J. Membrane Biol. 117, 189–199 (1990)

The Journal of
Membrane Biology

Large-Conductance Ion Channel Measured by Whole-Cell Voltage Clamp in Single Cardiac Cells: Modulation by β-Adrenergic Stimulation and Inhibition by Octanol

L. Pott and S. Mechmann
Institut für Zellphysiologie, Ruhr-Universität Bochum, D-4630 Bochum, Federal Republic of Germany

Summary. Membrane currents in single cardiac myocytes from adult guinea pigs were studied by means of the patch-clamp technique (whole-cell mode). During spontaneous or caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum openings of a novel ion channel with large unitary conductance (280 pS) can be recorded. The density of these channels and/or its open-state probability are unusually low. On average in the whole-cell mode simultaneous maximum superposition of only four channels is observed. Opening events of this channel require an intracellular Ca^{2+} transient. Activation by $[Ca^{2+}]_i$, however. seems to be indirect; maximum opening activity occurs with a delay of several hundred milliseconds after peak $[Ca^{2+}]_i$. Single-channel activity can be enhanced by a cyclic AMP dependent process via β-adrenergic stimulation of a cell. This can also be mimicked by caffeine, most likely via inhibition of phosphodiesterase. Octanol, an inhibitor of gap-junctional coupling in a variety of tissues, causes a concentration-dependent and reversible decrease in single-channel activity. Unitary conductance is not affected by octanol. The low density of these channels in cardiac membranes and their poor selectivity render any role in normal cardiac electrical activity unlikely. A possible relation of the channel to cardiac gap junctions is discussed.

Key Words cardiac myocytes · ion channel · gap junction · Ca-activated ion channel · maxichannel · β-adrenoceptor

Introduction

Cardiac cells possess a large number of different ion channels which contribute to the action potential, are involved in the control of contraction, serve to initiate or modulate spontaneous activity or have not yet defined functions (for review, *see* Noble, 1984; DiFrancesco & Noble, 1985; Gintant & Cohen, 1989). Furthermore, each cardiac cell is coupled to its neighbors via gap junctions containing cell-cell channels (*see* Page & Manjunath, 1986, for further literature).

Transitions between open and closed states of ion channels in cell membranes can be measured by means of the patch-clamp technique, which permits isolation of a small area of membrane containing a small number, ideally one, of channels from the usually large population of channels present in a cell (Hamill et al., 1981). In the whole-cell modification of the patch-clamp technique, on the other hand, macroscopic membrane currents representing the average properties of a large number of channels of one particular type can be measured.

In the present investigation openings of a novel large-conductance ion channel (LCC) in the membrane of cardiac myocytes have been identified. Single-channel open events have only been detected in the whole-cell configuration but not in membrane patches. The channel, having a unitary conductance of 280 pS in the present conditions, could be modulated by conditions causing a rise in intracellular cAMP, and it was inhibited by octanol ($\geq 10^{-5}$ M). A possible relation of this channel to gap junction hemichannels will be discussed. A preliminary report of this study has been published in abstract form (Mechmann & Pott, 1989).

Materials and Methods

Isolated Myocytes

The method of cell isolation and the conditions for long-term culture of atrial myocytes from hearts of adult guinea pigs have been described in detail previously (Bechem, Pott & Rennebaum, 1983). Briefly, hearts from guinea pigs of either sex weighing 200–300 g were perfused under sterile conditions in a laminar flow cabinet with a Ca^{2+}-free (0.1 mM EGTA) modified Tyrodes's solution for 5 min. This was followed by perfusion with a solution containing collagenase (1 mg/ml; Worthington CLS II) and elastase (Suspension Serva; 10 μl/ml) for 20–40 min. Atrial and ventricular tissue were separately dispersed and carefully washed with culture medium (M 199, Gibco or BM-86 Wissler, Boehringer Mannheim, both buffered with 20 mM HEPES to pH 7.2–7.4) supplemented with FCS (1%, Gibco) and gentamycin (25 μg/ml,

Gibco). The cells were transferred to plastic tissue culture dishes (35-mm diameter, Falcon) at a density of a few thousand cells per dish. The cultures were stored in an incubator at 37°C, 90% humidity and 1% CO_2. For the experiments atrial myocytes were used from day 0 to day 10 after isolation; their membrane capacitance ranged from 15 to 25 pF.

Solutions

For the measurements the culture medium was replaced by a solution containing (in mM): NaCl 140, CsCl 2.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, HEPES/CsOH 10.0, pH 7.4.

The pipette filling solution (ICS) for intracellular dialysis contained (in mM): Cs_3citrate 65, CsCl 10.0, NaCl 10.0, $MgCl_2$ 1.0, HEPES/CsOH 10, pH 7.4. This solution was supplemented with MgATP (4 mM) and EGTA as indicated for the individual experiments (*compare* Bechem & Pott, 1985). After replacement of the culture medium by the solution of the composition listed above, the volume of the dish was reduced by a Perspex ring, fitted into the dish, to which thin plastic tubes for inflow and suction of solution were attached. The effective volume of the chamber surrounded by this device was about 0.5 ml. This chamber was continuously perfused by gravity at a rate of approximately 2 ml · min^{-1}.

Current Measurement

Membrane currents were measured under voltage clamp by means of patch-clamp pipettes (whole-cell mode; Hamill et al., 1981). Patch-clamp pipettes were fabricated from Pyrex glass (o.d., 1.5 mm, i.d., 1.0 mm) and were filled with ICS of the above composition. The DC resistance of the filled pipettes ranged from 2 to 6 MΩ. Voltage and current measurements were performed by means of a patch-clamp amplifier (List LM/EPC 7). Signals were stored on analog tape (Racall 4DS), and later analyzed by means of an IBM-compatible AT computer equipped with an AD/DA-board (Data-Translation 2821). Experiments were performed at ambient temperature (21–23°C).

Results

Activation of Ion Channels by Calcium Release

Upon dialysis with the pipette solution listed above most of the myocytes developed spontaneous transient inward currents (I_{TI}) at constant negative holding potentials. It has been shown previously that this type of transient inward current is caused by cyclic Ca^{2+} release from the SR, the charge carrying mechanism being Na^+Ca^{2+} exchange (Mechmann & Pott, 1986; Lipp & Pott, 1988*a,b*). In most of the cells studied superimposed on the spontaneous transient inward current was sporadic activity of an ionic

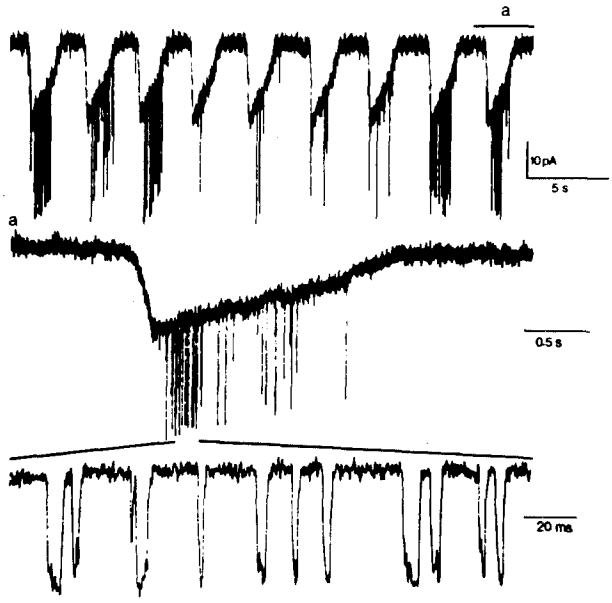

Fig. 1. Spontaneous cyclic Ca^{2+} release causes Na^+-Ca^{2+} exchange current and openings of a large-conductance ion channel. Atrial myocyte dialyzed for 6 min with ICS of the composition listed in Materials and Methods. Holding potential was −60 mV throughout. The middle trace marked *a* corresponds to the period of time marked by the horizontal line in the top trace

channel, as illustrated in Fig. 1. Although openings of this channel could be detected in the majority of myocytes displaying spontaneous transient inward currents, they were very infrequent events. During the period of time of 30 sec, represented by the trace in Fig. 1, only about 150 opening events could be identified. Furthermore, in this cell no superpositions of two or more channel openings were detected.

