]$ ,  $[\text{Na}^+]$ , and  $[\text{K}^+]$  were normally set to 1.2 mM, 140 mM, and 5.4 mM, respectively. To simulate

the addition of EGTA and BAPTA to the intracellular compartments from a patch pipette, the diffusion of  $\text{Ca}^{2+}$  and exogenous  $\text{Ca}^{2+}$  buffers (with or without bound  $\text{Ca}^{2+}$ ) among the pipette, subsarcolemmal spaces, dyadic spaces, and cytosol was described using ordinary differential equations where the flux of  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$  buffer between adjacent compartments is directly proportional to the concentration difference of  $\text{Ca}^{2+}$  or  $\text{Ca}^{2+}$  buffer between the compartments and inversely proportional to the time constant of  $\text{Ca}^{2+}$  or buffer exchange, as described previously [13]. To replicate the experimental conditions used to determine the effect of EGTA and BAPTA on  $I_{\text{Ca}}$  inactivation during voltage clamp, trains of six voltage pulses (200 ms, 0.1 Hz) from  $-80$  to  $0$  mV were used, and inactivation during the 6th pulse was analyzed [15]. The following ion concentrations were used for the extracellular compartment, in the pipette, and intracellular compartment:  $[\text{Ca}^{2+}]_e = 1$  mM;  $[\text{Na}^+]_e$  and  $[\text{K}^+]_e \approx 0$  ( $10^3 \times$  lower than normal, that is, 0.135 and 0.005 mM, resp.);  $[\text{Ca}^{2+}]_p = 0.5$  nM;  $[\text{Na}^+]_i$  and  $[\text{K}^+]_i \approx 0$  (0.01 and 0.14 mM, resp.), reflecting their dialysis via the pipette.  $\text{K}^+$  currents were disabled because they were blocked experimentally using  $\text{Cs}^+$  [15].

The simulations investigating the effect of  $\text{Ca}^{2+}$  efflux pathway distribution on intracellular  $\text{Ca}^{2+}$  dynamics under current clamp conditions were performed at 5 Hz steady-state stimulation, which corresponds to resting heart rate in the rat. The simulations investigating the effect of EGTA and BAPTA under current clamp were performed at 1 Hz steady-state stimulation, because  $\text{Ca}^{2+}$  overload was observed in the model cell at higher stimulation rates in the presence of these buffers.

Numerical computation of the system of 97 nonlinear differential equations was performed in MATLAB v.7.2 (MathWorks, Natick, MA, USA) using the solver for stiff system ODE-15s. The model equations were simultaneously solved using a time-step adjusted to keep the estimated relative error of inner variables below a threshold value of  $10^{-6}$ . To attain dynamic steady state in the model cell at both stimulation frequencies (1 Hz and 5 Hz), the model was paced for 600 s of equivalent cell life time. The basic units in which the equations were solved were the following: mV for membrane potential, mA for membrane currents, mM for ionic concentrations, s for time, and mL for volumes.

## 3. Results

*3.1. The Effect of  $\text{Ca}^{2+}$  Removal Pathway Distribution on  $\text{Ca}^{2+}$  Dynamics.* Figure 2 shows the effect of increasing the dyadic fraction of NCX (a), sarcolemmal  $\text{Ca}^{2+}$  pump (b), and SERCA (c), from 0 to 0.3, on whole cell transmembrane  $\text{Ca}^{2+}$  fluxes and dyadic and cytoplasmic  $\text{Ca}^{2+}$  transients during a steady-state beat at 5 Hz. Increasing dyadic NCX resulted in increased  $\text{Ca}^{2+}$  efflux via NCX, reflected as an increase in peak inward  $I_{\text{NaCa}}$ , as expected from exposure of 30% of NCX to the high  $\text{Ca}^{2+}$  in the dyadic space. This was accompanied by a small increase in the amplitude of the cytoplasmic  $\text{Ca}^{2+}$  transient, partly as a result of reduced  $\text{Ca}^{2+}$  removal via NCX

