Biochimica et Biophysica Acta, 1065 (1991) 269–271

ADONIS 0005273691002478

BBAMEM 70682

Rapid Report

Changes of the subsarcolemmal Na^+ concentration in internally perfused cardiac cells

Frieda V. Bielen, Helfried G. Glitsch * and Fons Verdonck

Interdisciplinary Research Centre, Catholic University of Leuven Campus Kortrijk, Kortrijk (Belgium)

(Received 25 March 1991)

Key words: Ion distribution; Dialyzed cell; Whole-cell recording; Sodium/potassium pump current; Sodium–calcium exchange current

Transients of Na^+/K^+ pump and of Na^+/Ca^{2+} exchange current occur during whole-cell recording from cardiac cells upon quick changes of active Na^+ efflux. The transients reflect a temporary loss of control of the subsarcolemmal Na^+ concentration. Even in the steady state the control is not complete is certain cells. Quantitative studies on ion transport by whole-cell recording are meaningful only if an adequate control of the submembranal ionic composition is demonstrated.

The use of the whole-cell recording technique [1] in single cells has improved the manipulation of the membrane potential and of the composition of the intra- and extra-cellular media during electrophysiological measurements. The technique that includes intracellular dialysis via wide-tipped patch pipettes has been widely applied to a variety of cardiac cells. Though the concentrations of substances transported across the cell membrane generally differ between the pipette solution and the cytosol [2] it is generally assumed that, for example, the subsarcolemmal Na^+ concentration ($[Na^+]_{ssl}$) of cardiac myocytes can be regulated by the Na^+ concentration of the pipette solution ($[Na^+]_{pip}$), at least in the steady state [3]. We report here that whole-cell recording from cardiac cells does not permit effective control of the $[Na^+]_{ssl}$. This is true not only for minutes following changes of the net sarcolemmal Na^+ flux but also for the steady state in certain isolated heart cells. The conclusion originates from measurements of two currents activated by Na^+_{ssl} and carried by ion transporters, i.e. the Na^+/K^+ pump and the Na^+/Ca^{2+} exchange current.

The experiments were mainly performed on single rabbit cardiac Purkinje cells and ventricular myocytes. The cells were isolated by a conventional enzymatic procedure described before [4]. Purkinje cells were 141.2 ± 3.2 μm in length and 16.1 ± 0.5 μm in diameter (mean ± S.E., $n = 72$) whereas ventricular cells were 156.2 ± 3.3 μm in length and 29.3 ± 0.8 μm in width ($n = 46$). These dimensions are close to published measurements [4,5] and similar to those of guinea-pig and rat cardiomyocytes [5,6]. The cells were internally dialyzed by means of patch pipettes with a resistance of 1–4 MΩ, commonly used for whole-cell recording from cardiac cells [7–9]. The fire-polished patch pipettes were filled with (mM): 80 cesium aspartate, 20 CsOH, 5 NaOH, 10 EGTA, 40 Hepes, 5 $MgCl_2$, 5 glucose, 5 MgATP, 5 disodium creatine phosphate (pH 7.2). In two experiments the Na^+ concentration of the patch pipette-solution was raised to 50 mM by replacing Na^+ for Cs^+.

The superfusion medium contained (mM): 144 NaCl, 0–10.8 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, 10 Hepes (adjusted with NaOH to pH 7.4) and 2 mM $BaCl_2$ in order to suppress sarcolemmal K^+ conductances. The superfusion solution could be changed at the cell surface within 250 ms via multibarrelled pipettes near the cell studied. Whole-cell recording was performed at 31–34 °C by means of a voltage-clamp amplifier (Axoclamp 2A). The holding potential was set to −20 mV throughout the experiments in order to inactivate sarcolemmal Na^+ and Ca^{2+} conductances. The Na^+/K^+-pump current (I_p) was identified as the current activated by extracellular K^+ (K_0^+) in Ba^{2+}-containing solution. Under these conditions the K_0^+-activated current is identical to the current inhibited by cardiac steroids which are specific blockers of the

* On leave from the Department of Cell Physiology, Ruhr-University, D-4630 Bochum 1, F.R.G.

Correspondence: H.G. Glitsch, Department of Cell Physiology, Ruhr-University, D-4630 Bochum 1, F.R.G.