As has been shown previously, (Mechmann & Pott, 1986; Lipp, Mechmann & Pott, 1987) higher and more sustained activity of LCC can be observed following caffeine-induced Ca^{2+} release.

Figure 2 shows a representative current recording illustrating the effect of fast application of caffeine. During spontaneously occurring I_{TI} few openings could be detected. Superfusion of the cell with caffeine-containing (10 mM) solution caused an inward current of similar peak amplitude as spontaneous I_{TI}. At this concentration caffeine causes release of Ca^{2+} from the SR-stores (Weber & Herz, 1968) and prevents re-uptake, most likely by keeping the release channels in an active state (Rousseau & Meissner, 1989). A much higher activity of LCC was observed in the presence of caffeine. As channel activity was only observed following Ca^{2+} release from the SR, Ca^{2+} is likely to be involved in channel gating. The action of $[Ca^{2+}]_i$ on channel activity, however, is slow. The expanded trace in Fig. 2

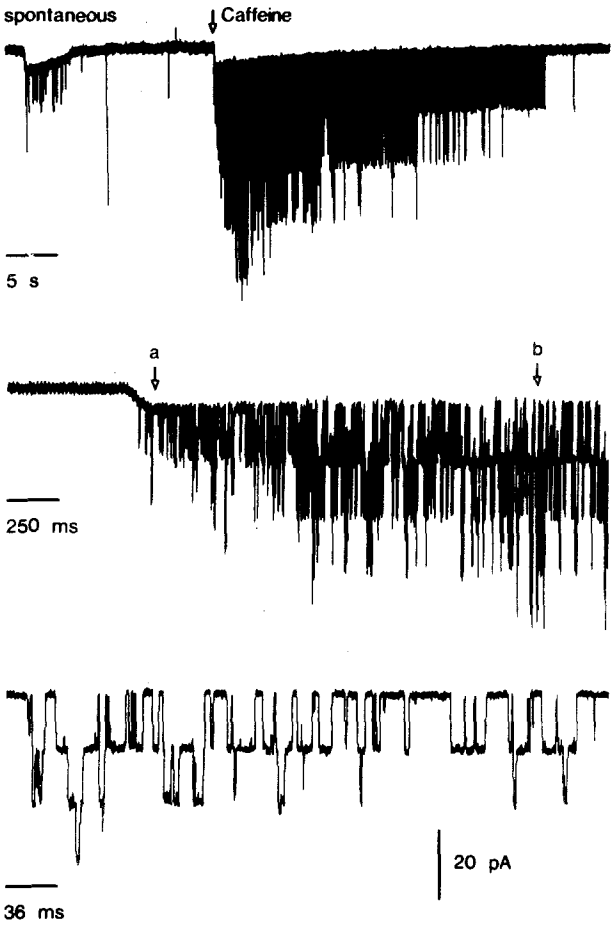

Fig. 2. Channel activity evoked by caffeine-induced Ca^{2+} release from the sarcoplasmic reticulum. Membrane potential was held at −50 mV throughout. Caffeine-containing solution (10 mM) was applied by pressure ejection from a micropipette located in a distance of around 20 μm from the cell. The arrows labeled *a* and *b* in the middle trace mark the peak of Ca^{2+}-release-dependent inward current, *a*, and maximum of channel activity, *b*

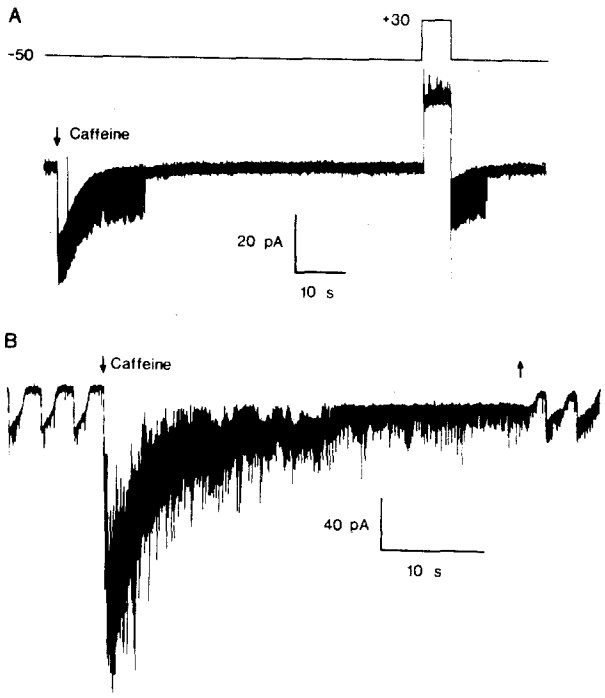

Fig. 3. Variability of channel activity in different myocytes. Caffeine was applied as described to two different myocytes. (*A*) Apparent transient activation of a single channel (no superpositions of channel openings are detectable). After 20 sec in the presence of caffeine, channel activity ceased. In the presence of caffeine brief periods of opening activity can be induced by strong depolarizations (e.g., 5 sec to +30 mV). (*B*) Transient simultaneous activation of at least 20 channels. Caffeine-containing solution (10 mM) was superfused during the period of time between the two arrows. Note spontaneous inward currents and channel activity before and after superfusion with caffeine

clearly shows that the maximum of channel activity lagged behind the peak of the transient inward current by about 2 sec. The latter has been shown under very similar conditions to be linearly and instantaneously related to $[Ca^{2+}]_i$ (Beuckelmann, Pott & Wier, 1989); thus, peak I_{ti} corresponds to peak $[Ca^{2+}]_i$. A delay between peak $[Ca^{2+}]_i$ and maximum activity was a consistent finding (*see also* Figs. 1 and 4) and suggests Ca^{2+}-dependent slow reaction(s) and not just Ca^{2+}-binding to an activation site to be involved in activating channel gating. In this cell the caffeine-evoked Ca^{2+} release caused a maximum of four simultaneous openings, which was the average maximum figure from more than 200 atrial myocytes. Note however, that in the continuous presence of caffeine channel activity slowly decayed. Sometimes LCC activity ceased after some tens of seconds, whereas in the majority of cells activity of an apparently small number of channels can be recorded for 10–20 min.