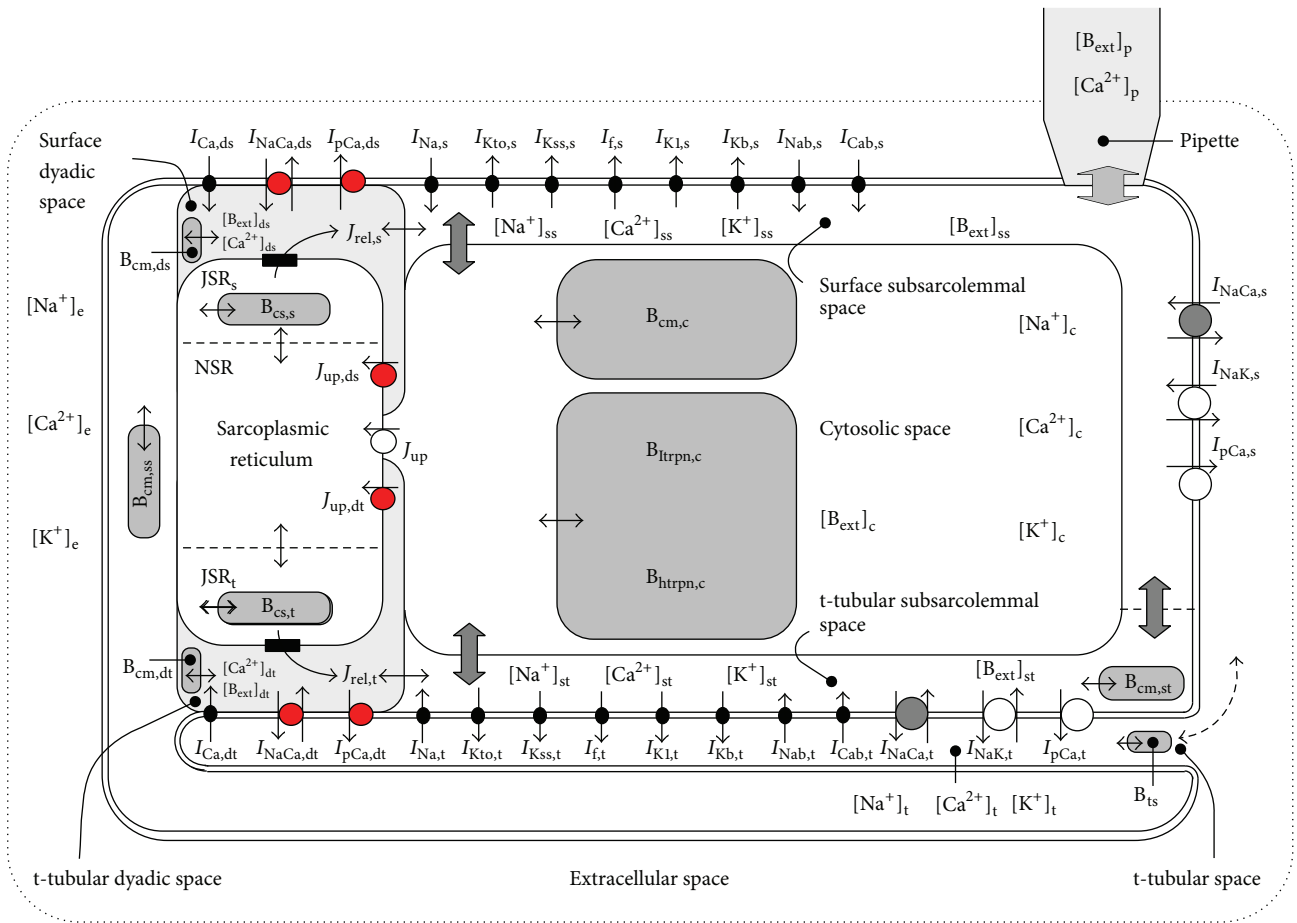


FIGURE 1: Schematic diagram of the rat ventricular cell compartmental model used in the present study. The description of the electrical activity of surface membrane (s, ds at surface dyads) and t-tubular membrane (t, dt at t-tubular dyads) comprises formulations of the following ion currents: fast sodium current ( $I_{Na}$ ), L-type calcium current ( $I_{Ca}$ ), transient outward potassium current ( $I_{Kto}$ ), steady-state outward potassium current ( $I_{Kss}$ ), inward rectifying potassium current ( $I_{K1}$ ), hyperpolarization-activated current ( $I_f$ ), background currents ( $I_{Kb}$ ,  $I_{Nab}$ , and  $I_{Cab}$ ), sodium-calcium exchange current ( $I_{NaCa}$ ), sodium-potassium pump current ( $I_{NaK}$ ), and calcium pump current ( $I_{pCa}$ ). The intracellular space contains the cytosolic space (c), surface and t-tubular subsarcolemmal spaces (ss, st), surface and t-tubular dyadic spaces (ds, dt), network and junctional compartments of sarcoplasmic reticulum (NSR, JSR<sub>s</sub>, and JSR<sub>t</sub>), endogenous  $Ca^{2+}$  buffers (calmodulin ( $B_{cm}$ ), troponin ( $B_{htrpn}$ ,  $B_{ltrpn}$ ), and calsequestrin ( $B_{cs}$ )), and exogenous  $Ca^{2+}$  buffer (e.g., BAPTA or EGTA ( $B_{ext}$ )).  $B_{ts}$  denotes the nonspecific  $Ca^{2+}$  buffer associated with luminal part of t-tubular membrane.  $J_{up}$  represents  $Ca^{2+}$  flow via SR  $Ca^{2+}$  pump and the small filled rectangles in JSR membrane ryanodine receptors. The small bidirectional arrows denote  $Ca^{2+}$  diffusion. Ion diffusion between the t-tubular and extracellular spaces is represented by the dashed arrow. The changes with respect to the previous model [13] are highlighted in red. The Matlab code of the model can be downloaded at: <http://www.it.cas.cz/en/d3/l033/biophysics-cardiac-cells>.

from the bulk cytoplasm. The increase of cytosolic  $Ca^{2+}$  and decrease of cytosolic NCX resulted in a small increase in  $Ca^{2+}$  removal via the sarcolemmal  $Ca^{2+}$  ATPase and SERCA (see  $I_{pCa}$  and  $J_{up}$ , resp.). The small prolongation of  $I_{Ca}$  is mainly the result of APD prolongation (see Figure 3), due primarily to the increase of  $I_{NaCa}$ , rather than to decreased CDI. Increasing the dyadic sarcolemmal  $Ca^{2+}$  pump fraction resulted in an increase in  $Ca^{2+}$  efflux via the pump but had little effect on the other  $Ca^{2+}$  fluxes or concentrations, reflecting its relatively minor role in  $Ca^{2+}$  handling. In contrast, increasing dyadic SERCA resulted in increased SR  $Ca^{2+}$  uptake that was accompanied by an increase in the amplitude of the dyadic and cytoplasmic  $Ca^{2+}$  transients as a result of increased SR

$Ca^{2+}$  release, and thus a small increase in  $Ca^{2+}$  extrusion via NCX and the sarcolemmal  $Ca^{2+}$  pump during the later stages of the  $Ca^{2+}$  transient.

The dyadic  $Ca^{2+}$  transient depends not only on  $Ca^{2+}$  entry into the dyad via  $I_{Ca}$  and SR  $Ca^{2+}$  release and the absence or presence of  $Ca^{2+}$  extrusion pathways at the dyad but also on  $Ca^{2+}$  diffusion from the dyad into adjacent cytoplasm. In the absence of  $Ca^{2+}$  extrusion pathways at the dyad, all of the  $Ca^{2+}$  leaves the dyad by diffusion, reaching a maximum value of  $Ca^{2+}$  flux from t-tubular dyadic space to t-tubular subsarcolemmal space of 11.23 fM/s at the peak of the dyadic  $Ca^{2+}$  transient. Increasing the fraction of NCX or sarcolemmal  $Ca^{2+}$  ATPase at the dyad to 0.3 had little effect

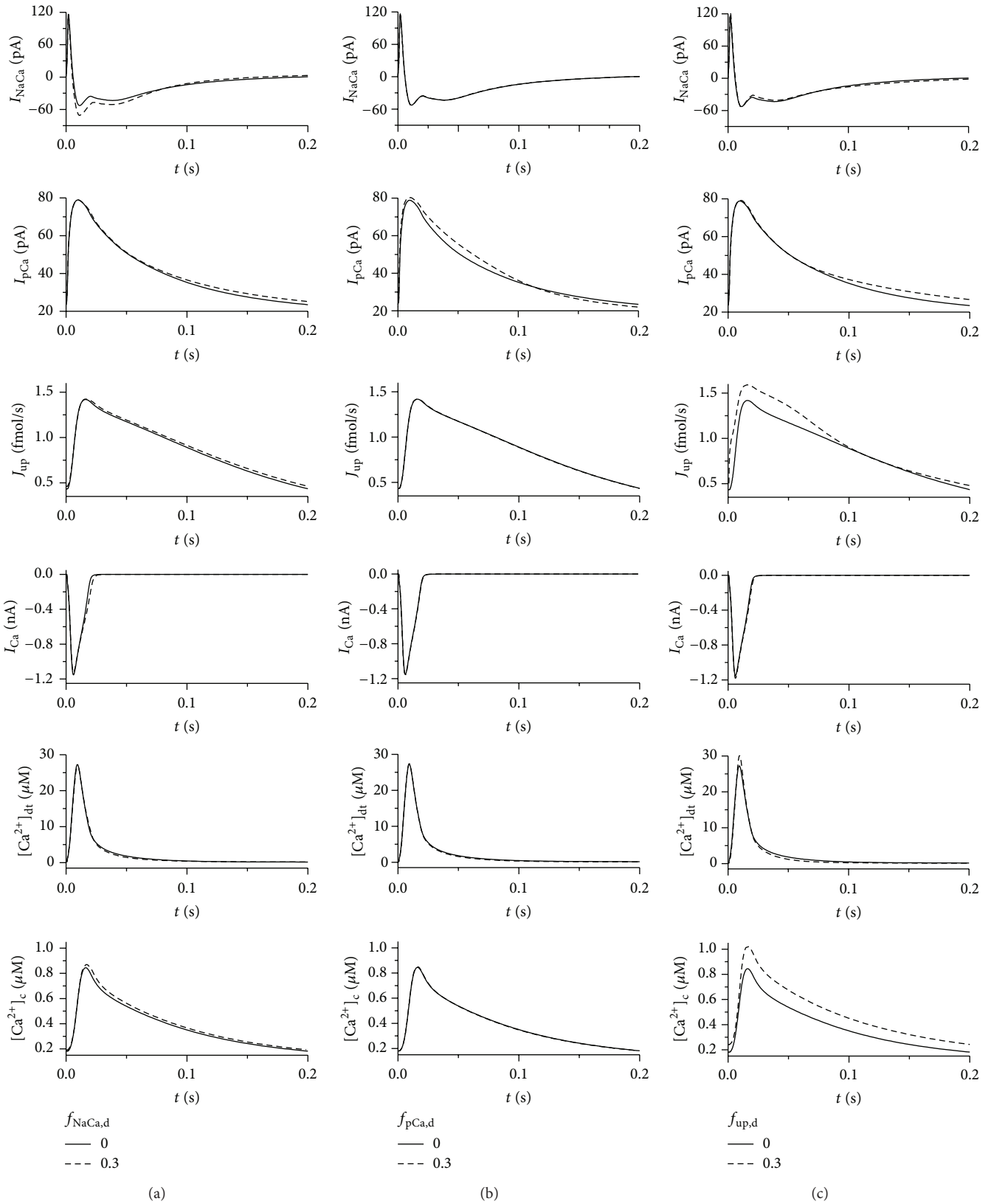


FIGURE 2: Effect of partial localization of NCX exchange (a), sarcolemmal Ca<sup>2+</sup> pump (b), and SERCA (c) at the dyads on the time course of total  $I_{NaCa}$ ,  $I_{pCa}$ ,  $J_{up}$ , and  $I_{Ca}$  and Ca<sup>2+</sup> transients in the t-tubular dyadic space ( $[Ca^{2+}]_{dt}$ ) and in the cytosol ( $[Ca^{2+}]_c$ ) during a steady-state cycle at 5 Hz (action potentials elicited by 1 ms current clamps). The solid and dashed lines, respectively, represent traces obtained in control conditions ( $f_{NaCa,d} = f_{pCa,d} = f_{up,d} = 0$ ) and when  $f_{NaCa,d}$ ,  $f_{pCa,d}$ , or  $f_{up,d}$  was separately increased to 0.3.

