Biochimica et Biophysica Acta, 1029 (1990) 167–172
Elsevier

BBAMEM 75036

Action potential duration and activation of ATP-sensitive potassium current in isolated guinea-pig ventricular myocytes

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(Received 11 May 1990)

Key words: Cardiac muscle; ATP; Potassium ion channel; Action potential; Metabolic stress

It is difficult to associate the ATP-sensitive potassium (K-ATP) channel of cardiac muscle with hypoxia/ischemia induced action potential shortening because this occurs before intracellular ATP falls to levels associated in vitro with channel opening. This leaves the cardiac K-ATP channel without any obvious physiological function. We have quantitatively examined the relationship between action potential duration and K-ATP channel activity in enzymatically isolated ventricular myocytes of the guinea-pig. In whole-cell voltage-clamp recording experiments when the K-ATP channel opener SR 44866 (2–10 μM) stimulated an outward membrane current greater than 50 pA at 0 mV membrane potential (the equivalent of 30 open K-ATP channels or 1% of the cell K-ATP channel population) action potential duration was reduced by more than 50%. In the majority of cell-attached membrane patch recordings metabolic inhibition stimulated K-ATP channel open probability of 1–2% which continued for long periods (7–25 min) before cell contracture and coincident major K-ATP channel activation (open probability 65%). Our quantitative analysis thus shows that physiologically relevant activity of K-ATP channels in cardiac muscle is confined to a very small percentage of the possible cell K-ATP current and thus intracellular ATP would not have to fall very far before the opening of K-ATP channels would influence cardiac excitability.

Introduction

With the available evidence it is not always easy to understand the physiological role of the ATP-sensitive potassium (K-ATP) channel in cardiac muscle. The obvious link between the channel and a product of cellular metabolism suggests that it might be activated under ischemic/hypoxic conditions [1–3], accounting for a time-dependent potassium current [4] and action potential shortening [5] which could be reversed by intracellular injection of ATP [6]. But a problem is that the channel appears to be too sensitive to ATP. Published values for the 50% inhibition of channel opening range from 30 to 200 μM ATP [1,7–9]. Modelling ischemic conditions with metabolic poisons evokes K-ATP channel opening in intact ventricular myocytes [1,2]. But this appears to be closely associated with rigor contracture [10] caused by the almost total disappearance of intracellular ATP which is really too late for a physiological function of the channel. Also shortening of the cardiac action potential has been recorded before significant reduction of intracellular ATP [11]. On the other hand, studies with 'K-channel opening' drugs have left no doubts that the activation of K-ATP channels in cardiac muscle will result in action potential shortening (see Ref. 12 for recent review). We thus have a situation where the effects of activation of K-ATP channels correspond to the required response, i.e., action potential shortening, but where the intracellular conditions apparently required for channel activation, i.e., very low levels of ATP, do not occur at the right time.

We felt that useful information might be obtained not if we asked how sensitive the K-ATP channel was to intracellular ATP but if we asked how sensitive the action potential duration was to activation of K-ATP channels. To this end we have used a 'K-channel opener' [13], avoiding the multifactorial influence of metabolic stress, to determine how much K-ATP channel current was needed to reduce ventricular action potential duration by half. This required the activation of only a very few K-ATP channels which supports the conclusions recently obtained by more indirect methods [14]. We therefore re-examined the question of when K-ATP

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channels might be activated under quasi-ischemic conditions by applying metabolic poisons to intact isolated myocytes.

Materials and Methods

Individual myocytes were obtained from guinea-pig hearts by standard methods [15]. All experiments were performed at room temperature (20–230 °C). Single-channel and whole-cell ion currents and voltages [16] were recorded with a Biologic RK300 amplifier (Grenoble, France) and stored on DAT cassette (Biologic DTR-1200 digital audio tape recorder). Stimulus protocols and data collection were controlled by a microcomputer (S200 interface, Cambridge Research Systems, U.K.; software provide by Intracel, Cambridge, U.K.). Voltage-clamp and single-channel records were replayed onto a Gould 2400 pen recorder (frequency response approx. 150 Hz) whereas action potentials were plotted (Graphtec MP3100 XY plotter, Tokyo, Japan).