The variability of channel activity in individual myocytes is illustrated in Fig. 3, which shows membrane currents from two different cells as affected by fast application of caffeine-containing solution (10 mM). Whereas in the cell in panel *A* only one open-channel current level was observed in the presence of caffeine (superimposed on inward I_{NaCa}), at least 20 channels were transiently active in panel *B*. In *A* a strong depolarization (5 sec to +30 mV) in the presence of caffeine resulted in a transient reactivation of opening activity. This is likely to reflect the additional Ca^{2+}-load via reverse mode Na^+-Ca^{2+} exchange (Lipp & Pott, 1988*b*). A direct effect of the depolarization as such, however, at present cannot be excluded. The examples traced in Fig. 3 represent two extremes. The behavior found more commonly was depicted in Fig. 2. In addition to the variability observed among different cells,

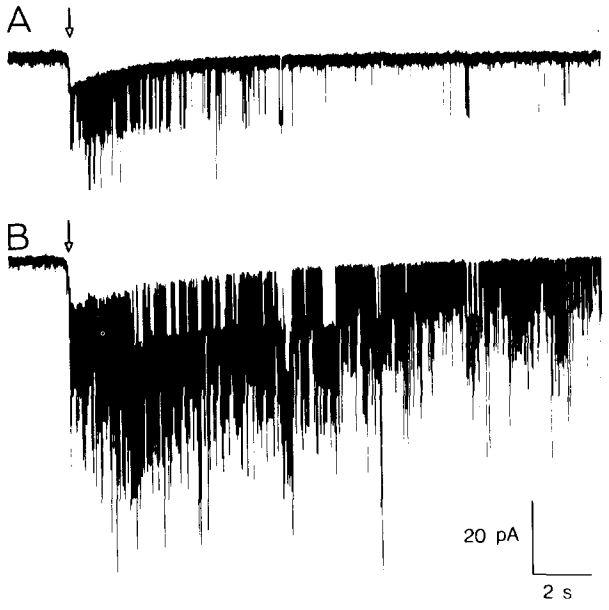

Fig. 4. Variability of channel activity in a single myocyte. Two superfusions with caffeine-containing solution at $t = 6$ min (*A*) and $t = 12$ min (*B*) after getting access to the inside of the cell. The caffeine-containing pipette was in identical positions for both traces, as was the applied pressure for ejecting solution

repeated application of caffeine in the same cell often resulted in very different levels of LCC activity. An example is illustrated in Fig. 4. The first superfusion with caffeine resulted in maximum superposition of three channels. After a period of 5 min in caffeine-free solution a second challenge with this drug, using an identical position of the flushing pipette and identical flow rate, resulted in much higher activity. Up to five channels are simultaneously open during peak of activity. The channel current integrated for a period of time between 1.5 and 2.5 sec after $t = 0$ (arrows) is 12-fold higher for trace *B* as compared to *A*. Such a variability was a consistent finding. It was unpredictable, however, whether activity during the second caffeine-induced Ca^{2+}-release was higher than during the first one or vice versa. The sample traces shown in Figs. 3 and 4 demonstrate the great variability of LCC activity which impairs study of kinetic aspects of this channel.

In agreement with a previous study (Lipp et al., 1987) the conductance of the channel in the present conditions was determined as 284 ± 34 pS ($n = 12$, *see also* Fig. 5*D*). In a range of membrane potentials between −100 and +80 mV the open-channel *I/V* relation was linear. The reversal potential was determined as +13 ± 5 mV. The selectivity of the channel for monovalent cations was rather poor. A detailed analysis of the selectivity properties of this channel will be presented in a separate paper (S. Mechmann & L. Pott, *in preparation*).

Apart from the 280 pS main conductance, the channel exhibited smaller conductance levels in most experiments. The sublevel most frequently detected, had 50% of the main conductance (Fig. 5). The 50% level was identified in virtually all experiments, its probability amounting to about 5% of the main level. A plot of both the current carried by the fully open channel and the 50% state, respectively, yields almost identical reversal potentials of 17 and 15 mV, respectively (Fig. 5*C*). A 25% level was detected in only about 10% of the current recordings. A sample trace is shown in Fig. 6. Its overall probability was less than 1% of the fully open level. Apart from these subconductance levels occasionally transitions to conductance states other than 50 and 25% were observed. These, however, were observed too seldom to permit a clear identification as sublevels of LCC, or a different type of channel, respectively.

The facts that small numbers of active channels were reproducibly recorded in the whole-cell mode and that long-lasting elevations of $[Ca^{2+}]_i$, much longer than a physiological Ca^{2+} transient, were necessary to observe these single-channel current events, suggest a contribution to cardiac excitation to be unlikely. A contribution to $[Ca^{2+}]_i$-release-dependent transient inward current can also be excluded. This current can be recorded also without LCC activity superimposed. Note that LCC unitary conductance differs by about one order of magnitude from the $[Ca^{2+}]_i$-activated unselective cation channel (Colquhoun et al., 1981; Ehara, Noma & Ono, 1988), which is being discussed in the context of contributing to arrhythmogenic transient inward current.

Modulation of Channel Activity by β-Adrenergic Stimulation

The tool used in the present investigation to reproducibly induce measurable activity of LCC was caffeine. Apart from its action on sarcoplasmic reticulum release channel (Rousseau & Meissner, 1989) this substance, like other methylxanthines, acts as an inhibitor of phosphodiesterase and, therefore, may stimulate the cAMP-dependent regulatory pathway. In order to investigate whether such an effect is involved in activation or modulation, respectively, of LCC, we evaluated channel current following administration of caffeine by integrating channel current during appropriate periods of time. The time-dependent change of channel