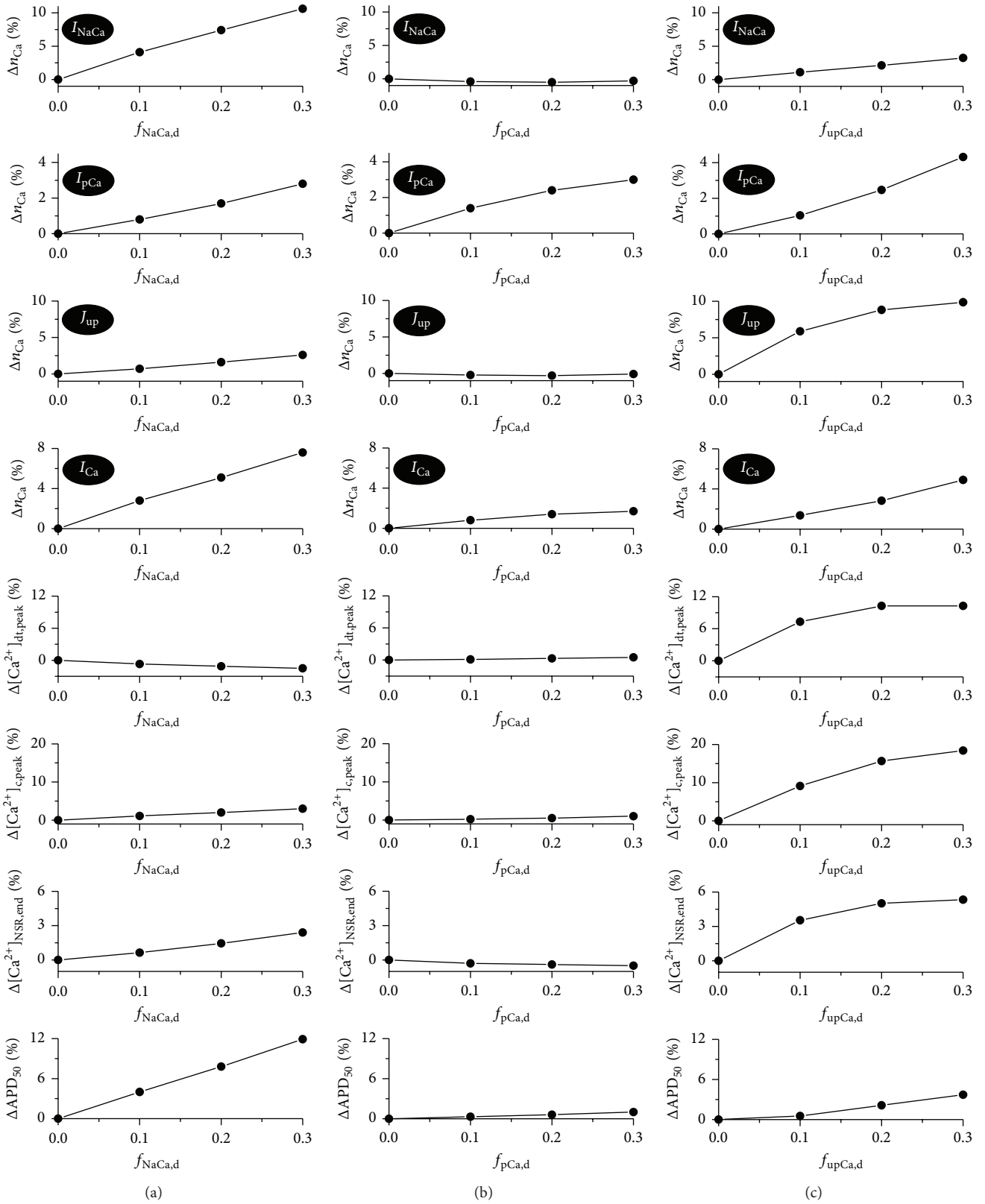


FIGURE 3: Effect of partial localization of NCX exchange (a), sarcolemmal  $Ca^{2+}$  pump (b), and SERCA (c) at the dyads on the amount of  $Ca^{2+}$  ( $n_{Ca}$ ) transferred through  $I_{NaCa}$ ,  $I_{pCa}$ ,  $J_{up}$ , and  $I_{Ca}$ ; the peak value of  $Ca^{2+}$  transients in the t-tubular dyadic space ( $[Ca^{2+}]_{dt,peak}$ ) and in the cytosol ( $[Ca^{2+}]_{c,peak}$ ); the end-diastolic level of  $Ca^{2+}$  concentration in the network SR ( $[Ca^{2+}]_{NSR,end}$ ); and action potential duration at 50% repolarization ( $APD_{50}$ ) during a steady-state cycle at 5 Hz. The fraction of all three transporters at the dyads was increased separately from control ( $f_{NaCa,d} = f_{pCa,d} = f_{up,d} = 0$ ) to 0.1, 0.2, and 0.3. All values are expressed as a percentage relative to those obtained in control conditions.

on this flux, while increasing the fraction of SERCA at the dyad to 0.3 increased its maximum value to 12.4 fM/s, as a result of the increase in the amplitude of the dyadic  $\text{Ca}^{2+}$  transient. The fraction of dyadic  $[\text{Ca}^{2+}]$  leaving the dyad by diffusion decreased from 1 under control conditions to 0.81 when the fraction of SERCA at the dyad was increased to 0.3.

Figure 3 shows the percentage changes in integrated steady-state  $\text{Ca}^{2+}$  fluxes,  $\text{Ca}^{2+}$  concentrations, and action potential duration as the dyadic fraction of NCX (a), sarcolemmal  $\text{Ca}^{2+}$  pump (b), and SERCA (c) was increased in 0.1 steps from 0 to 0.3. This shows gradation of the changes described above with increasing dyadic fractions of these  $\text{Ca}^{2+}$  removal pathways. It is worth noting that increasing dyadic NCX, in addition to the changes described above, led to a small increase in SR  $\text{Ca}^{2+}$  content, as a result of increased  $\text{Ca}^{2+}$  uptake via SERCA as a consequence of a larger cytosolic  $\text{Ca}^{2+}$  transient induced partly by lower extradyadic fraction of NCX. Changing the dyadic fraction of the sarcolemmal  $\text{Ca}^{2+}$  pump had little effect on other aspects of  $\text{Ca}^{2+}$  handling, as noted above. Figure 3 also shows the increase in SR  $\text{Ca}^{2+}$  content that resulted from the increase in dyadic SERCA and shows increased  $\text{Ca}^{2+}$  influx via  $I_{\text{Ca}}$  as a result of reduced CDI due to more rapid  $\text{Ca}^{2+}$  removal from the dyad by SERCA and a slight increase in action potential duration (APD) as a result of the changes in  $I_{\text{Ca}}$  and  $I_{\text{NaCa}}$ .