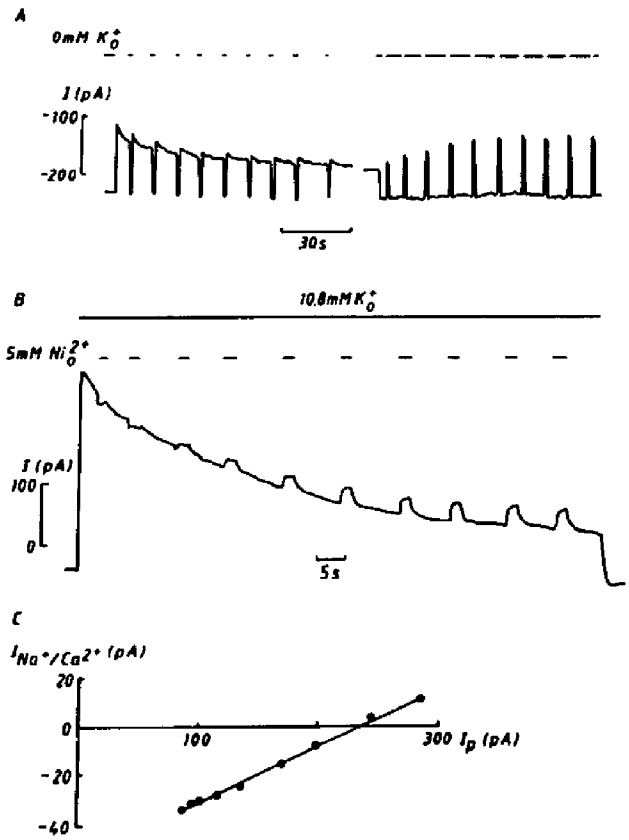

Fig. 1. Effect of alterations in transmembrane Na^+ efflux on Na^+/K^+-pump current (A, B) and Na^+/Ca^{2+}-exchange current (B) in single cardiac Purkinje cells. Holding potential: −20 mV; $[Na^+]_{pip} = 15$ mM. (A, B) Chart recordings of whole-cell current in two Purkinje cells. (A) Upper bars mark lowering $[K^+]_0$ from 10.8 to 0 mM. Initial pipette resistance = 1.1 MΩ. Interruption of the current trace indicates omission of 45 s of record. (B) Upper line shows presence of 10.8 K_0^+, short bars mark the presence of Ni_0^{2+} (5 mM). Initial pipette resistance = 3.2 MΩ. (C) Relation between Ni_0^{2+}-sensitive $I_{Na^+/Ca^{2+}}$ exchange current and Na^+/K^+-pump current I_p during pump reactivation. Data taken from B.

Na^+/K^+ pump [10,11]. The Na^+/Ca^{2+} exchange current ($I_{Na^+/Ca^{2+}}$) was measured as Ni^{2+}-sensitive current [12].

