Simultaneous recording of Indo-1 fluorescence and Na^+/Ca^{2+} exchange current reveals two components of Ca^{2+}-release from sarcoplasmic reticulum of cardiac atrial myocytes.

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Simultaneous measurements of intracellular Ca^{2+}-concentration ($[Ca^{2+}]_i$) using Indo-1 and the current generated by electrogenic Na^+/Ca^{2+}-exchange ($I_{Na/Ca}$) have been performed on atrial myocytes from hearts of adult guinea-pigs. Whereas the fluorescence-measurements provide information on global $[Ca^{2+}]_i$, $I_{Na/Ca}$ which is a linear function of Ca^{2+}-concentration, indicates subsarcolemmal $[Ca^{2+}]$. Under conditions in which intracellular Ca^{2+}-transients due to Ca^{2+}-release from the sarcoplasmic reticulum (SR) have been artificially slowed, a deviation between the two different Ca^{2+}-signals can be found. During onset of release signals Ca^{2+}-concentration seen by the membrane is higher than global $[Ca^{2+}]_i$. Our results provide evidence that in atrial myocytes, lacking a T-system, Ca^{2+}-induced Ca^{2+}-release occurs first from a subsarcolemmal compartment of the SR. The resulting Ca^{2+}-transient serves to trigger Ca^{2+}-release from deeper SR-compartments.

Cardiac myocyte; Ca^{2+}-release; Sarcoplasmic reticulum; Indo-1; Na^+/Ca^{2+}-exchange; Guinea-pig heart)

1. INTRODUCTION

Cardiac contraction is controlled by a phasic rise of $[Ca^{2+}]_i$ primarily caused by Ca^{2+}-release from the sarcoplasmic reticulum [1]. There is compelling evidence that the signal that causes opening of the release channels in the SR-membrane, is a rise in free $[Ca^{2+}]_i$ due to Ca^{2+}-entry through voltage-gated Ca^{2+}-channels (Ca^{2+}-induced Ca^{2+}-release, [2-5]). In myocytes, particularly of supraventricular origin, peripheral couplings, i.e. close associations of SR-vesicles with the surface membrane seem to function as equivalents to triads or diads in t-tubule containing muscle cells [6]. If the membrane signal is transmitted to the SR release channels via Ca^{2+}-ions, these peripheral structures should detect such a signal earlier than deeper layers of the SR and therefore should release Ca^{2+} earlier than the latter. Using simultaneous measurement of Na^+-Ca^{2+} exchange current [7-9] as a signal indicating subsarcolemmal Ca^{2+}-concentration and fluorescence changes of the Ca^{2+}-sensitive dye Indo-1 [10,11] as a signal indicating global cytosolic Ca^{2+}-concentration, such spatial inhomogeneities are found in the present study. Our measurements for the first time permit the identification of two distinct components of Ca^{2+}-release in an excitable cell with fast Ca^{2+}-signalling. One of these is likely to represent a peripheral release compartment, transmitting the signal due to transmembrane Ca^{2+}-entry to central regions of the cell.

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2. MATERIALS AND METHODS

2.1. *Materials*

Atrial myocytes from hearts of adult guinea-pigs were enzymatically isolated and cultured as described previously [12]. The cells were grown on thin coverslips in medium M199 supplemented with FCS (2%) and gentamycin (25 mg/ml). Cells were used from day 1 to 5 after isolation. Most of the cells were completely spherical within one day of culture. Cells with a diameter between 10 and 20 μm were selected for the experiments. Their membrane capacitance was less than 20 pF. External solution contained (mM): NaCl 140, CsCl 2.0, $CaCl_2$ 2.0, $MgCl_2$ 2.0, TTX 10 μM, Hepes/CsOH 10.0, pH 7.4. In order to maintain the capability of the SR to accumulate and release Ca^{2+}, the external solution was supplemented with $1-5\times10^{-10}$ M isoprenaline [13], a concentration which upon acute application did not significantly increase I_{Ca}-amplitude. Patch electrodes [14] were filled with the following solution: Cs_3-citrate 60, NaCl 10.0, CsCl 10.0, $MgCl_2$ 1.0, MgATP 5.0, Indo-1 (K-salt) 0.1, Hepes/CsOH 10.0, pH 7.4. Pipette resistance ranged from 1.5 to 2.5 MΩ. Citrate-based internal solution was used, to get prolonged release-signals (e.g. [8,15]) and to prevent focal Ca^{2+}-release, previously described to occur in cardiac myocytes [16]. The coverslips containing the myocytes were mounted in a sandwich chamber which was placed on the stage of an inverted microscope and continuously perfused with external solution (22-24°C).

