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ATP-sensitive K^+ channels in heart muscle cells first open and subsequently close at maintained anoxia

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Abstract In ventricular myocardial cells of the guinea pig and the mouse, anoxia caused after a mean latency of 439 ± 141 s and 129 ± 23 s (mean ± S.E.M.), respectively, a large current through K_{ATP}-channels. This current disappeared within several seconds when reoxygenating the cells but decayed also completely at maintained anoxia. The kinetics of the latter process, however, were much slower and obeyed an approximately monoexponential time course with time constants in the range of 30 s. The results suggest that in the ischaemic myocardium K_{ATP}-channels contribute only to the initial phase of extracellular K^+ accumulation.

Key words: Patch clamp; Heart cell; K_{ATP} channel; Anoxia

1. Introduction

The first five to eight minutes of myocardial ischaemia are accompanied by a rapid extracellular K^+ accumulation which is followed by a second phase of slow rise and a third phase of intermediate rise [1–4]. One possible mechanism underlying this K^+ accumulation is the opening of ATP-sensitive K^+ channels (K_{ATP}-channels) which has been described to appear in isolated heart cells by application of inhibitors of the oxidative metabolism [5–8] or by anoxia [9]. The most likely opening mechanism is a sufficient drop of the cytosolic ATP (or the ATP/ADP ratio). Progressive opening of these channels, however, can neither explain the triphasic time course of K+ accumulation in the ischaemic myocardium nor why preferentially the first phase is sensitive to the specific blocker of K_{ATP}-channels glibenclamide. Here we show that K_{ATP}-channels open under anoxic conditions in a transient fashion and in a time range which fits with a predominant action of these channels in the first minutes of ischaemia.

2. Materials and methods

Ventricular myocytes were isolated from the mouse and guinea pig heart as described previously [9,10]. Patch clamp experiments were performed in a chamber which has been designed to establish a layer of ultra pure argon above the bath to isolate atmospheric air [11] leaving free access for the patch pipette. Control Tyrode solution, as used in the bath, was substrate-free and contained (mM) 150.0 NaCl, 5.4 KCl, 3.6 $CaCl_2$, 1.2 $MgCl_2$, 10.0 HEPES, 0.005 nitrendipine, pH 7.4 (NaOH). Anoxic Tyrode solution was prepared according to Allshire et al. [12]. Whole cell ionic currents were recorded with a patch clamp technique using an RK 300 patch-clamp-amplifier (Biologic, Claix, France). Glass pipettes (1–2 MΩ) were pulled from borosilicate glass and their tips were heat polished. The pipette solution contained (mM): 150.0 KCl, 5.0 HEPES, and 10 EGTA, pH 7.3 with KOH. Criteria for the acceptance of a guinea pig cell for an experiment were a regular shape with clear cross striation and a positive current at −45 mV. A mouse cell was accepted for an experiment if it was rod-shaped and developed regular transient outward currents [13]. Series resistance compensation was adjusted such that any ringing was avoided leaving about 20% uncompensated. Inspite of this compensation, it should be stressed that all currents in this report with amplitudes larger than 10 nA are certainly an underestimation due to limitations of the voltage clamp. The holding potential was generally set to −80 mV. Single channel currents were measured in cell-attached patches by means of a EPC-7 amplifier (List Electronic, Darmstadt, Germany). The cells were bathed in substrate free Tyrode solution in which K^+ (high K^+ Tyrode) was increased to 10.8 mM. Under those conditions a resting potential of both types of cells at ~ −65 mV was measured. All potentials given for cell-attached patches were corrected for this mean resting potential, i.e. the indicated potentials were close to the true transmembrane potentials. Patch pipettes (2.4 MΩ) were pulled from thick-walled borosilicate glass, heat polished, coated with Sylgard 184 (Dow Corning, USA), and filled with high K^+ Tyrode solution. All experiments were carried out at 37°C.

Recording and analysis of the data was performed with the ISO2 software developed in this lab (MFK Computer, Frankfurt, Germany). Whole cell currents were filtered at 10 kHz (4-pole Bessel) and single channel currents at 3 kHz. All traces were recorded with the sampling rate of 10 kHz (12-bit resolution).