We also explored the effect of simultaneously increasing the dyadic fraction of the three  $\text{Ca}^{2+}$  removal pathways to 0.3 to investigate the effect of competition between the extrusion pathways at the dyad (not shown). This increased  $\text{Ca}^{2+}$  removal by NCX, SL  $\text{Ca}^{2+}$  ATPase, and SERCA by 10.3%, 8.9%, and 13.7%, respectively, and increased the  $\text{Ca}^{2+}$  load of the cell. End-diastolic  $[\text{Ca}^{2+}]$  in the network SR (NSR) increased by 9.13%, while that in the cytoplasm increased by 45.6% to 275 nM; the peak of the dyadic  $\text{Ca}^{2+}$  transient at the t-tubules increased by 9.9%, while the peak of the cytoplasmic  $\text{Ca}^{2+}$  transient increased by 30.2%. Action potential duration at 50% repolarization ( $\text{APD}_{50}$ ) increased by 16%, and  $\text{Ca}^{2+}$  influx via  $I_{\text{Ca}}$  increased by 11.8%. These data highlight the interaction between the different  $\text{Ca}^{2+}$  efflux pathways and their location, showing that changes in the location of a single pathway can alter the activity of the other  $\text{Ca}^{2+}$  flux pathways, as well as  $\text{Ca}^{2+}$  concentrations in subcellular spaces and the electrophysiology of the cell.

**3.2. The Effect of Buffering  $\text{Ca}^{2+}$  in Different Compartments on  $\text{Ca}^{2+}$  Dynamics.** EGTA and BAPTA have been widely used experimentally to buffer bulk cytoplasmic  $\text{Ca}^{2+}$  and cytoplasmic plus dyadic  $\text{Ca}^{2+}$ , respectively. Given the effects of changing the distribution of  $\text{Ca}^{2+}$  efflux pathways on  $\text{Ca}^{2+}$  dynamics described above, we have investigated the effects of these buffers on  $\text{Ca}^{2+}$  dynamics in the model (at  $f_{\text{NaCa,d}} = f_{\text{pCa,d}} = f_{\text{up,d}} = 0$  and  $f_{\text{Ca,d}} = 1$ ).

Figure 4 shows the effect of 10 mM EGTA (a) or BAPTA (b) on the same  $\text{Ca}^{2+}$  fluxes and concentrations shown in Figure 2. In support of the idea that these buffers differentially affect  $[\text{Ca}^{2+}]$  in the dyadic and cytoplasmic spaces, EGTA

decreased the amplitude of the dyadic  $\text{Ca}^{2+}$  transient by ~17%, but it almost completely abolished the cytoplasmic  $\text{Ca}^{2+}$  transient, decreasing its amplitude by >90%. In contrast, BAPTA decreased the dyadic  $\text{Ca}^{2+}$  transient by ~86% and completely inhibited the cytosolic  $\text{Ca}^{2+}$  transient. As a result, these buffers had different effects on  $\text{Ca}^{2+}$  fluxes.

EGTA decreased  $\text{Ca}^{2+}$  extrusion via NCX during the initial 170 ms of the cycle as a result of the decreased  $\text{Ca}^{2+}$  transient in the dyadic and subsarcolemmal spaces. However, subsequent  $\text{Ca}^{2+}$  extrusion via NCX, until the end of the cycle, was higher than in the absence of EGTA, as a result of higher  $[\text{Ca}^{2+}]$  in both spaces ( $[\text{Ca}^{2+}]_{\text{dt,end}}$ : 38 nM versus 26 nM in control,  $[\text{Ca}^{2+}]_{\text{c,end}}$ : 47 nM versus 34 nM in control) due to  $\text{Ca}^{2+}$  release from the buffer. Similar changes were observed for the SL  $\text{Ca}^{2+}$  pump and SERCA, although for these extrusion pathways the increase of  $\text{Ca}^{2+}$  extrusion during the later phase of the cycle is greater than its decrease during the  $\text{Ca}^{2+}$  transient. Similarly, the faster  $\text{Ca}^{2+}$  buffer BAPTA decreased  $\text{Ca}^{2+}$  extrusion via NCX during the initial phase of the cycle (80 ms). However, subsequent  $\text{Ca}^{2+}$  extrusion via NCX was substantially greater in the presence of BAPTA as a result of the higher  $[\text{Ca}^{2+}]$  in both spaces ( $[\text{Ca}^{2+}]_{\text{dt,end}}$ : 95 nM versus 26 nM in control,  $[\text{Ca}^{2+}]_{\text{c,end}}$ : 92 nM versus 34 nM in control) due to  $\text{Ca}^{2+}$  released from the buffer. A similar change was observed for the SL  $\text{Ca}^{2+}$  pump. The different effect of EGTA and BAPTA on  $I_{\text{Ca}}$  reflects their different efficiency to reduce  $\text{Ca}^{2+}$  transient in the dyads and thus to suppress  $\text{Ca}^{2+}$ -induced inactivation of  $I_{\text{Ca}}$ .

The concentration dependence of the effects of EGTA and BAPTA is illustrated in Figure 5, which shows the percentage changes in the amount of  $\text{Ca}^{2+}$  transferred by each efflux pathway (calculated as the integral of the corresponding flux during a steady-state cycle at 1 Hz),  $\text{Ca}^{2+}$  concentrations in intracellular spaces, and AP duration. Over the concentration range investigated,  $\text{Ca}^{2+}$  extrusion via SERCA is increased by EGTA and decreased by BAPTA, while the opposite holds for extrusion via NCX. The complex relationship between [buffer] and  $\text{Ca}^{2+}$  flux via SERCA is due to the dynamics of  $[\text{Ca}^{2+}]$  changes in the dyads and the cytosol during the two phases described for Figure 4, which are different for BAPTA and EGTA. In general terms,  $\text{Ca}^{2+}$  flux via SERCA is reduced during the  $\text{Ca}^{2+}$  transient, since  $\text{Ca}^{2+}$  transient amplitude is reduced by the buffer; following the  $\text{Ca}^{2+}$  transient,  $\text{Ca}^{2+}$  flux via SERCA is enhanced as  $\text{Ca}^{2+}$  is released from the buffer; this results in an increase of  $[\text{Ca}^{2+}]_{\text{NSR,end}}$  (by 16% for EGTA and 143% for BAPTA). Figure 5 also shows that, while 10 mM EGTA increases  $\text{Ca}^{2+}$  influx via  $I_{\text{Ca}}$  and the associated  $\text{APD}_{50}$  slightly (4.2% and 1.8%, resp.), BAPTA at the same concentration affects both profoundly (181% and 227%, resp.) as a result of increased  $\text{Ca}^{2+}$  entry via  $I_{\text{Ca}}$  due to a marked decrease in CDI as a result of the smaller dyadic  $\text{Ca}^{2+}$  transient.