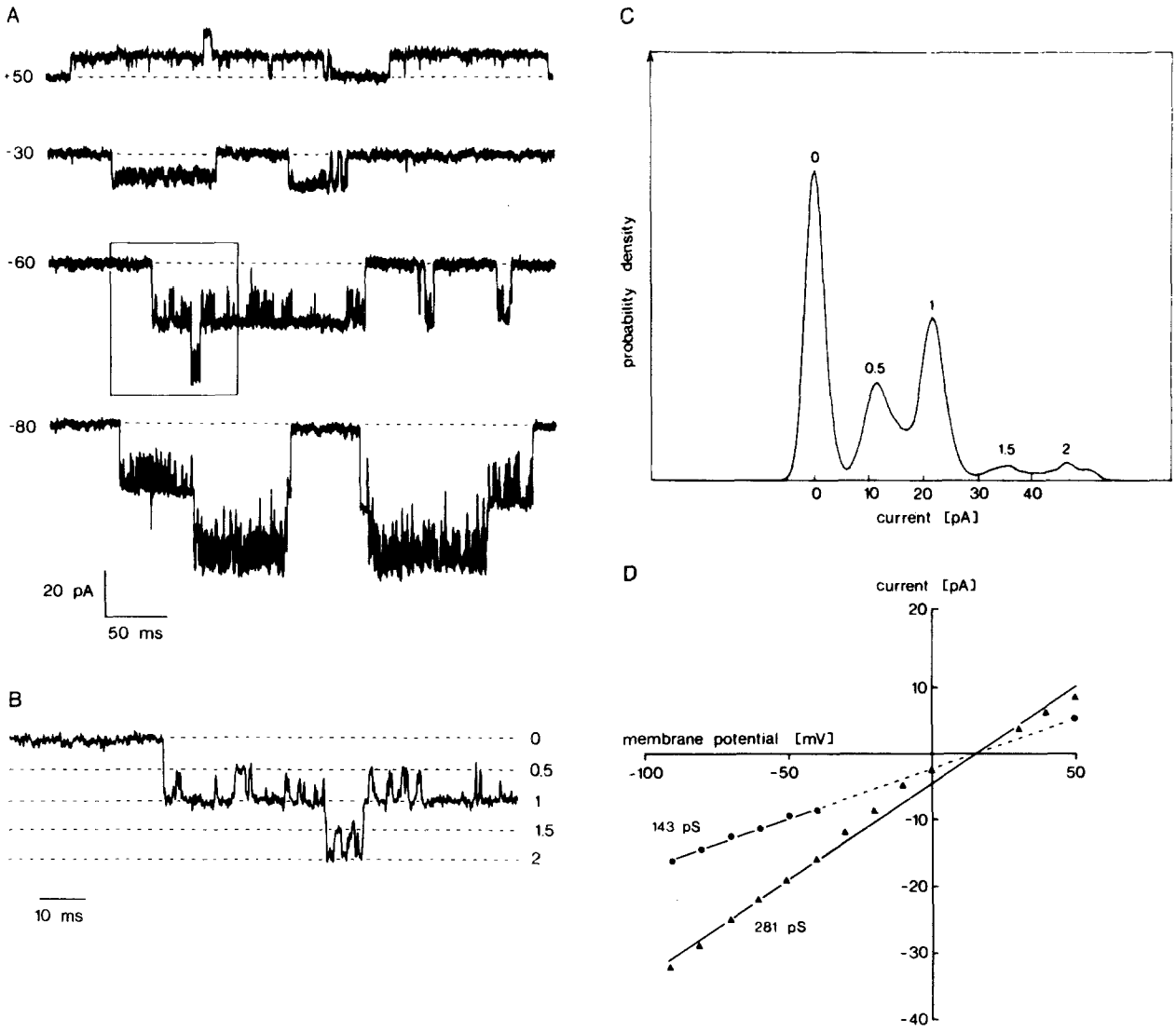

Fig. 5. 50% subconductance of LCC. (*A*) Sample traces of channel openings at the membrane potentials indicated. (*B*) Expanded section corresponding to the box in *A*. (*C*) Amplitude histogram generated from a trace of 2 sec in duration (−60 mV). (*D*) Plots of current against membrane potential for 100% conductance state (▲) and 50% sublevel (●). Plotted data were derived from maxima of amplitude histograms. The straight lines are least-square fits of the data points

current was compared to the increase of the amplitude of L-type Ca^{2+} current (I_{Ca}), which can be used as a qualitative monitor for an increase of intracellular cAMP concentration (e.g., Trautwein et al., 1986). A representative experiment is illustrated in Fig. 7. I_{Ca} was elicited by stepping from the holding potential (−50 mV) to +10 mV (40 msec; 0.1 sec^{-1}). I_C, the channel current, was integrated over periods of time of 30 sec. About 30 sec after superfusion with caffeine-containing solution was started, I_{Ca} began to increase from 45 to 150 pA within about $2\frac{1}{2}$ min. This increase of I_{Ca} is paralleled by an increase of I_C from 0.6 to 2.3 pA during the first two 30-sec periods reaching a level of 3.3 pA after 4 min. As shown in Figs. 2 and 3 this result, however, is not representative. In the majority of experiments I_C reached a maximum briefly after a caffeine challenge and then decayed or even ceased after a few minutes. These diverging results are possibly due to the fact that $[Ca^{2+}]_i$, the primary activator of LCC, reaches a maximum briefly after SR Ca^{2+} release and then decays to a level which might be different in different myocytes, whereas intracellular cAMP upon inhibition of phosphodiesterase, slowly rises to an equilibrium concentration which is reached

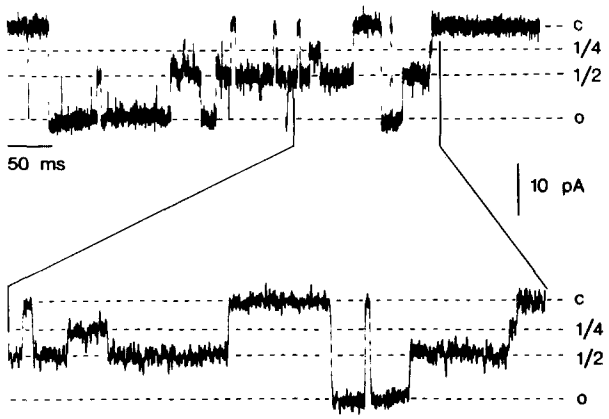

Fig. 6. Sample trace showing simultaneous occurrence of 50 and 25% subconductance levels (holding potential −55 mV). *c* and *o* denote fully closed and fully open states

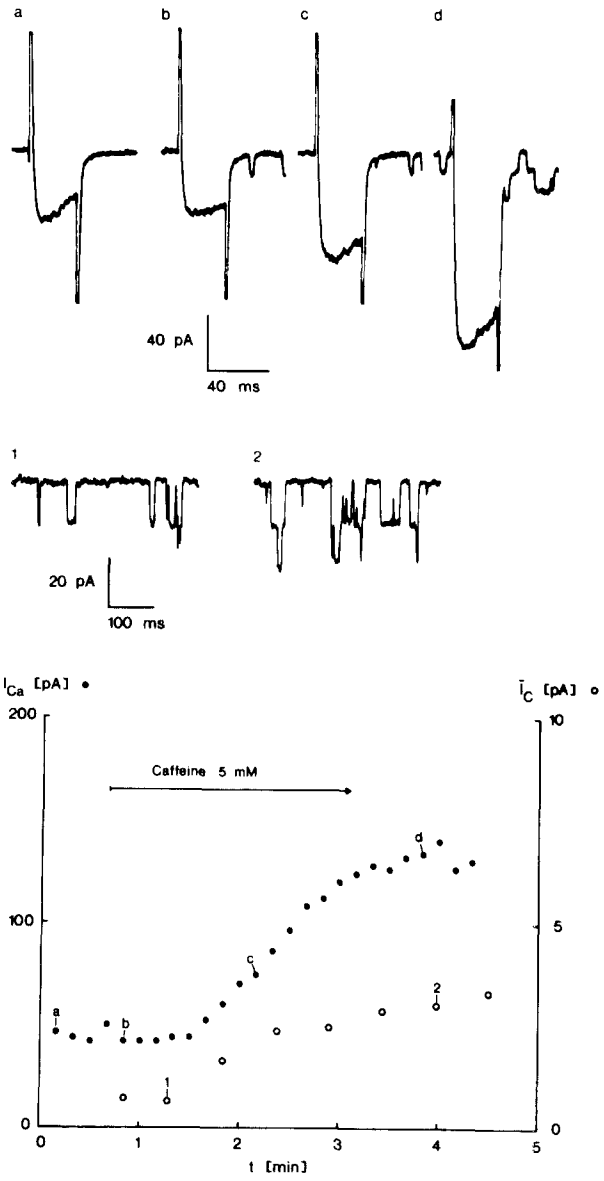

Fig. 7. Plot of Ca^{2+} current evoked by step depolarizations from holding potential of −50 to +10 mV (I_{Ca}, *o*, left ordinate) and integrated channel current (I_C, *o*, right ordinate) against time (t = 0 is arbitrary and corresponds to about 5 min after establishing the whole-cell recording configuration). Perfusion of the cell with caffeine-containing solution is indicated by the horizontal bar. The data points labeled *a–d* and *1* and *2* correspond to the current traces

only after several minutes. The superposition of the variable time courses of the two putative factors affecting LCC activity might cause differences in the overall time course of LCC activity.