2.2. *Measurements of membrane current and $[Ca^{2+}]_i$*

Current measurements were performed in whole cell mode [14] using a patch-clamp amplifier (EPC-7, List, Darmstadt). After a few minutes of loading, Indo-1-fluorescence was measured from a field slightly larger than the size of the cell. Excitation wavelength was 360 nm; emission was measured at 405 and 485 nm wavelength using two

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photomultipliers, $[Ca^{2+}]_i$ was calculated from the ratio of fluorescence ($R = F_{380}/F_{340}$) after background subtraction using the equation $[Ca^{2+}]_i = K_d(R - R_{min})/(R_{max} - R)$. In vivo determination of R_{min} and R_{max} was performed analogous to the procedure described previously for fura-2 [4]. For K_d a value of 213 nM was used [10].

3. RESULTS

In Fig. 1 membrane current following activation of L-type Ca^{2+}-current and corresponding changes of $[Ca^{2+}]_i$ are illustrated. The myocyte was depolarized at 0.2 s^{-1} from −50 to +5 mV causing a Ca^{2+} inward current of 240 pA peak amplitude (not shown). During a train of 20 identical step depolarizations every second was followed by an inward current after repolarization, paralleled by an intracellular Ca^{2+}-transient, $[Ca^{2+}]_i$ reaching a peak level of around 1.2 μM (A). It has been shown previously that the inward current in the present conditions exclusively reflects electrogenic Na^+-Ca^{2+} exchange (I_{NaCa}, e.g. [8,17,18]), caused by SR Ca^{2+}-release. The contribution of the Ca^{2+}-current itself to the Ca^{2+}-transient is very small. Every second Ca^{2+}-current in this myocyte failed to trigger release (B). Virtually no inward current following repolarization was recorded under that condition, and $[Ca^{2+}]_i$ rose from its basal level of 110 nM to only around 200 nM. For the isolated Na^+-Ca^{2+} exchange current in the absence of a functional SR a linear dependence on $[Ca^{2+}]_i$ has been documented over a wide range of concentrations [19,20]. In Fig. 1C inward current following repolarization has been plotted against $[Ca^{2+}]_i$ (from A), resulting in a completely linear relation in the range of concentrations (≤ 1 μM) which can be reliably measured by the fluorescent dye. Thus, for the decay of the transient, i.e. late after release, the Ca^{2+}-concentration detected by the internal face of the membrane, or the exchanger respectively, is likely to correspond to the global Ca^{2+}-concentration indicated by the indo-measurement.

By variation of the depolarization parameters (duration and/or frequency), in the majority of myocytes conditions could be found, that resulted in release signals, the rising phase of which was clearly separated from the triggering I_{Ca}. In Fig. 2 membrane current (A, top) and $[Ca^{2+}]_i$ (A, bottom) during a train of depolarizing voltage steps eliciting I_{Ca} have been traced. The first pulse was followed by a large Ca^{2+}-transient and the corresponding inward current, the rising phase of which was fused with I_{Ca} (a). The subsequent voltage steps either failed to elicit release or caused release signals of variable amplitudes and time courses (b–d). The rising phases of the current transients which were clearly separated from I_{Ca}, displayed two components (b,d) particularly prominent in d. A plot of change of current vs $[Ca^{2+}]_i$ for the relaxation of the pair of transients labelled 'a' again yields a straight line between around 100 nM and 1 μM which intersects close to the origin (B,a). Above 1 μM a flattening is observed, possibly due to saturation of the exchanger and/or the