These data are consistent with the idea that EGTA and BAPTA have differential effects on  $\text{Ca}^{2+}$  buffering in the dyad and bulk cytoplasm and, thus, on CDI of  $I_{\text{Ca}}$ , as suggested from the experimental data. We, therefore, investigated in

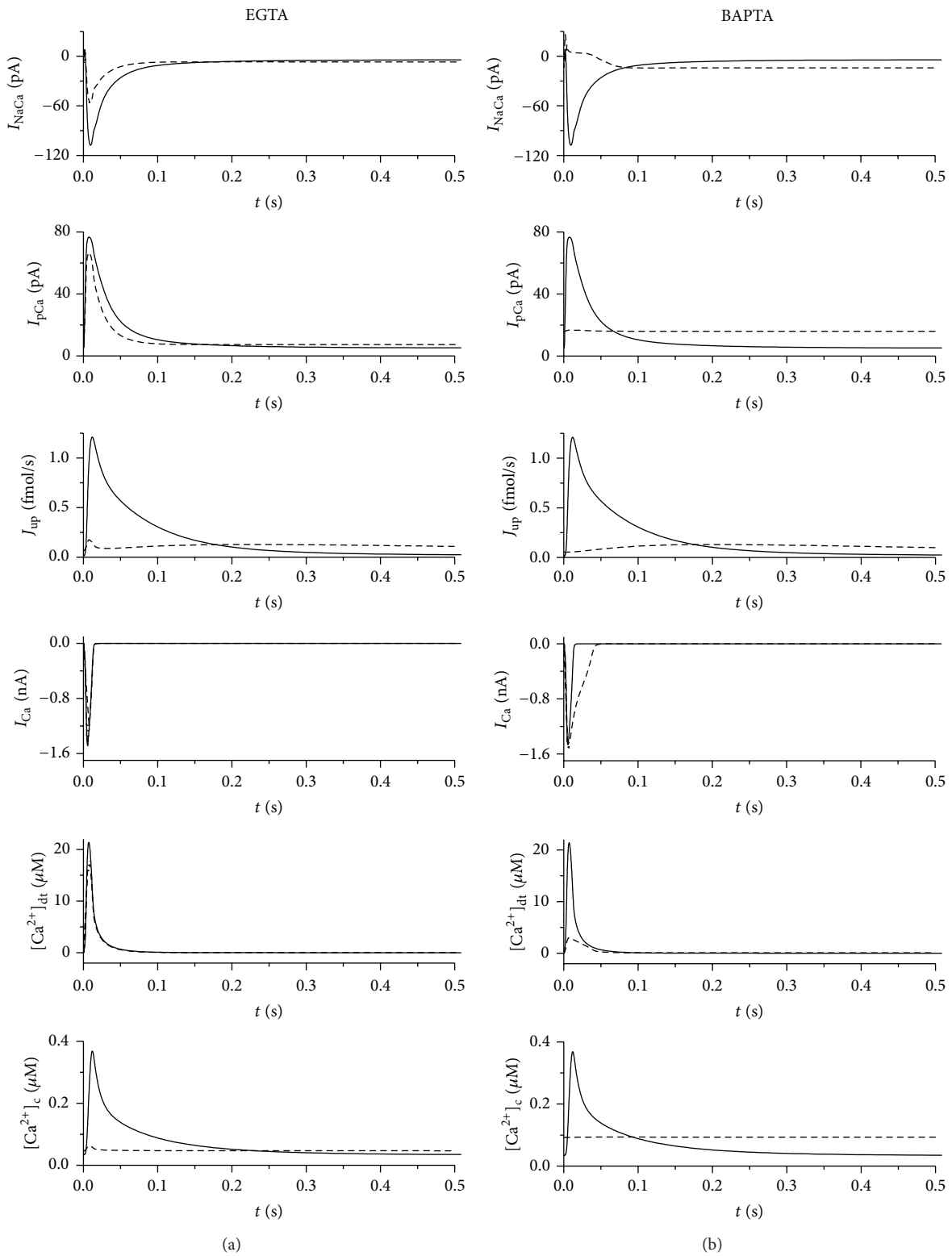


FIGURE 4: Effect of 10 mM EGTA (a) and 10 mM BAPTA (b) on the time course of total  $I_{NaCa}$ ,  $I_{pCa}$ ,  $J_{up}$ , and  $I_{Ca}$  and  $Ca^{2+}$  transients in the t-tubular dyadic space ( $[Ca^{2+}]_{dt}$ ) and in the cytosol ( $[Ca^{2+}]_c$ ) during the first 0.5 s of steady-state cycle at 1 Hz (action potentials elicited by 1 ms current clamps). The solid and dashed lines show, respectively, the traces obtained in control conditions and in the presence of the buffers in the intracellular space.

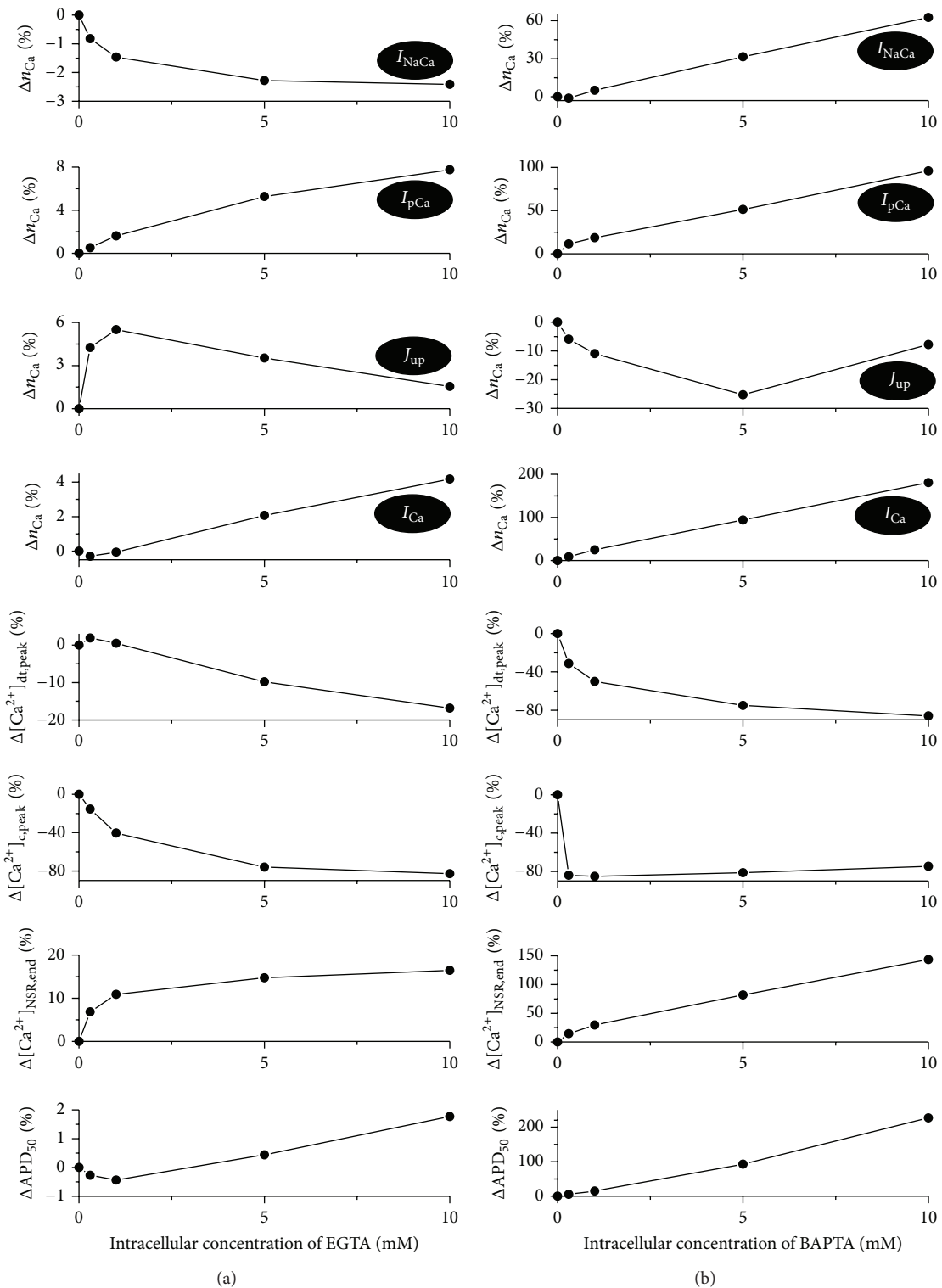


FIGURE 5: Effect of EGTA (a) and BAPTA (b) on the amount of  $Ca^{2+}$  ( $n_{Ca}$ ) transferred through  $I_{NaCa}$ ,  $I_{pCa}$ ,  $J_{up}$ , and  $I_{Ca}$ , the peak value of  $Ca^{2+}$  transients in the t-tubular dyadic space ( $[Ca^{2+}]_{dt,peak}$ ) and in the cytosol ( $[Ca^{2+}]_{c,peak}$ ), the end-diastolic level of  $Ca^{2+}$  concentration in NSR ( $[Ca^{2+}]_{NSR,end}$ ), and  $APD_{50}$  during a steady-state cycle at 1 Hz. All values are expressed as a percentage relative to those obtained in control conditions. Positive values of  $\Delta n_{Ca}$  related to  $I_{NaCa}$ ,  $I_{pCa}$ , and  $J_{up}$  indicate an increase of  $Ca^{2+}$  extrusion while positive values related to  $I_{Ca}$  indicate increased  $Ca^{2+}$  entry. Buffer concentrations were 0.3, 1, 5, and 10 mM.