Additional stimulation of the cAMP-dependent regulatory pathway by isoprenaline in 8 out of 20 experiments caused an increase in channel activity which was closely related in time to the addition of the β-receptor agonist. One example is illustrated in Fig. 8. LCC activity was evoked by superfusion with caffeine at $t = -2$ min. Activity, assayed by integrating periods of 2 sec was fairly constant for about 5 sec. Superfusion with caffeine plus isoprenaline (5×10^{-7} M) starting at $t = 6$ sec results in an increase of mean channel activity by about 400% within 6 sec. No depolarizing clamp steps were applied in this experiment in order to exclude the possibility that increased $[Ca^{2+}]$-entry via L-type channels contributes to the increase in LCC activity.

Cyclic AMP dependent protein kinase has a number of different target proteins at various sites of cardiac myocytes (for review, *see* Robinson-Steiner & Corbin, 1986). In the sarcolemma, apart from the well-known role of this enzyme in regulating L-type I_{Ca} (e.g., Trautwein et al., 1986), modulation of cell-to-cell coupling following β-adrenergic stimulation or injection of cAMP, respectively, have been documented (e.g., DeMello, 1986). Although single-channel measurements in cell pairs have provided information on the properties of the channel connecting two cells (*see* Discussion for further literature) it is not known at present whether or not putative hemichannels (connexons) are able to open under certain conditions. Nor is it known what exactly happens during decoupling or recoupling of cells or during modulation of coupling by cAMP-dependent kinase.

Inhibition of Channel Activity by Octanol

In order to test the working hypothesis of LCC representing opening activity of gap-junctional hemichannels, the effect of octanol was studied. It has

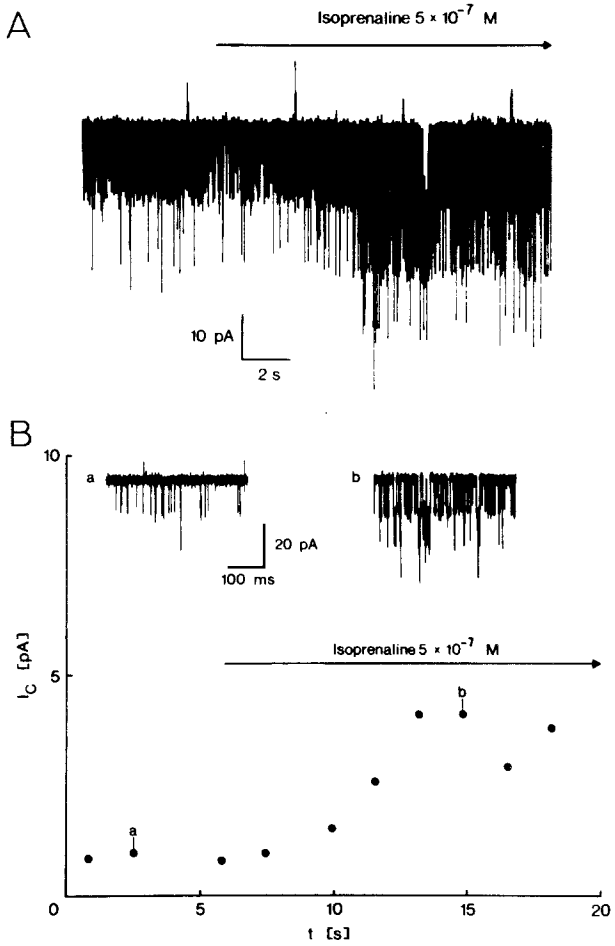

Fig. 8. Effect of isoprenaline (5×10^{-7} M) on channel activity. (*A*) Low speed recording of membrane current of a myocyte held at −50 mV. The recording starts 2 min after switching to caffeine-containing solution (10 mM, bath perfusion). (*B*) Integrated channel activity (from *A*). Channel activity was integrated over periods of 1 sec; sections containing artifacts (e.g., upward deflections in top trace) were discarded

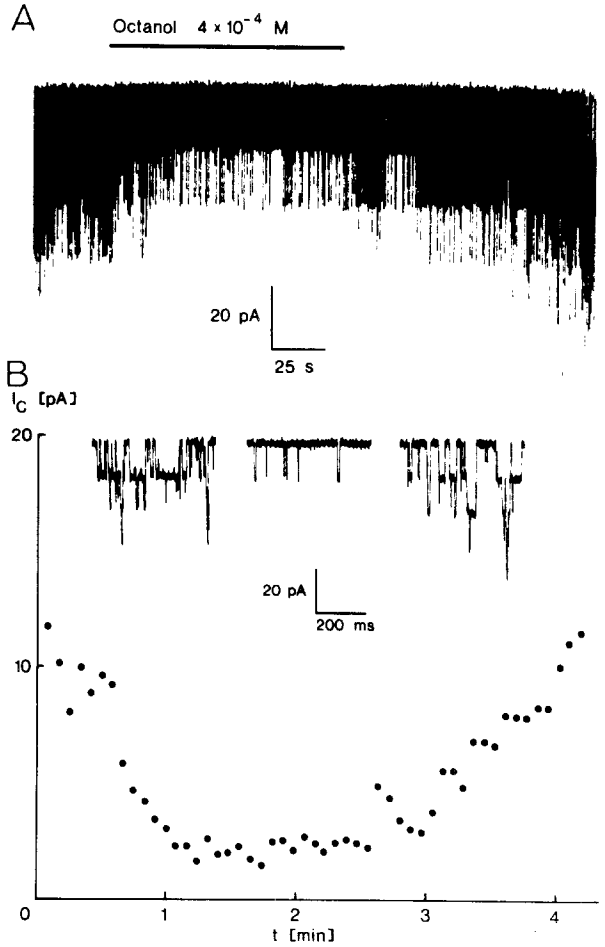

Fig. 9. Reduction of channel activity by octanol. (*A*) LCC openings were evoked by bath perfusion with caffeine-containing solution (starting 2 min before the beginning of the displayed trace). Octanol was applied from a micropipette containing both the alcohol and 10 mM caffeine. (*B*) Integrated channel activity (5 sec per point) from *A*. The insets are Sample traces before, during and after application of the alcohol. Holding potential was −50 mV

been shown previously that long-chain alcohols such as octanol or heptanol cause decoupling in a variety of cell types (Délèze & Hervé, 1983; Niggli et al., 1989). The mechanism of this decoupling, however, is not well understood. Figure 9 illustrates that superfusion of a cell with octanol (4×10^{-4} M), in the presence of caffeine, causes a reduction of LCC activity to about 10% of the control level. The action, which is a reduction of the open-state probability, is completely reversible. The step size of the current events remains unaffected. This is confirmed by analyzing the effect of OCT at different membrane potentials (Fig. 10). The slope conductance between −80 and +20 mV of 281 pS is the same with and without the alcohol. Thus, OCT somehow affects gating of LCC rather than its conductance properties. The concentration-response curve for this effect of OCT which is shown in Fig. 11, can be approximated by a simple binding curve with half-maximum inhibition at 4.5×10^{-5} M. These results might indicate some relation of LCC to cardiac gap-junctional channels. The action of OCT, however, is not at all specific. As depicted in Fig. 12 the amplitude of L-type I_{Ca} evoked by stepping from −50 to 0 mV is reversibly reduced to 20% by superfusion with a solution containing OCT (4×10^{-4} M). Furthermore the fast TTX-sensitive Na current evoked by stepping from −70 to −40 mV was also reduced by OCT in that range of concentrations (*not shown, compare* Hirche, 1985).