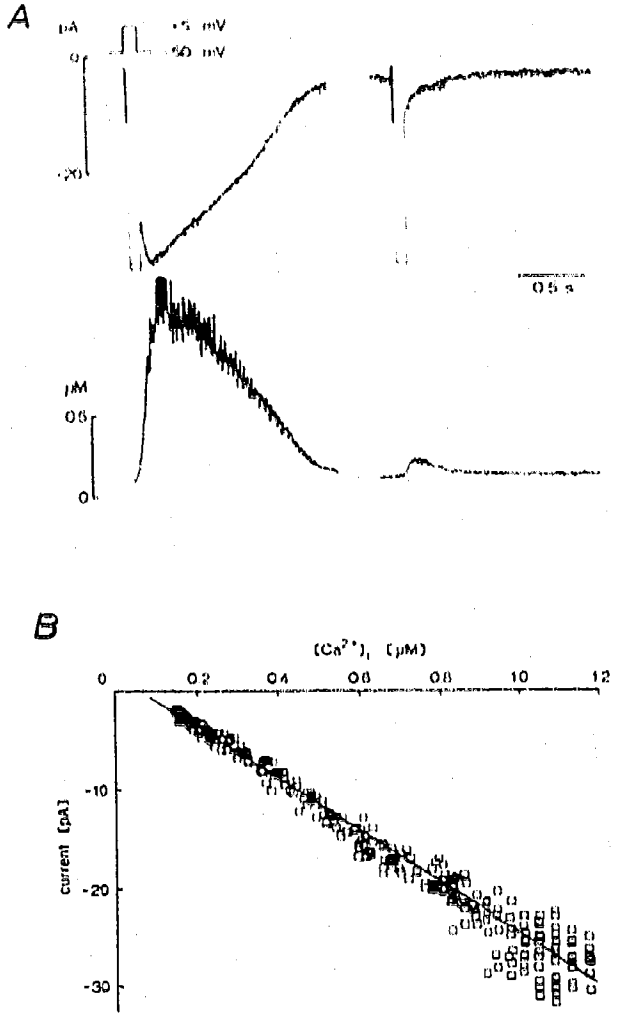

Fig. 1. (A) Correlation between release-dependent inward current and $[Ca^{2+}]_i$. Membrane current (top traces) and intracellular Ca^{2+}-concentration (bottom traces) following activation of I_{Ca} by depolariation from −50 to +5 mV (step duration 50 ms; frequency 0.2 s^{-1}). I_{Ca}-amplitude was constant (240 pA; off scale) throughout a train of 20 pulses. Currents and $[Ca^{2+}]_i$-signals alternated between the two representative responses illustrated. (B) Plot of change of membrane current against $[Ca^{2+}]_i$ for the period of time marked by the arrows in (A). One point per 10 ms from the data-set has been plotted. The straight line was calculated by linear regression for 200 nM $\leq [Ca^{2+}]_i \leq 1$ μM.

Ca^{2+}-indicator which has a K_d for Ca^{2+} in the order of 200 nM [10]. For the falling phase of signal-pairs b and d identical slopes of this relation were determined (open circles in B,b and B,d). If, in the same co-ordinates, the rising phases are plotted (closed symbols), considerable deviations from this relation are observed. A fit of the first 20 data points of set b yields a slope of -4.1×10^{-2} pA/nM as compared to -1.8×10^{-2} pA/nM for the falling phase. An identical line was drawn through the data in d.

For clarity the maxima of the small transient (c) and the first component of d, have been included in graph