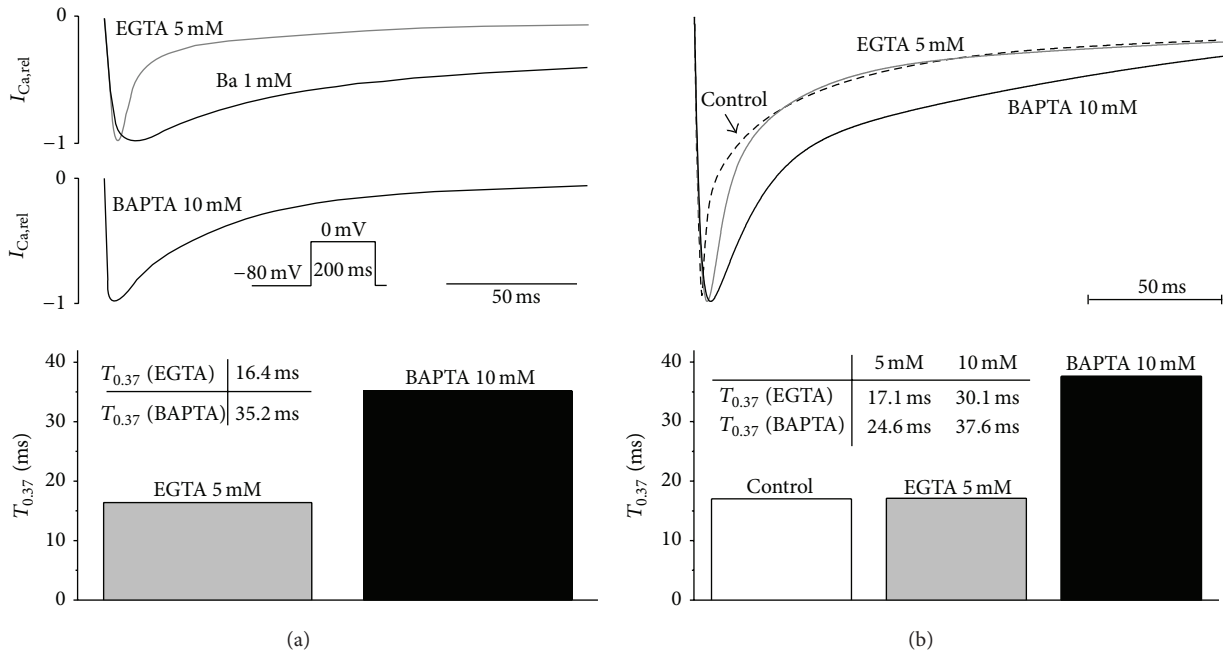


FIGURE 6: (a) Inactivation of  $I_{Ca}$  in the presence of intracellular EGTA (5 mM) or BAPTA (10 mM) in rat ventricular myocytes (adopted from [15]);  $I_{Ca}$  was recorded during a 200 ms depolarising pulse from  $-80$  mV to  $0$  mV at room temperature. The kinetics of  $I_{Ca}$  inactivation were characterized by the time required for the current to decay to 0.37 of its peak amplitude ( $T_{0.37}$ ). The lower panel shows that  $I_{Ca}$  inactivation was substantially slowed in the presence of 10 mM BAPTA:  $T_{0.37} = 35.2$  ms versus 16.4 ms in the presence of 5 mM EGTA. 1 mM Ba was used to show the time course of  $I_{Ca}$  in the absence of  $Ca^{2+}$ -dependent inactivation. (b) Reconstruction of experimentally observed effect of EGTA and BAPTA on  $I_{Ca}$  inactivation in the model. The top panel shows superimposed normalized responses of  $I_{Ca}$  in control conditions and with 5 mM EGTA and 10 mM BAPTA. The lower panel shows corresponding values of  $T_{0.37}$ .

more detail whether the model replicates the effect of these buffers on  $I_{Ca}$  and the mechanism of  $Ca^{2+}$  buffering.

Figure 6 shows the effect of EGTA (used experimentally to buffer bulk cytoplasmic  $Ca^{2+}$ ) and BAPTA (used to buffer cytoplasmic and dyadic  $Ca^{2+}$ ) on  $I_{Ca}$  in the model, compared with the experimental data. Figure 6(a) shows experimental records of  $I_{Ca}$  in the presence of EGTA and BAPTA (top, 1 mM Ba was used to determine the time course of  $I_{Ca}$  in the absence of CDI) and the time required for the current to decay to 0.37 of the peak amplitude during inactivation (below). Corresponding records and data from the model obtained under the same conditions (see Section 2) are shown in Figure 6(b), showing that the model accurately replicates the experimental data.

To understand these changes in more detail, we investigated the differences in concentration of  $Ca^{2+}$ -bound and  $Ca^{2+}$ -free buffer between the dyadic and adjacent subsarcolemmal spaces ( $\Delta[B_{ext} - Ca^{2+}]$  and  $\Delta[B_{ext} - free]$ ) in the upper panels of Figure 7). The simulations revealed that the rates of buffer exchange (both  $Ca^{2+}$ -bound and  $Ca^{2+}$ -free) between the dyadic and adjacent subsarcolemmal spaces are critical to the efficacy of  $Ca^{2+}$  buffers in the dyadic space. Although both buffers inhibited the cytosolic  $Ca^{2+}$  transient (Figure 4), only BAPTA inhibited effectively the rise of  $Ca^{2+}$  in the dyadic space, thus causing significant inhibition of CDI of  $I_{Ca}$ . The principal reason for the different potencies of BAPTA and EGTA in reducing the dyadic  $Ca^{2+}$

transient was their different rates of  $Ca^{2+}$  binding (EGTA:  $k_{on} = 5000 \text{ mM}^{-1} \text{ s}^{-1}$ ; BAPTA:  $k_{on} = 500000 \text{ mM}^{-1} \text{ s}^{-1}$ ). As a consequence, the concentration gradients of free BAPTA and  $Ca^{2+}$ -BAPTA and free EGTA and  $Ca^{2+}$ -EGTA between the dyadic space and bulk cytosol were substantially different after the onset of depolarisation. In the case of BAPTA, fast onset of large gradients provided large driving forces, causing rapid movement of  $Ca^{2+}$ -BAPTA molecules out of the dyadic space and of free BAPTA into the dyadic space, so that BAPTA appeared to act as a fast “shuttle.” The slower rate of  $Ca^{2+}$  binding to EGTA and the consequent development of substantially smaller free EGTA and  $Ca^{2+}$ -EGTA gradients between the dyadic space and cytosol reduced the ability of EGTA to affect dyadic  $[Ca^{2+}]$  and, thus,  $Ca^{2+}$ -induced inactivation of  $I_{Ca}$ . The corresponding  $[Ca^{2+}]$  in the dyads and its effect on CDI of  $I_{Ca}$  are shown in the other panels of Figure 7.