3. Results

Fig. 1 illustrates typical responses of ionic currents in a guinea pig and a mouse myocyte at maintained anoxia. When clamping from −80 to +40 mV under control conditions (a), the guinea pig cell showed at the beginning of the test pulse an inward Na current spike followed by some delayed rectifier current. Anoxia caused after several minutes the development of a large time-independent K^+ current (b) that has been described in a previous report to be carried by K_{ATP}-channels which close within a few seconds after reoxygenation [9]. Herein, the cells were not reoxygenated at the time of substantial current amplitude. During the following minutes of maintained anoxia, the large K_{ATP}-channel current decayed until disappearance (c). The full time course of the holding and the late test current clearly shows that the extra current, generated by the opening of K_{ATP}-channels, appeared and disappeared in a continuous manner and reversed near −80 mV, quite as expected for K^+ selective channels. In addition, at the time when the K_{ATP}-channel current decreased a current with less specific conductance (nonspecific current) developed which becomes obvious by the downwardly deflecting current at −80 mV. This current was regularly accompanied by a progressive hypercontracture and the death of the cell. Guinea pig cells developed the nonspecific current at the latest after the decay of the K_{ATP}-channel current, typically earlier. In the mouse cell of Fig. 1, the control trace (a) is dominated by the transient outward

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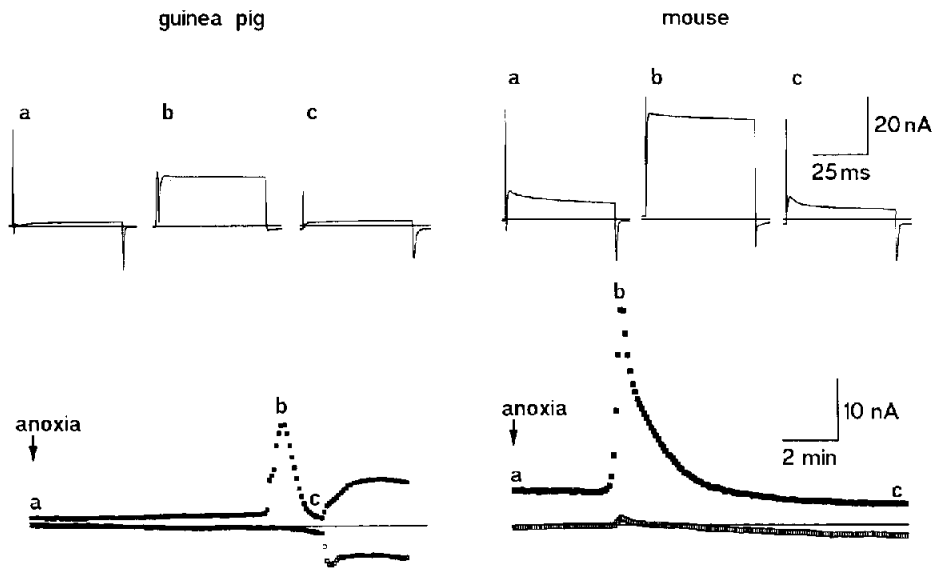

Fig. 1. Typical long time courses of whole cell current at −80 mV (lower curve) and +40 mV (late current in pulses of 50 ms, upper curve) in a guinea pig and mouse cell. The cells were pulsed at a frequency of 1 Hz and current amplitudes were plotted in intervals of five seconds. At characteristic points of the experiment, individual current traces are shown above: control (a), at the time of maximally developed K_{ATP}-channel current (b), and after the decay of this current (c).

current (I_{to}) which itself isdown-regulated by anoxia [14]. Analog to the guinea pig cell, the mouse cell also generated an anoxia-induced time-independent current (b) which subsequently decayed in the following minutes. In mouse cells, however, the nonspecfic current was either absent or, if present, of smaller amplitude and more separated from the decay phase of the K_{ATP}-channel current than in guinea pig cells.

The time course of the K_{ATP}-channel current peak was quantified in 12 guinea pig and 31 mouse myocardial cells by (i) the latency until the first appearance of K_{ATP}-channel current after starting anoxia, (ii) the peak amplitude of this current, (iii) the duration at 50% of the maximum amplitude, (iv) the rise time of the current measured as the time elapsing between the 10% and 90% amplitude, and (v) the decay time constant by fitting single exponentials to the time course starting at the 66% level. In guinea pig cells, the values for the five parameters were 439 ± 141 s, 24.0 ± 5.0 nA, 256 ± 49 s, 39 ± 9 s, and 34 ± 6 s, respectively. In the mouse cells, the K_{ATP}-channel current appeared significantly earlier (latency 129 ± 23 s) and faster (10% to 90% rise time 27 ± 6 s) and lasted only over shorter periods (50% duration 174 ± 15 s) whereas the amplitude (20.3 ± 1.4 nA) and the decay time constant (30.7 ± 5.0 s; $n = 10$) were similar. Out of a total of 71 mouse cells, 26 cells did not develop a K_{ATP}-channel current within the first 304 ± 65 s of anoxia. The latencies in the mouse cells were distributed approximately in an exponential manner, quite similar as described previously in guinea pig myocytes [9].