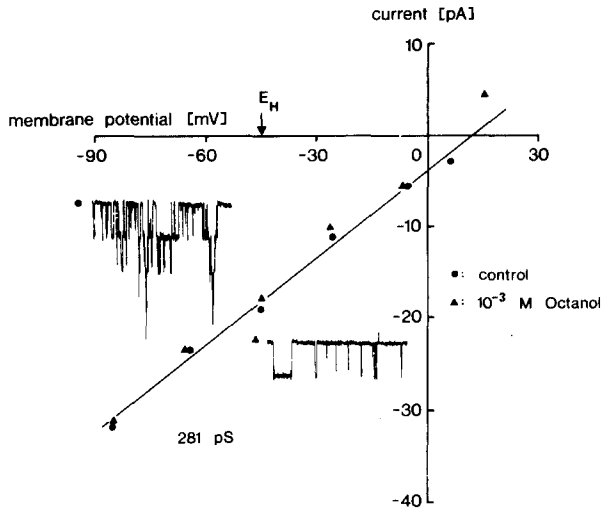

Fig. 10. Lack of effect of octanol on channel conductance. Channel current amplitude in the absence (●) and in the presence (▲) of octanol (10^{-3} M) has been plotted against membrane potential. The straight line represents a linear regression of the control data (281 pS; E_{rev} = 12 mV). The sample traces were recorded at −45 mV

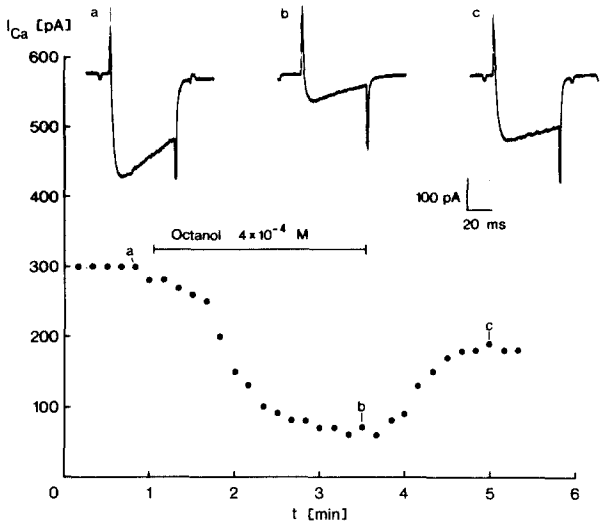

Fig. 12. Effect of octanol on L-type Ca^{2+} current. Peak I_{Ca} has been plotted against time. I_{Ca} was elicited by voltage steps from −50 to 0 mV (duration 50 msec; frequency 0.1 sec^{-1}). Caffeine was superfused starting 2 min before the beginning of the plot. Octanol (4×10^{-4} M) was superfused during the time illustrated by the horizontal bar

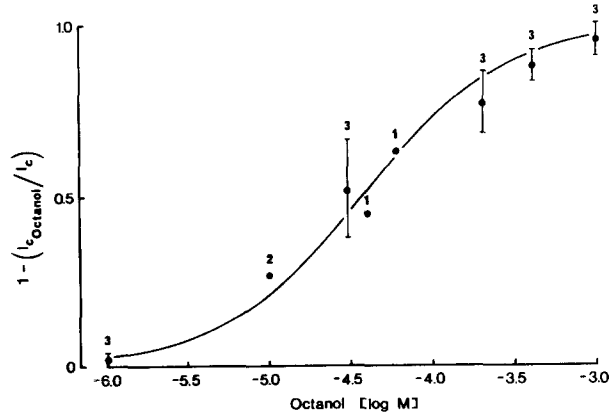

Fig. 11. Concentration-response curve of the inhibitory action of octanol on LCC. Inhibition expressed as $1-(I_{Coctanol}/I_C)$, with $I_{Coctanol}$ denoting the integrated channel current in the presence of octanol, and I_C the integrated channel current before application of the alcohol. I_C was determined during a 30-sec period before superfusion with the OCT-containing solution. $I_{Coctanol}$ was determined during a period of 30 sec when the action of the alcohol appeared to be in a steady state (*compare* Fig. 9). Holding potential was either −45 or −50 mV. The data points represent single measurements, mean values, or mean values ± SEM, respectively. The numbers of different cells are indicated. The curve was calculated using simple saturation kinetics with half-maximum inhibition at 4.5×10^{-5} M

Discussion

The major findings of the present investigation can be summarized as follows: A novel ion channel with unusually large conductance can be measured in isolated cardiac myocytes in the whole-cell mode of the patch-clamp technique, but not in isolated membrane patches. Each cell seems to possess only a small number of these channels. The channel is activated by a rise in $[Ca^{2+}]_i$ in an indirect way. Its activity can be modulated by β-adrenergic stimulation, and it is inhibited by octanol in the external solution.

In the present investigation a citrate-based solution for internal dialysis of the cells was used. Dialysis with such a solution has been shown to provide a condition for measuring stable Ca^{2+}-release-dependent inward current (Mechmann & Pott, 1986; Lipp et al., 1987; Lipp & Pott, 1988*a,b*; Beuckelmann et al., 1989; Boller & Pott, 1989). Furthermore, long-lasting activity of LCC hitherto has only been described under this condition. Observations of LCC opening events, however, are not limited to myocytes dialyzed with such a solution. We have detected activity of an ion channel in myocytes dialyzed with Cs-aspartate solution without citrate following caffeine-induced Ca^{2+} release (*not shown*). Furthermore in a previous publication from a different laboratory single-channel activity in whole-cell clamped cardiac myocytes resembling LCC activity has been detected (Callewaert, Cleemann & Morad,

1989). This safely excludes that channel activity of the type described here represents an effect of citrate as such.

Apart from the studies cited above (*see also* Penner, Matthews & Neher, 1988) resolution of discrete single-channel current events in the whole-cell patch-clamp configuration is unusual. It requires an extremely low density of the particular species of channel in the membrane and/or a very low probability of opening. In the present study cells with a capacity of the surface membrane in the order of 20 pF were used. Assuming a specific capacitance of 1 $\mu F \cdot cm^{-2}$, this corresponds to a membrane area of 2000 μm^2. On average, maximum superposition of five simultaneous openings was found. Although we have no data on open-state probabilities of the single channel, these figures suggest an extremely low density of LCC in the cardiac sarcolemma (*compare,* e.g., 3–5 μm^{-2} for Ca^{2+} channels in ventricular myocytes; Pelzer et al., 1986).