After whole-cell recording for 20 min, which largely exceeds the calculated time required for equilibration of small ions between pipette solution and cell interior via diffusional exchange [3], the trace shown in Fig. 1A was recorded. Following 2.5 min of superfusion with K^+-free solution a large outward current transient was elicited at 10.8 mM K_0^+ in a Purkinje cell equilibrated with a patch-pipette solution containing 15 mM Na^+. The inward deflections of the current elicited by short pulses of K^+-free solution (Fig. 1A, left part) show that the outward current is indeed the Na^+/K^+-pump current which declines exponentially towards a final constant amplitude during continuous superfusion with the K^+-containing medium. The time constant of the decline averaged 22 ± 2 s ($n = 12$) at 10.8 mM K_0^+, but was considerably larger at 1 mM K_0. Small I_p transients could be detected even after 0 mM K_0^+ pulses of only 1 s duration. The I_p transient was found in more than 60 cells tested and was independent of the cation species used as an external pump activator (K_0^+, Cs_0^+, NH_4^+ or Tl_0^+). The transient was also observed in each of five guinea-pig ventricular cells (138.6 ± 10.9 μm in length, 29.2 ± 2.7 μm in width). If the initial overshooting of I_p were due to an increase in $[Na^+]_{ssl}$ during the preceding period of time in K^+-free solution, it should be possible to measure an increasing I_p by application of short K_0^+ pulses during superfusion with a K^+-free medium. This is expected because K^+-free solution blocks Na^+/K^+ exchange via the Na^+ pump. As passive Na^+ influx persists, $[Na^+]_{ssl}$ should increase if there were no effective control of $[Na^+]_{ssl}$ by intracellular dialysis via the patch pipette. An increased $[Na^+]_{ssl}$ in turn should augment I_p upon reactivation of the Na^+/K^+ pump in K^+-containing medium [7,13]. The right part of Fig. 1A demonstrates such an increasing I_p during superfusion with K^+-free solution. The current stabilizes after 1 min of superfusion at a value about twice the steady-state amplitude of I_p in the solution containing 10.8 mM K^+. If the I_p transient indicates a temporary loss of $[Na^+]_{ssl}$ control by intracellular dialysis $[Na^+]_{ssl}$-dependent currents different from I_p should likewise change during the transient. Therefore, we studied the Na^+/Ca^{2+} exchange current ($I_{Na^+/Ca^{2+}}$) identified as the Ni_0^{2+}-sensitive current. Following intracellular dialysis for 10 min and superfusion with a K^+-free medium for 8 min the record shown in Fig. 1B was taken. Similar to the finding displayed in Fig. 1A application of a solution containing 10.8 mM K^+ evoked a large I_p transient. Ni_0^{2+} (5 mM) was additionally applied for about 2 s every 10 s. The Ni_0^{2+}-sensitive current reversed from outward (3 Na^+ leaving the cell for 1 Ca^{2+} coming in) to inward (1 Ca^{2+} leaving the cell for 3 Na^+ entering) during the I_p transient. These changes of both I_p and $I_{Na^+/Ca^{2+}}$ are clearly in line with the idea of a decreasing $[Na^+]_{ssl}$. Furthermore, the linear relationship between I_p and $I_{Na^+/Ca^{2+}}$ (Fig. 1C) agrees with their dependence on one changing, common factor, which is most likely $[Na^+]_{ssl}$. The current record shown in Fig. 2 suggests that even in the steady-state $[Na^+]_{ssl}$ is not controlled by $[Na^+]_{pip}$, at least in certain cells. Switching from 10.8 to 1 mM K_0^+ (at a) induced a sudden inward shift of the membrane current. This is probably due to a decrease of I_p because the Na^+/K^+ pump is substantially less activated at the lower K^+ concentration [14]. However, I_p increased again during the following 3 min and shifted the membrane current towards the level measured previously at 10.8 mM K_0^+ (compare b to a). Reapplication of the medium containing 10.8 mM K^+ (at b) caused a rapid outward shift of the current followed by a decline to a final amplitude not

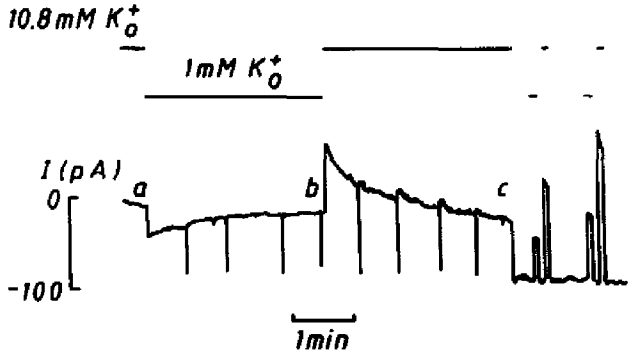

Fig. 2. Effect of 1 mM and 10.8 mM K_0^+ on Na^+/K^+-pump current in K^+-containing (a to c) or K^+-free superfusion solution. Record of whole-cell current from a Purkinje cell. Upper bars indicate $[K^+]_0$. Downward deflections of the current trace between a and c were evoked by brief 0 K_0^+ pulses and represent the I_p amplitude. Holding potential: −20 mV; $[Na^+]_{pip}$ = 15 mM; initial pipette resistance = 2.7 MΩ.