In analogy to the experiments with anoxia, also the uncoupler 2,4 dinitrophenol (DNP) induced after a delay a large K_{ATP}-channel current which decayed in the time range of minutes after reaching a peak. In four guinea pig cells, we calculated for the latency, the peak amplitude, the 50% duration, and the rise time 109 ± 25 s, 37 ± 7 nA, 425 ± 85 s, and 68 ± 39 s, respectively. Apart from appearing significantly earlier, the transient opening of K_{ATP}-channels induced by DNP was indistinguishible from that during anoxia.

If K_{ATP}-channels carry the transient K^+ current induced here by either anoxia or DNP, this current should be sensitive to the specific blocker of these channels, glibenclamide. Fig. 2A illustrates an experiment in a mouse myocyte where glibenclamide was administered twice during anoxia; during the first rising phase of the current for a period of 40 s and as a short pulse during the second rising phase after washing out the drug (arrow). The long application of the drug blocked the K_{ATP}-channel current completely whereas the short pulse generated only a notch in the plot. After reaching a maximum, the current at +40 mV started to decay. Approximately at the same time, this cell developed the nonspecific current. The efficacy of glibenclamide on the anoxia-induced current was confirmed in eight mouse cells. Fig. 2B illustrates in a mouse cell that the K_{ATP}-channel current is caused indeed by anoxia since reoxygenation at the time of substantial current switched it off within a few seconds, exactly in the same way described previously in guinea pig myocytes [9]. The same result was obtained in nine other cells. In order to rule out that the effect of glibenclamide was pretended by a contamination of oxygen upon the solution change, analog experiments were carried out by inducing the K_{ATP}-channel current with DNP. A pulse-like application of the drug during the rising phase (arrow) caused an immediate reduction of the current at +40 mV. During the progress of washing out the drug, the current further increased, passed a maximum, and subsequently decayed. In this cell, the nonspecific current started to develop approximately during the peak. A glibenclamide-induced decrease of the K_{ATP}-channel current was observed in seven out of seven mouse cells.

Fig. 3 illustrates in a cell-attached patch of a guinea pig cell that the increase and the decrease of the anoxia-induced K^+ current is caused by a corresponding increase and decrease of the open probability of K_{ATP}-channels. Plotted is the time course of the product of the channel number N and the open probability P_o of the K_{ATP}-channels. About 1 min after the onset of anoxia, the first unitary outward currents became