Since experimental data suggest that dyadic function is different at the t-tubules and surface membrane, with more rapidly inactivating  $I_{Ca}$  at the t-tubules [15], we also used the model to explore the effect of these buffers at the two sites. Figure 7 shows that, in control conditions, the dyadic  $Ca^{2+}$  transient during a voltage clamp pulse is larger at the t-tubules than at the surface membrane ( $293 \mu\text{M}$  versus  $216.7 \mu\text{M}$ ). This is due to a faster activation of SR  $Ca^{2+}$  release at the t-tubules caused by a faster rise of  $Ca^{2+}$  in t-tubular dyads at the beginning of the pulse. The main reason is the different

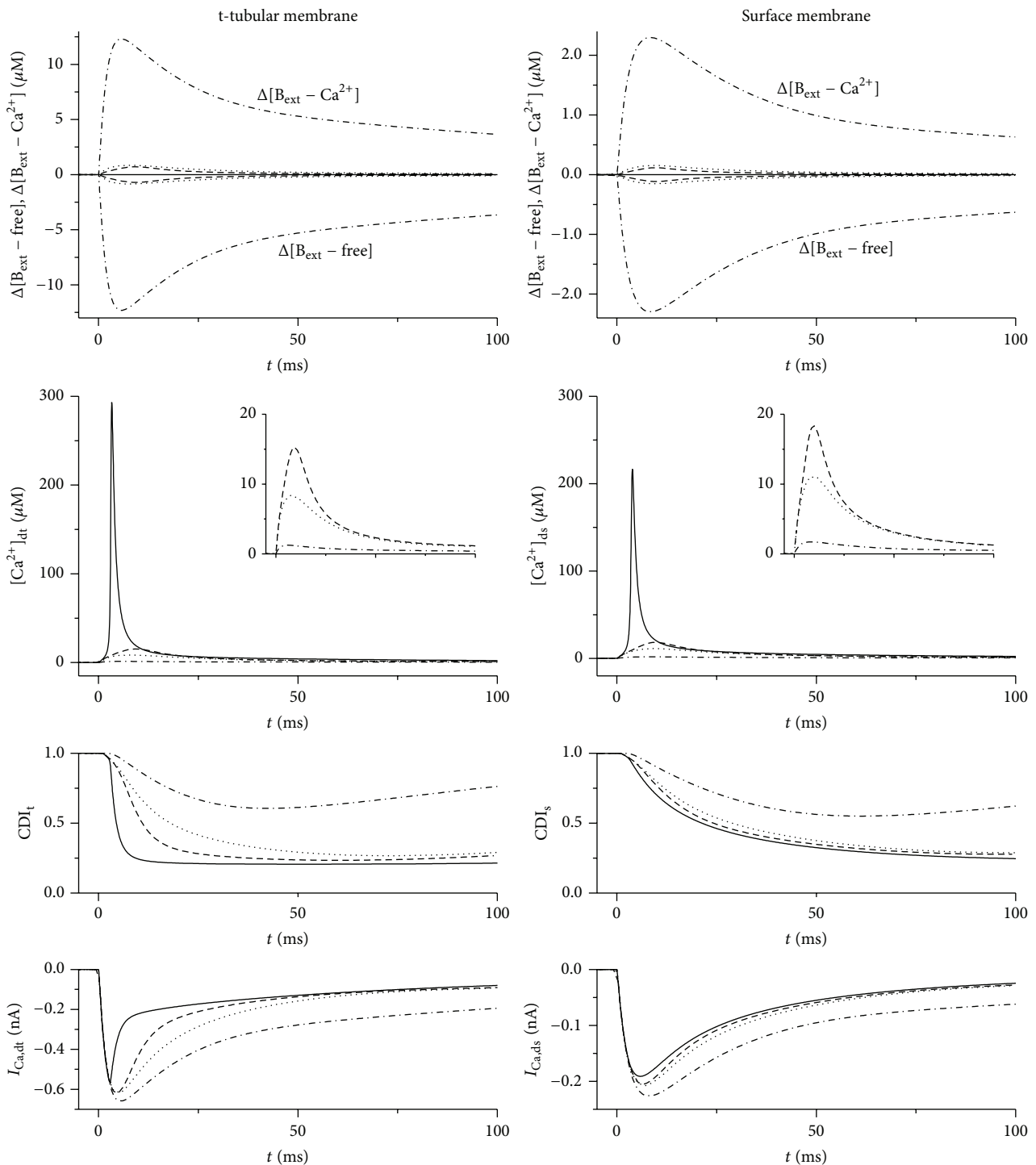


FIGURE 7: Effect of EGTA and BAPTA on intracellular  $\text{Ca}^{2+}$  and  $I_{\text{Ca}}$  inactivation at the t-tubular and surface membranes during a voltage clamp pulse from  $-80$  to  $0$  mV in the model. The upper panels show the differences between concentrations in the dyadic and subsarcolemmal spaces of the buffers with bound  $\text{Ca}^{2+}$  ( $\Delta[\text{B}_{\text{ext}} - \text{Ca}^{2+}] = [\text{B}_{\text{ext}} - \text{Ca}^{2+}]_{\text{d}} - [\text{B}_{\text{ext}} - \text{Ca}^{2+}]_{\text{s}}$ ) and unbound  $\text{Ca}^{2+}$  ( $\Delta[\text{B}_{\text{ext}} - \text{free}] = [\text{B}_{\text{ext}} - \text{free}]_{\text{d}} - [\text{B}_{\text{ext}} - \text{free}]_{\text{s}}$ ). The other panels show  $\text{Ca}^{2+}$  transients in both dyadic spaces ( $[\text{Ca}^{2+}]_{\text{dt}}$ ,  $[\text{Ca}^{2+}]_{\text{ds}}$ ),  $\text{Ca}^{2+}$ -dependent inactivation of t-tubular and surface membranes  $I_{\text{Ca}}$  ( $\text{CDI}_{\text{t}}$ ,  $\text{CDI}_{\text{s}}$ ), and time courses of related current components ( $I_{\text{Ca,dt}}$  and  $I_{\text{Ca,ds}}$ ). The solid, dashed, dotted, and dashed-dotted lines show the data obtained in control conditions and in the presence of 5 mM EGTA, 10 mM EGTA, and 10 mM BAPTA, respectively. The insets in the graphs of  $[\text{Ca}^{2+}]_{\text{dt}}$  and  $[\text{Ca}^{2+}]_{\text{ds}}$  show the dyadic  $\text{Ca}^{2+}$  transients in the presence of  $\text{Ca}^{2+}$  buffers at a higher gain.

volumes of t-tubular and surface subsarcolemmal spaces in the model (fractional volumes:  $fV_{s,t} = 0.35$  and  $fV_{s,s} = 0.65$ , resp.), which were set to be proportional to the area of each membrane that is nonjunctional: 52% of t-tubular membrane and 92.3% of surface membrane [13, 14]. Both buffers reduced the dyadic  $\text{Ca}^{2+}$  transient (Figure 7), but to a greater extent at the t-tubules, so that in the presence of buffer the  $\text{Ca}^{2+}$  transient was even smaller in the t-tubular dyads than in the surface dyads. This is a consequence of changes in  $I_{\text{Ca}}$  and, hence, CICR, mainly as a result of reduced t-tubular  $I_{\text{Ca}}$  due to  $\text{Ca}^{2+}$  depletion in the t-tubular lumen [13]. This  $\text{Ca}^{2+}$  depletion is enhanced in the presence of exogenous buffer because of slowed inactivation of t-tubular  $I_{\text{Ca}}$ .