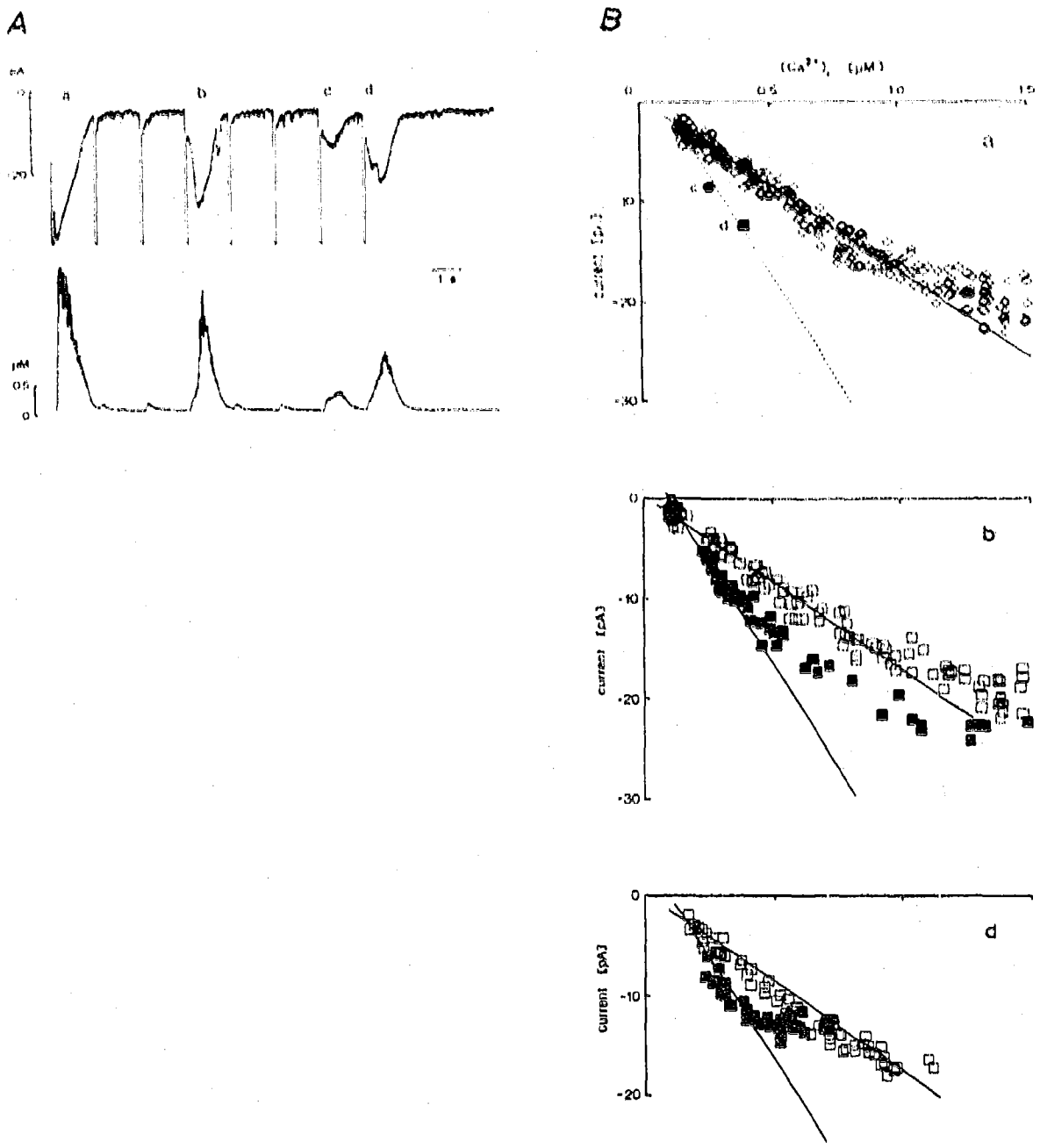

Fig. 2 Two components of Ca^{2+}-release. (A) A train of step depolarizations was applied from −50 to +5 mV (duration 100 ms; frequency 0.7 s^{-1}), resulting in failures and variable release signals (a–c), one with a distinct two-component rising phase (d). The decay phases of signals a, b and d have been analysed using plots of change in current against $[Ca^{2+}]_i$ (open symbols in (B)). A straight line was calculated through the data points of event a for $0.2\,\mu M \leq [Ca^{2+}]_i \leq 1.0\,\mu M$. An identical line was drawn through the sets of data corresponding to event c and d. The rising phases of events b and d have been symbolized by the closed squares in the corresponding graphs. A straight line was calculated through the first 20 pairs of data from b. This line was copied to graphs a and d. In A the peak current of c and of the first component of d are indicated by the closed symbols. (The rapid downward deflection in the current trace b is due to opening of a large-conductance ion channel described previously [26]).

B,a (closed symbols). The same straight line as in graphs b and d can be drawn through these data. Thus, the deviation from the I vs $[Ca^{2+}]_i$ plot of the falling phase is a property of the first component of the release signal, which is represented in isolation by the transient marked 'c'. Furthermore this component is also hidden in the rising phase of Ca^{2+}-transients lacking two clearly identifiable components.