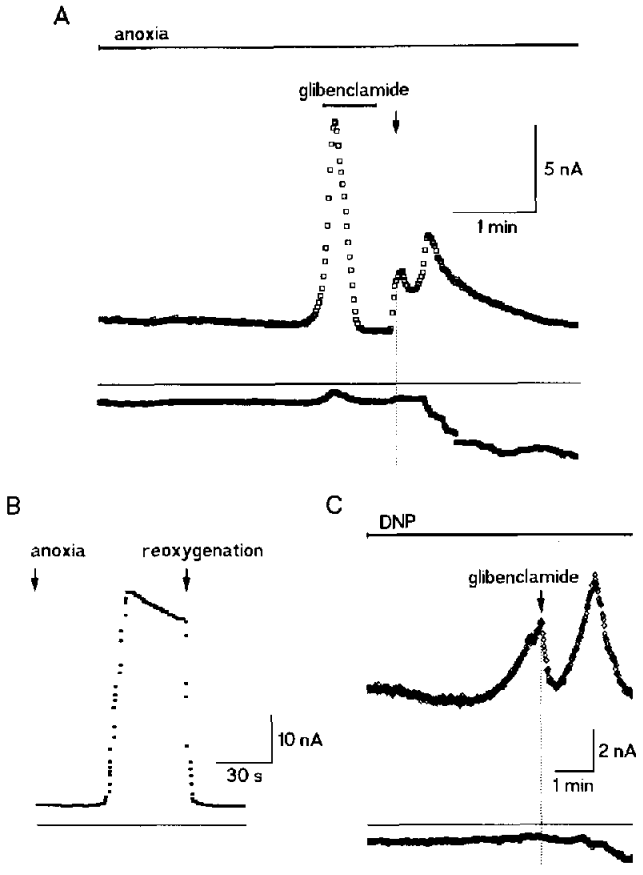

Fig. 2. Identification of K_{ATP}-channels to carry the large transient K^+ current induced by blocking oxidative metabolism. Mouse myocytes were clamped from −80 to +40 mV (pulse duration 50 ms; pulsing frequency 1 Hz). (A) Glibenclamide (100 μM) blocks the anoxia-induced K_{ATP}-channel current. Plotted are the late current at +40 mV (upper trace) and the holding current at −80 mV (lower trace). The horizontal line indicates the zero current level. Glibenclamide was administered first for a period of 40 s to block the K_{ATP}-channel current completely and second, after washing out the drug, by a short interval (2 s) during the second increase of the current (arrow). (B) Reoxygenation closed anoxia-induced current within seconds. The late current at +40 mV is plotted. The time difference between the data points is one second. (C) Glibenclamide (100 μM) affects the K^+ current induced by DNP (10 μM). The late current at +40 mV (upper trace) and the holding current at −80 mV (lower trace) are plotted. Glibenclamide was administered for a short interval (2 s) during the increase of the K_{ATP}-channel current (arrow).

prominent (top left trace). Until minute 4, NP_o progressively increased (top middle trace) to reach values of up to 5. Then, in analogy to the whole cell currents, NP_o of the K_{ATP}-channels started to decline again until only one channel remained open at the end of the experiment (top right trace). All properties of the individual channels were consistent with those of K_{ATP}-channels [5,6,9] and are therefore not further considered here. Reversible opening of elementary K_{ATP}-channels was observed in three guinea pig cells and five mouse cells.

Since part of the mouse myocytes (30%) survived the period of opening and closing of K_{ATP}-channels, it could be tested in these cells whether or not closing is associated with an irreversible alteration of the channels. Fig. 4 illustrates an experiment in which a transient opening of K_{ATP}-channels could be elicited twice by two subsequent periods of anoxia separated by a period of normoxia. During this period, the transient outward current I_{to} recovered slowly [14]. The second anoxic period caused a similar transient opening of K_{ATP}-channels as the first one. Typically the latency was shorter lasting in this cell only 1 min. The insets show that I_{to} was present even after the end of the second transient opening of K_{ATP}-channels which may be taken as an indicator for a fairly intact cell. This panel clearly demonstrates that closing of K_{ATP}-channels during the decay phase is not an irreversible alteration in the channels but a reversible mechanism.

4. Discussion

Since the discovery of K_{ATP}-channels in the myocardium [6,15] there has been an extensive debate as to whether or not these channels contribute to the extracellular accumulation of K^+ during ischaemia. The main objections against a relevant contribution of K_{ATP}-channels are: (i) half maximum inhibition of the channel activity is reached at tens to hundreds of μM ATP [16,17] whereas measured ATP levels in the ischaemic myocardium are in the range of several mM [18–20] which would allow only a few channels to open. In turn this would not be sufficient to explain the large K^+ efflux; (ii) glibenclamide, a specific blocker of K_{ATP}-channels, blocks the K^+ efflux in the ischaemic myocardium only during the first steep phase [4].

In this study we show that K_{ATP}-channels close again during maintainedanoxia and that the period of their opening is in the range of several minutes. Guinea pig cells regularly did not survive the period of the decay of the K_{ATP}-channel current but developed a large nonspecific conductance associated with hypercontracture. This may explain why previous studies did not detect the closure of the channels by evaluating this nonspecific current as break of the seal. We speculate at the present that a cytosolic Ca^{2+} load, via a presently unknown mechanism, triggers hypercontracture which in turn causes membrane defects thereby enhancing both Ca^{2+} load and hypercontracture in a vicious circle. Mouse ventricular myocytes responded in a different way: In numerous experiments the decay of the K_{ATP}-channel current was separated well from the nonspecific current. The reason for this species difference is not clear. The fact that in mouse cells the decay of the K_{ATP}-channel current was observed also in the absence of nonspecific current and hypercontracture proves that closure of the K_{ATP}-channels is independent of the mechansims underlying both of these phenomena. The survival of mouse cells allowed also another experiment: the K_{ATP}-channel current could be elicited repeatedly by a second period of anoxia (Fig. 4). This showed that the closure of the channels during anoxia was not generated by an irreversible alteration of the channel molecules. From the possibility of a repeated performance of the transient opening of K_{ATP}-channels at repeated anoxia and the rapid closure of the channels following reoxygenation, we favour the idea that the open probability of the channels is controlled by the cytosolic (submembrane) ATP level and that this level may drop in individual cells to very low values (< 10 μM). ATP in this concentration range is essential for sufficient phosphorylation of K_{ATP}-channels to open [21,22]. The putative sequence of events in the cell after the onset of anoxia is: block of oxidative ATP-synthesis, inhibition of glycolysis (mechanism still un-