very different from that measured after 3 min at 1 mM K_0^+ (compare c to b). The initial rapid outward shift is probably caused by the increased activation of the Na^+ pump at the higher $[K^+]_0$. The nearly identical final I_p amplitudes at the two different K_0^+ concentrations (compare b to c) strongly suggest that $[Na^+]_{ssl}$ in the steady state is higher at 1 than at 10.8 mM K_0^+. If this is correct the I_p amplitudes at 1 or 10.8 mM K_0^+ should be quite different at constant $[Na^+]_{ssl}$. Starting from c in Fig. 2 the cell was superfused with a K^+-free medium except for short successive pulses with solutions containing either 1 or 10.8 mM K^+ to activate I_p. It seems unlikely that $[Na^+]_{ssl}$ was markedly increased at the beginning of the second of two successive pulses. Under these conditions the I_p amplitude at 1 mM K_0^+ amounted to only 42% of the I_p value at 10.8 mM K_0^+. One the average of 14 cells the 'steady-state' I_p current at 1 mM K_0^+ amounted to 66 ± 3% of the I_p amplitude observed at 10.8 mM K_0^+, whereas, during superfusion with a K^+-free medium, I_p activated by short pulses of 1 mM K_0^+ was only 34 ± 1% of that measured at 10.8 mM K_0^+.

The measured I_p transients indicate changes of $[Na^+]_{ssl}$ following alterations of active Na efflux during whole-cell recording from cardiac cells with a common shape by means of patch pipettes with the typical resistance of 1 to 4 MΩ. The variations of $[Na^+]_{ssl}$ persisted for minutes in all cells studied. Measurable differences in $[Na^+]_{ssl}$ at various $[K^+]_0$ were found even in the steady state, at least in certain cells. The technique of whole-cell recording permits, in principle, the control of the cytosolic ionic composition underneath the cell membrane. The present results emphasize the necessity to test whether such a control is in fact achieved under the respective experimental conditions. Adequate tests are most important in quantitative studies on ion transports through the cell membrane. The mechanisms involved in the (transient) loss of control of $[Na^+]_{ssl}$ are not yet fully understood. A $[Na^+]_{pip}$ of 50 mM used in two Purkinje cells resulted in a smaller I_p overshoot and a more rapid decline to a higher final I_p amplitude after switching from a K^+-free solution to a medium containing 10.8 mM K^+, if compared to the current traces shown in Figs. 1 A, B. The slow increase of I_p at 1 mM K_0^+ (Fig. 2, a to b) was nearly abolished. Thus the Na^+ concentration gradient between patch pipette and cytosol seems to be a major factor, but the present measurements cannot exclude the existence of a subsarcolemmal 'fuzzy space' [15].

References

1 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
2 Mathias, R.T., Cohen, I.S. and Oliva, C. (1990) Biophys. J. 58, 759–770.
3 Mogul, D.J., Singer, D.H. and Ten Eick, R.E. (1989) Biophys. J. 56, 565–577.
4 Scamps, F. and Carmeliet, E. (1989) Am. J. Physiol. 257, C1086–C1092.
5 Mitra, R. and Morad, M. (1985) Am. J. Physiol 249, H1056–H1060.
6 Powell, T. and Twist, V.W. (1976) Biochem. Biophys. Res. Commun. 72, 327–333.
7 Glitsch, H.G., Krahn, T. and Pusch, H. (1989) Pflügers Arch. 414, 52–58.
8 Kameyama, M., Hofmann, F. and Trautwein, W. (1985) Pflügers Arch. 405, 285–293.
9 Leblanc, N. and Hume, J.R. (1990) Science 248, 372–376.
10 Gadsby, D.C. and Nakao, M. (1989) J. Gen. Physiol. 94, 511–537.
11 Glitsch, H.G., Pusch, H., Schumacher, T. and Verdonck, F. (1982) Pflügers Arch. 394, 256–263.
12 Kimura, J., Miyamae, S. and Noma, A. (1987) J. Physiol. (Lond.) 384, 199–222.
13 Nakao, M. and Gadsby, D.C. (1989) J. Gen. Physiol. 94, 539–565.
14 Gadsby, D.C. (1980) Proc. Natl. Acad. Sci. USA 77, 4035–4039.
15 Lederer, W.J., Niggli, E. and Hadley, R.W. (1990) Science 248, 283.