As an alternative the possibility has to be considered that LCC activity is particularly low in the conditions of the present investigation. In that case, however, any other experimental condition used in the past in patch-clamp and conventional voltage-clamp studies on isolated cardiac cells should be even less favorable for activating LCC. Small numbers of channels should have been detected as single-channel events, larger numbers as corresponding contamination of other membrane current systems studied. (Note that simultaneous opening of only 100 channels—still a small number—at a holding potential of −50 mV, which is often used, e.g., for investigations of Ca^{2+} currents, would contribute a current on the order of 2 nA in magnitude. Such a linear leak-current would completely dominate the *I/V* characteristics of a cardiac cell).

In cardiac myocytes isolated enzymatically from different tissues of various mammalian and amphibian species, a large number of different ion channels with known or in some cases unknown function have been identified in recent years. In the context of the present investigation two types of ion channels have to be considered: an unselective cation channel, first described in cultured neonatal rat myocytes (Colquhoun et al., 1981) and, more recently also in adult ventricular cells (Ehara et al., 1988). The unitary conductance of this channel has been determined as 30–40 pS by Colquhoun et al. (1981) in neonatal myocytes and as about 15 pS under somewhat different conditions in adult ventricular cells by Ehara et al. (1988). Thus, the conductance of that type of channel is about one order of magnitude less as compared to LCC. Furthermore, those channels could be reproducibly detected in isolated membrane patches, which suggests their density to be in the region of 1 μm^{-1}. These two properties render any relation of LCC to the previously described unselective cation channel very unlikely.

Kameyama et al. (1984) reported the existence of a large-conductance K-selective channel in ventricular myocytes which is activated by high intracellular Na^+ concentrations which are hardly reached under any physiological or pathophysiological condition. Half-maximum activation occurred at around 60 mM $[Na^+]_i$. This channel, the function of which is not at all understood, is completely insensitive to changes of $[Ca^{2+}]$ at the cytosolic face of the membrane. Thus, despite the very similar unitary conductance, an identity with LCC can be excluded.

Taking into consideration the properties of this channel, a physiological role during the cardiac cycle would be difficult to explain. The inhibitory effect of octanol suggests LCC to be related to the gap-junctional channel which under physiological conditions does not exist as a *trans*-membrane channel connecting the cytoplasm and the extracellular space but as a dimeric channel connecting the cytoplasm of two adjacent myocytes. Ultrastructural work has shown that enzymatic isolation goes along with internalization of the gap junctions (Mazet, Wittenberg & Spray, 1985). On the other hand a certain number of preformed connexons is likely to exist in the surface membrane of single cells. At least in neonatal rat myocytes electrical coupling takes place within a few minutes if two cells are brought into close contact (Rook, Jongsma & van Ginneken, 1988). This can hardly be explained by *de novo* synthesis of the channel-forming protein. The questions, however, arise (i) whether or not the hemichannel can show gating and (ii) whether this can be provoked by a rise in free $[Ca^{2+}]_i$. The latter seems to be paradoxical, since it has been demonstrated previously that Ca^{2+} overload causes decoupling of cardiac cells (e.g., Dahl & Isenberg, 1980; DeMello, 1986; Maurer & Weingart, 1987). One of the primary events causing decoupling seems to be a change of the geometry of the two hemichannels involved (Unwin, Zampighi, 1980).The latter authors have shown, using high-resolution electron microscopy that the change of geometry can be described by a slight rotation of the tilted six subunits forming a connexon. In this or any similar model decoupling due to a rise in free $[Ca^{2+}]_i$ goes along with a strong conformational change of the two connexons, brought about by the decoupling agent. It is conceivable at least that Ca^{2+} binding to a single (closed) connexon, with a low probability causes transitions to a 'coupled', i.e., open conformation.

The large conductance of about 280 pS would be compatible with single-channel measurements of cell-to-cell channels in various types of paired cells

by means of double patch clamp: 120 pS in rat lacrimal gland by Neyton and Trautmann, 1985; 165 pS in chicken ventricular myocytes by Veenstra and DeHaan, 1986; and 130 pS in mouse pancreas by Somogyi and Kolb, 1989. Hepatocyte gap junctions reconstituted into lipid bilayers had a conductance of 150 pS (Spray et al., 1986). The figures quoted above were the main conductance states. Smaller sublevels were found in most of those studies. If the cell-to-cell channel represented two hemichannels forming aqueous pores in series, the conductance of the latter should be larger by a factor of two. A very recent publication on paired guinea pig ventricular myocytes, using decoupling by means of heptanol, clearly demonstrated a major conductance around 37 pS of the cell-to-cell channel (Rüdisüli & Weingart, 1989). In cell pairs from neonatal rat heart the larger conductance state was found to be very similar (43 pS, Rook et al, 1988; Rook, Jongsma & de Jonge, 1989). In order to account for the much larger conductance of LCC and for the discrepancy between these small conductance levels and those quoted above one should have to assume that, depending on cell species and experimental condition, different conductance states can be stabilized and appear as major state. If, e.g., the hemichannel were locked in the 25% sublevel found only very occasionally in the present study, its conductance would be in the order of $\geq$70 pS, resulting in a theoretical conductance of $\geq$35 pS for two channels in series.

Our interpretation of LCC gating in terms of opening of single connexons at present is not directly supported by experimental evidence. Neither the blocking effect of octanol nor the stimulating β-adrenergic effect are specific to such a mechanism. There is not even a common view on the mechanism how long-chain alcohols cause decoupling. This could theoretically occur either via a continuous reduction of the conductance of the dimeric channel from some finite value to zero without visible unitary steplike events (Somogyi & Kolb, 1989) or by all-or-none closure of individual channels (Spray et al., 1986; Veenstra & DeHaan, 1988; Rüdisüli & Weingart, 1989). The latter would be compatible with the present observation.

In order to either corroborate or discard our hypothesis further experiments using more specific tools are required.

This work was supported by the Deutsche Forschungsgemeinschaft (FG Konzell).