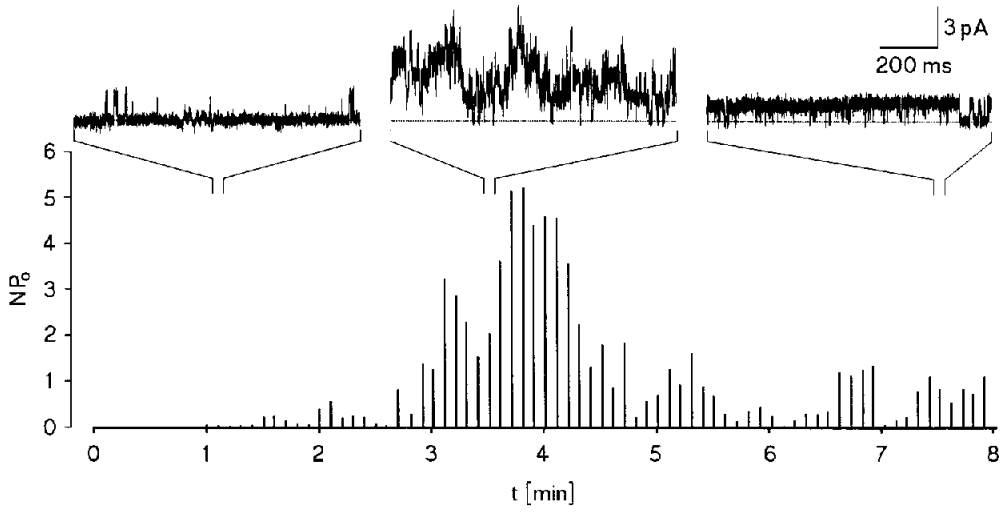

Fig. 3. Anoxia causes opening and closure of elementary K_{ATP}-channels. The current was measured in a cell-attached patch of a guinea pig cell. The patch was held at −20 mV throughout. Filter frequency 3 kHz. The time axis starts at the beginning of anoxia. The current was recorded in traces of 950 ms duration at a frequency of 0.83 Hz. Every 6 s, NP_o was calculated in a 950-ms interval by dividing the mean current by the single channel current of 1.6 pA. Three representative traces (top) at the indicated times are illustrated.

known) leading to massive opening of the channels, further decrease of ATP thereby stopping phosphorylation by protein kinase A and closing of K_{ATP}-channels. Not clear is why the latency until the first opening of K_{ATP}-channels during anoxia is of such great variance as observed here and also in a previous report [9]. Different positions of the cells in the chamber may explain latency differences at the most in the range of a few minutes but certainly not in the range of tens of minutes. We speculate that this variance among the cells is also present in the ischaemic myocardium where it might explain the controversy why millimolar ATP levels are measured in the ischaemic myocardium whereas in the individual cells only much lower levels allow for massive opening of K_{ATP}-channels. The clustered transient opening of K_{ATP}-channels during the first minutes of anoxia also fits with the only effect of glibenclamide on the early time course of K^+ accumulation during ischaemia [4].

The longer latency of the anoxia-induced K_{ATP}-channel current in individual cells compared to the rapid first rise in the extracellular K^+ concentration in the ischaemic myocardium may be explained by the limited speed of washing out the oxygen in our experimental chamber. Oxygen in the bath solution was removed via both solution exchange and diffusion to the argon atmosphere established above the solution. The speed of both processes strongly depends on the degree of convection in the bath solution which is certainly also a function of the position of the cell within the chamber. Since the solution entered the chamber on the one side and left it on the other, an upper limit estimate for the decay of oxygen in the chamber is to assume a perfect mixture of the present normoxic (150 mm Hg) and the inflowing anoxic solution. In order to reach 0.22 mm Hg, the half maximum oxydation of cytochrome oxidase [23], the normoxic solution has to be diluted by some more than 29-fold. The chamber volume was replaced twice per minute yielding 4.5 min for this process. Since in some of the mouse myocytes latencies of less than a minute were found, a faster decay of oxygen at distinct sites in the chamber was certainly possible. On average, however, the latency to reach sufficiently low levels of oxygen was some longer to be in the range of minutes. This is in contrast to the ischaemic myocardium where the oxygen present in the tissue is rapidly consumed by the mass of cells thereby blocking more rapidly the cytochrome oxidase reaction.