#### 4. Discussion

The first part of the present study was designed to investigate the effect of  $\text{Ca}^{2+}$  efflux pathway distribution on the  $\text{Ca}^{2+}$  dynamics of the cardiac ventricular myocyte. The reason for such an approach is that relatively little is known about the distribution of these pathways or its functional significance. For example, biochemical studies suggest that SERCA is located throughout the SR [11]. However, immunohistochemical studies have shown it predominantly at the Z-line and, thus, close to the t-tubules and the site of SR  $\text{Ca}^{2+}$  release [12]. The functional significance of such a location is unclear; it would be expected to result in futile  $\text{Ca}^{2+}$  cycling, unless this is minimized by rapid  $\text{Ca}^{2+}$  diffusion away from the site of release. However, functional data shows that  $\text{Ca}^{2+}$  entry during the latter part of  $I_{\text{Ca}}$  can load the SR with  $\text{Ca}^{2+}$  that is released in the subsequent contraction [16, 17]; this suggests that  $\text{Ca}^{2+}$  entering the cell via  $I_{\text{Ca}}$ , which occurs predominantly at the t-tubules, is easily accessible to SERCA. Similarly, it has been suggested that  $\text{Ca}^{2+}$  released from the SR has “privileged access” to NCX [3]. This is consistent with the mainly t-tubular location of NCX, which places it in close proximity to the majority of SR  $\text{Ca}^{2+}$  release channels, which are also found predominantly at the t-tubules. However, this might be expected to be disadvantageous by producing futile  $\text{Ca}^{2+}$  cycling and enhancing arrhythmias caused by spontaneous SR  $\text{Ca}^{2+}$  release, although it may also contribute to autoregulation (see Section 1).

The present study shows that location of a  $\text{Ca}^{2+}$  efflux pathway at the dyad results in increased  $\text{Ca}^{2+}$  uptake by that pathway, as a result of exposure to the high dyadic  $[\text{Ca}^{2+}]$ . The quantitative impact of changes in distribution was  $\text{SERCA} > \text{NCX} > \text{sarcolemmal } \text{Ca}^{2+} \text{ ATPase}$ , as expected from their relative importance in  $\text{Ca}^{2+}$  removal from the cell cytoplasm [18]. Interestingly, a reduction in  $\text{Ca}^{2+}$  removal from the cell cytoplasm by one pathway (as a result of its redistribution to the dyad) resulted in an increase in the cytoplasmic  $\text{Ca}^{2+}$  transient and an increase in  $\text{Ca}^{2+}$  removal by the other two pathways. These data illustrate, therefore, (a) the competition between  $\text{Ca}^{2+}$  efflux pathways in a particular cellular compartment and (b) changes in another compartment due to the redistribution of  $\text{Ca}^{2+}$  efflux by one pathway.

The data also suggest that changes in distribution may alter cell function, for example, by altering the amplitude of the cytoplasmic  $\text{Ca}^{2+}$  transient and, hence, contraction, as a result of changes in SR  $\text{Ca}^{2+}$  content and/or  $\text{Ca}^{2+}$  extrusion from the cytoplasm; changes in dyadic  $\text{Ca}^{2+}$  may also alter the amount of  $\text{Ca}^{2+}$  released in response to  $I_{\text{Ca}}$ . An increase in SR  $\text{Ca}^{2+}$  content—as, for example, when NCX in the dyad was increased—would be expected to be proarrhythmic, particularly when coupled to the increased NCX adjacent to the site of  $\text{Ca}^{2+}$  release. However, what is perhaps most striking is the stability of the cell to the imposed changes: even large (30%) changes in the distribution of  $\text{Ca}^{2+}$  efflux pathways led to relatively small disturbances of cell  $\text{Ca}^{2+}$  dynamics. This may reflect diffusional redistribution of  $\text{Ca}^{2+}$  between cell compartments and the presence of multiple flux pathways, which may be advantageous and protective in conditions such as HF in which changes in protein expression, distribution, and activity may occur.

Given these data, it was of interest to investigate the effect of perturbing cell  $\text{Ca}^{2+}$  handling using a different method. We chose to investigate the effect of the exogenous  $\text{Ca}^{2+}$  buffers EGTA and BAPTA, since they are used experimentally to buffer  $\text{Ca}^{2+}$  in different compartments of the cell—the bulk cytoplasm and the cytoplasm and dyad, respectively. Although the precise mechanism is unclear, the observation that BAPTA, but not EGTA, inhibits CDI of  $I_{\text{Ca}}$  supports the idea that they buffer  $\text{Ca}^{2+}$  in different compartments [19, 20].

Addition of EGTA or BAPTA to the model cell resulted in changes of  $I_{\text{Ca}}$  that were very similar to those observed experimentally [15]. In addition to providing credibility for the model, this also provides support for the use of these buffers in experiments, as described above. More interestingly, however, the model enabled us to investigate the mechanism of action of these buffers and suggested that, rather than acting as static buffers, they acted as shuttles. Thus, rapid binding of  $\text{Ca}^{2+}$  to BAPTA resulted in large concentration gradients, which caused rapid movement of  $\text{Ca}^{2+}$ -BAPTA molecules out of the dyadic space and of free BAPTA into the dyadic space, so that BAPTA appeared to act as a fast “shuttle.” The slower rate of  $\text{Ca}^{2+}$  binding to EGTA and the consequent development of substantially smaller free EGTA and  $\text{Ca}^{2+}$ -EGTA gradients between the dyadic space and cytosol reduced the ability of EGTA to affect dyadic  $[\text{Ca}^{2+}]$  and, thus, CDI of  $I_{\text{Ca}}$ .

The present work also suggests that EGTA and BAPTA have different effects on CDI of  $I_{\text{Ca}}$  at the t-tubular and surface membrane dyads. The effect of both buffers on CDI and, thus, on  $I_{\text{Ca}}$ , CICR, and the  $\text{Ca}^{2+}$  transient is greater at the t-tubular dyad than at the surface dyad (Figure 7), because of the more marked CDI at the t-tubules under control conditions. However, in the presence of EGTA, CDI remains greater at the t-tubular dyad than at the surface membrane, while it is similar at the two sites in the presence of BAPTA.

It has been suggested that the more marked CDI of  $I_{\text{Ca}}$  at the t-tubules results in t-tubular  $I_{\text{Ca}}$  that has the configuration required for an effective trigger for CICR, while the slower inactivating  $I_{\text{Ca}}$  at the surface membrane

has the configuration required to load the SR with  $\text{Ca}^{2+}$  for subsequent release [5]. The greater CDI of  $I_{\text{Ca}}$  at the t-tubules may also mean that they are an important site for  $\text{Ca}^{2+}$  autoregulation (see Section 1). Thus, effective coupling of CDI of  $I_{\text{Ca}}$  to SR  $\text{Ca}^{2+}$  release may result in an effective trigger for CICR and effective regulation of cell  $\text{Ca}^{2+}$  balance, controlled by SR  $\text{Ca}^{2+}$  release; conversely, extradyadic  $I_{\text{Ca}}$  may inactivate more slowly, thus loading the SR with  $\text{Ca}^{2+}$ , suggesting that this  $\text{Ca}^{2+}$  may access extradyadic SERCA, and would play less of a role in autoregulation, as a result of being less effectively modulated by SR  $\text{Ca}^{2+}$  release.

## Conflict of Interests

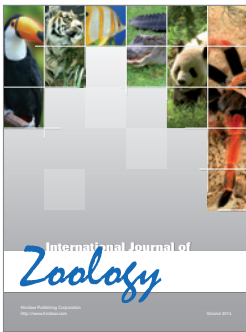
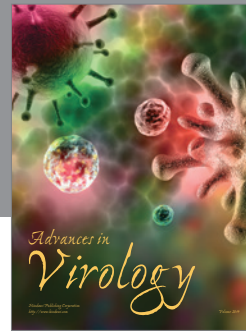
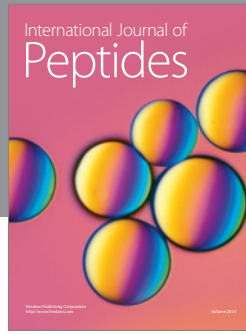
The authors declare that there is no conflict of interests regarding the publication of this paper.

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