References

Bechem, M., Pott, L. 1985. Removal of Ca current inactivation in dialysed guinea-pig atrial cardioballs by Ca chelators. *Pfluegers Arch.* **404:**10–20

Bechem, M., Pott,L., Rennebaum, H. 1983. Atrial muscle cells from hearts of adult guinea-pigs in culture: A new preparation for cardiac cellular electrophysiology. *Eur. J. Cell. Biol.* **31:**366–369

Beuckelmann, D.J., Pott, L., Wier, W.G. 1989. Transient inward current and intracellular calcium concentration in guinea-pig ventricular myocytes. *J Physiol (London)* **314:**110P *(Abstr.)*

Boller, M., Pott, L. 1989. β-Adrenergic modulation of transient inward current in guinea-pig cardiac myocytes: Evidence for regulation of Ca^{2+}-release from sarcoplasmic reticulum by a cyclic AMP-dependent mechanism. *Pfluegers Arch.* **415:**276–288

Callewaert, G., Cleemann, L., Morad, M. 1989. Caffeine-induced Ca^{2+} release activates Ca^{2+} extrusion via Na^{-}-Ca^{2+} exchanger in cardiac myocytes. *Am. J. Physiol.* **257:**C147–C152

Colquhoun, D., Neher, E., Reuter, H., Stevens, C.F. 1981. Inward current channels activated by intracellular Ca in cultured cardiac cells. *Nature (London)* **294:**752–754

Dahl, G., Isenberg, G. 1980. Decoupling of heart muscle cells: Correlation with increased cytoplasmic calcium activity and with changes of nexus ultrastructure. *J. Membrane Biol.* **53:**63–75

Délèze, J., Hervé, J.C. 1983. Effect of several uncouplers of cell-to-cell communication on gap junction morphology in mammalian heart. *J. Membrane Biol.* **74:**203–215

DeMello, W.C. 1986. Interaction of cyclic AMP and Ca^{2+} in the control of electrical coupling in heart fibers. *Biochim. Biophys. Acta* **888:**91–99

DiFrancesco, D., Noble, D. 1985. A model of cardiac electrical activity incorporating ionic pumps and concentration changes. *Phil. Trans. R. Soc. London B.* **307:**353–398

Ehara, T., Noma, A., Ono, K. 1988. Calcium-activated non-selective cation channel in ventricular cells isolated from adult guinea-pig hearts. *J. Physiol. (London)* **403:**117–133

Gintant, G.A., Cohen, I.S. 1989. Advances in cardiac cellular electrophysiology: Implications for automaticity and therapeutics. *Annu. Rev. Pharmacol. Toxicol.* **28:**61–81

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Hirche, G. 1985. Blocking and modifying actions of octanol on Na channels in frog myelinated nerve. *Pfluegers Arch.* **405:**180–187

Kameyama, M., Kakei, M., Sato, R., Shibasaki, T., Matsuda, H., Irisawa, H. 1984. Intracellular Na^{+} activates a K^{+} channel in mammalian cardiac cells. *Nature (London)* **309:**354–356

Lipp, P., Mechman, S., Pott, L. 1987. Effects of calcium release from sarcoplasmic reticulum on membrane currents in guinea pig atrial cardioballs. *Pfluegers Arch.* **410:**121–131

Lipp, P., Pott, L. 1988*a*. Transient inward current in guinea-pig atrial myocytes reflects a change of sodium-calcium exchange current. *J. Physiol. (London)* **397:**601–630

Lipp, P., Pott, L. 1988*b*. Voltage dependence of sodium-calcium exchange current in guinea-pig atrial myocytes determined by means of an inhibitor. *J. Physiol. (London)* **403:**355–366

Maurer, P., Weingart, R. 1987. Cell pairs isolated from adult guinea pig and rat hearts: Effects of $[Ca^{2+}]_i$ on nexal membrane resistance. *Pfluegers Arch.* **409:**394–402

Mazet, F., Wittenberg, B.A., Spray, D.C. 1985. Fate of intercellular junctions in isolated rat cardiac cells. *Circ. Res.* **56:**195–204

Mechmann, S., Pott, L. 1986. Identification of Na-Ca exchange

current in single cardiac myocytes. *Nature (London)* **319:**597–599

Mechmann, S., Pott, L. 1989. Inhibition of a large-conductance ion channel in guinea-pig cardiac myocytes by octanol. *J. Physiol. (London)* **417:**157P *(Abstr.)*

Neyton, J., Trautmann, A. 1985. Single-channel currents of an intercellular junction. *Nature (London)* **317:**331–335

Niggli, E., Rüdisüli, A., Maurer, P., Weingart, R. 1989. Effects of general anesthetics on current flow across membranes in guinea pig myocytes. *Am. J. Physiol.* **256:**C273–C281

Noble, D. 1984.The surprising heart: A review of recent progress in cardiac electrophysiology. *J. Physiol. (London)* **353:**1–50

Page, E., Manjunath, C.K. 1986. Communicating junctions between cardiac cells. *In:* The Heart and Cardiovascular System. H.A. Fozzard, E. Haber, R.B. Jennings, A.M. Katz, and H.E. Morgan, editors. pp. 573–600. Raven, New York

Pelzer, D., Cavalié, A., McDonald, T.F., Trautwein, W. 1986. Macroscopic and elementary currents through cardiac calcium channels. *In:* Progress in Zoology. H.C. Lüttgau, editor. Vol. 33, pp. 83–98. Gustav-Fischer-Verlag, Stuttgart—New York

Penner, R., Matthews, G., Neher, E. 1988. Regulation of calcium influx by second messengers in rat mast cells. *Nature (London)* **334:**499–504

Robinson-Steiner, A.M., Corbin, J.D. 1986. Protein phosphorylation in the heart. *In:* The Heart and Cardiovascular System. H.A. Fozzard, E. Haber, R.B. Jennings, A.M. Katz, and H.E. Morgan, editors. pp. 887–910. Raven, New York

Rook, M.B., Jongsma, H.J., de Jonge, B. 1989. Single channel currents of homo- and heterologous gap junctions between cardiac fibroblasts and myocytes. *Pfluegers Arch.* **414:**95–98

Rook, M.B., Jongsma, H.J., van Ginneken, A.C.G. 1988. Properties of single gap junctional channels between isolated neonatal rat heart cells. *Am. J. Physiol.* **255:**H770–H782

Rousseau, E., Meissner, G. 1989. Single cardiac sarcoplasmic reticulum Ca^{2+}-release channel: Activation by caffeine. *Am. J. Physiol.* **256:**H328–H333

Rüdisüli, A., Weingart, R. 1989. Electrical properties of gap junction channels in guinea-pig ventricular cell pairs revealed by exposure to heptanol. *Pfluegers Arch.* **415:**12–21

Somogyi, R., Kolb, H.-A. 1989. Cell-to-cell channel conductance during loss of gap junctional coupling in pairs of pancreatic acinar and Chinese hamster ovary cells. *Pfluegers Arch.* **412:**54–65

Spray, D.C., Saez, J.C., Brosius, D., Bennett, M.V.L., Hertzberg, E.L. 1986. Isolated liver gap junctions: Gating of transjunctional currents is similar to that in intact pairs of rat hepatocytes. *Proc. Natl. Acad. Sci. USA* **83:**5494–5497

Trautwein, W., Kameyama, M., Hescheler, J., Hofmann, F. 1986. Cardiac calcium channels and their transmitter modulation. *In:* Progress in Zoology. H.C. Lüttgau, editor. Vol. 33, pp. 163–182. Gustav Fischer Verlag, Stuttgart—New York

Unwin, N., Zampighi, G. 1980. Structure of the junction between communicating cells. *Nature (London)* **283:**545–549

Veenstra, R.D., DeHaan, R.L. 1986. Measurement of single channel currents from cardiac gap junctions. *Science* **233:**972–974

Veenstra, R.D., DeHaan, R.L. 1988. Cardiac gap junction channel activity in embryonic chick ventricle cells. *Am. J. Physiol.* **254:**H170–H180

Weber, A., Herz, R. 1968. The relationship between caffeine contracture of intact muscle and the effect of caffeine on reticulum. *J. Gen. Physiol.***52:**750–759.

Received 15 December 1989; revised 16 April 1990