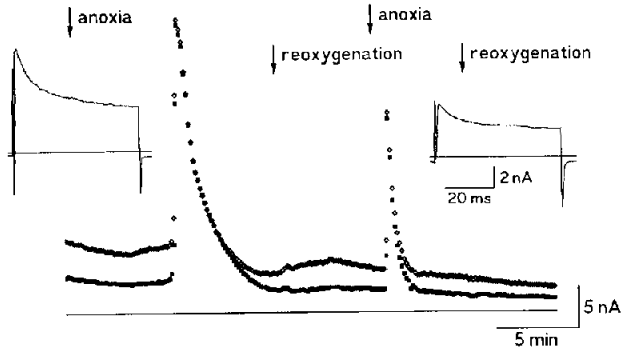

Fig. 4. Mouse myocytes may survive anoxia-induced K_{ATP}-channel currents. Long time course of the peak (upper trace) and late (lower trace) current at +40 mV. Anoxia generated the K_{ATP}-channel current. After its complete decay, the cell was reoxygenated and some minutes later anoxia induced a respective current a second time. The insets show individual current traces at the beginning (left) and end (right) of the long time course.

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References

[1] Hirche, H.J., Franz, C., Bös, L., Bissig, R., Lang, R. and Schramm, M. (1980) J. Mol. Cell. Cardiol. 12, 579–593.
[2] Weiss, J.N. and Shine, K.I. (1982) Am. J. Physiol. 242, H619–H628.
[3] Knopf, H., Theising, R., Moon, C.H. and Hirche, Hj. (1990) J. Mol. Cell. Cardiol. 22, 1259–1272.
[4] Wilde, A.A.M., Escande, D., Schumacher, C.A., Thuringer, D.,

Mestre, M., Fiolet, J.W.T. and Janse M.J. (1990) Circ. Res. 67, 883–843.
[5] Noma, A. and Shibasaki, T. (1985) J, Physiol. 363, 463 480.
[6] Trube, G. and Hescheler, J. (1984) Pflügers Arch. 401, 178–184.
[7] Weiss, J.N. and Lamp, S.T. (1987) Science 238, 67–69.
[8] Weiss, J.N. and Lamp, S.T. (1989) J. Gen. Physiol. 94, 911–935.
[9] Benndorf, K., Bollmann, G., Friedrich, M. and Hirche, H. (1992) J. Physiol. 454, 339–357.
[10] Benndorf, K. (1993) Pflügers Arch. 422, 506–515.
[11] Stern, M.D., Silverman, H.S., Houser, S.R., Josephson, R.A., Capogrossi, M.C., Nichols, C.G., Lederer, W.J. and Lakatta, E.G. (1988) Proc. Natl. Acad. Sci. USA 85, 6954–6958.
[12] Allshire, A., Piper, H.M., Cuthbertson, K.S.R. and Cobbold, P.H. (1987) Biochem. J. 244, 381–385,
[13] Benndorf, K. and Nilius, B. (1988) Gen. Physiol. Biophys. 7, 449–466.
[14] Thierfelder, S., Hirche, H. and Benndorf, K. (1994) Pflügers Arch., in press.
[15] Noma, A. (1983) Nature 305, 147–148.
[16] Lederer, W.J. and Nichols, C.G. (1989) J. Physiol. 419, 193–211.
[17] Nichols, C.G. and Lederer, W.J. (1990) J. Physiol. 423, 91–110.
[18] Hearse, D.J. (1979) Am. J. Cardiol. 44, 1115–1121.
[19] Matthews, P.M., Radda, G.K. and Taylor, D.J. (1981) Biochem. Soc. Trans. 9, 236–237.
[20] Allen, D.J., Morris, P.G., Orchard, C.H. and Pirolo, J.S. (1985) J. Physiol. 361, 185–204.
[21] Ribalet, B., Ciani, S. and Eddlestone, G.T. (1989) J. Gen. Physiol. 94, 693–717.
[22] Takano, M., Qin, D. and Noma, A. (1990) Am. J. Physiol. 258, H45–H50.
[23] Wittenberg, B.A. and Wittenberg, J.B. (1985) J. Biol. Chem. 260, 6548–6554.