4. DISCUSSION

Our results provide strong evidence that in atrial myocytes the membrane signal (Ca^{2+}-current) is transmitted into an intracellular Ca^{2+}-signal via CICR from superficial stores. The resulting subsarcolemmal Ca^{2+}-wave causes further release from deeper regions of the SR, also via CICR. This process is discontinuous giving rise, at least occasionally, to two distinct components of the exchange current. As compared to physiological Ca^{2+}-transients of atrial cells, which reach a peak within less than 30 ms [21], the signals measured in the present study are considerably slower. This slowing, which is a prerequisite for detecting the phenomena described above, is due to the loading of the cell with a high concentration of a mobile Ca^{2+}-chelator [15,22]. It does not represent an effect of citrate as a chemical. Similar slow transients were obtained upon replacement of citrate by an equivalent concentration of free ATP, which has rather similar Ca^{2+}-chelating properties [15,23].

The observation of two-component release signals is not due to focal Ca^{2+}-release described previously [16,24]. In 32 out of 42 cells that were studied using simultaneous measurement of I_{NaCa} and Indo-1-fluorescence and in 74 out of 92 cells which were investigated by current measurements only, two components could be identified. In none of those myocytes any evidence for more than two components was found. In case of focal inhomogeneities of Ca^{2+}-release, with some statistical probability more than two components should be detected. Spatial gradients with some similarity to those described in the present study for cardiac myocytes are likely to be involved also in other types of excitable and non-excitable cells, that use Ca^{2+}-signalling controlled by both Ca^{2+}-entry and Ca^{2+}-release for regulating physiological processes (see e.g. [25] for review).

REFERENCES

[1] Reiter, M. (1988) Pharmacol. Rev. 40, 189–217.
[2] Nabauer, M., Callewaert, G., Cleemann, L. and Morad, M. (1989) Science 800–803.
[3] Fabiato, A. (1985) J. Gen. Physiol. 85, 247–289.
[4] Beuckelmann, D.J. and Wier, W.G. (1988) J. Physiol. 405, 233–255.
[5] Tanabe, T., Mikami, A., Numa, S. and Beam, K.G. (1990) Nature 344, 451–453.
[6] Johnson, E.A. and Sommer, J.R. (1967) J. Cell Biol. 33, 103–129.
[7] Kimura, J., Noma, A. and Irisawa, H. (1986) Nature 319, 596–597.
[8] Mechmann, S. and Pott, L. (1986) Nature 319, 597–599.
[9] Eisner, D.A. and Lederer, W.J. (1989) in: Sodium-Calcium Exchange (Allen, T.J.A., Noble, D. and Reuter, H. eds) pp. 178–207, Oxford University Press, Oxford.
[10] Grynkiewicz, G., Poenie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
[11] Tsien, R.Y. (1989) Annu. Rev. Neurosci. 12, 227–253.
[12] Bechem, M., Pott, L. and Rennebaum, H. (1983) Eur. J. Cell Biol. 31, 366–369.
[13] Boller, M. and Pott, L. (1989) Pflügers Arch. 415, 276–288.
[14] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
[15] Lipp, P. and Pott, L. (1990) Circ. Res. (submitted)
[16] Berlin, J.R., Cannell, M.B. and Lederer, W.J. (1989) Circ. Res. 65, 115–126.
[17] Lipp, P., Mechmann, S. and Pott, L. (1987) Pflügers Arch. 410, 121–131.
[18] Egan, T.M., Noble, D., Noble, S.J., Powell, T., Spindler, A.J. and Twist, V.W. (1989) J. Physiol. 411, 639–661.
[19] Barcenas-Ruiz, L., Beuckelmann, D.J. and Wier, W.G. (1987) Science 238, 1720–1722.
[20] Miura, Y. and Kimura, J. (1989) J. Gen. Physiol. 93, 1129–1145.
[21] Bals, S., Bechem, M., Paffhausen, W. and Pott, L. (1990) Cell Calcium 11, 385–396.
[22] Lipp, P. and Pott, L. (1988) J. Physiol. 397, 601–630.
[23] Pott, L. and Lipp, P. (1990) J. Physiol. (abstr.)
[24] Wier, W.G., Cannell, M.B., Berlin, J.R., Marban, E. and Lederer, W.J. (1987) Science 235, 325–328.
[25] Berridge, M.J., Cobbold, P.H. and Cuthbertson, K.S.R. (1988) Phil. Trans. R. Soc. Lond. B 320, 325–343.
[26] Pott, L. and Mechmann, S. (1990) J. Membr. Biol. 117, 189–199.