Single ion channel open probability was calculated as follows: single-channel unitary current amplitude (i) was obtained from patch current amplitude histograms. The total number of channels present in the membrane patch (N) was recorded from excised inside-out membrane patches. We could thus calculate the maximum possible K-ATP current ($N \cdot i$). For 15 second segments of continuous cell-attached recording the channel-open probability was obtained by comparing the recorded patch current relative to the maximum possible current.

For cell-attached and inside-out membrane patch recording the pipette contained (in mM): NaCl 140; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; Hepes 10: pH was adjusted to 7.4 with NaOH. For cell-attached patches the bath solution contained (in mM): KCl 140; $MgCl_2$ 1.4; EGTA 5; Hepes 10; pH adjusted to 7.4 with KOH. Excised patches were bathed in a zero divalent cation solution which contained (in mM): KCl 140; EDTA 5; Hepes 10; pH adjusted to 7.4 with KOH. It should be noted that none of these solutions contained glucose or other metabolic substrates. Whole-cell recording experiments were performed with a bath solution which contained (in mM): NaCl 140; KCl 5; $CaCl_2$ 2; $MgCl_2$ 1; glucose 10; Hepes 10; pH 7.4 with NaOH, the pipette solution contained (in mM): K-aspartate 80; KCl 60; NaCl 10; $MgCl_2$ 1, EGTA 2; MgATP 2 or 10; Hepes 10; pH 7.4 with KOH.

Whereas 2,4-dinitrophenol (DNP) was directly dissolved in bath solution, rotenone and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) were prepared as stock solutions in dimethyl sulfoxyde (DMSO) and then added to the bath solution. SR 44866 (EMD 52692: Merck Darmstadt), 6-cyano-2,2-dimethyl-4-(1,2-dihydro-2-oxo-1-pyridyl)chromene, was synthesised by Sanofi Recherche (Montpellier, France) and a stock solution (10 mM) was prepared in 100% ethanol.

Results

Effect of K-ATP channel activation upon action potential duration and macroscopic current amplitude

To be able to follow as closely as possible the changes in whole-cell membrane current and action potential duration during activation of K-ATP channels with SR 44866 [13] we developed the following protocol. The cells were voltage-clamped at 0 mV and approximately every 35 s they were switched into 0 pA current-clamp, an action potential was evoked after 5 s and the cells were then returned to voltage-clamp at 0 mV. SR 44866 was only applied to myocytes which showed a stable action potential duration. Fig. 1 illustrates a typical experiment. Under control conditions the outward membrane current measured at 0 mV (I-0 mV) just before switching to current-clamp was 130 pA (Fig. 1B1) and the action potential recorded 5 s later had a duration of 625 ms (Fig. 1A1) measured at 0 mV (APD-0mV). In this example the application of 5 μM SR 44866 resulted in a progressive increase of I-0mV in parallel with a decrease of APD-0 mV which after 0.8, 2.0, 5.5 and 7.2 min were 150, 200, 320 and 395 pA (Fig. 1B2–5) and 495, 270, 58 and 0 ms (Fig. 1A2–5), respectively.

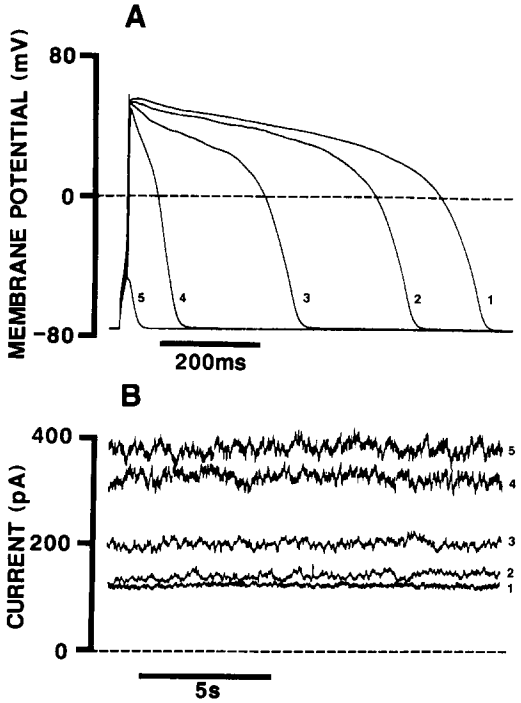

Fig. 1. Effect of 5 μM SR 44866 upon action potentials (A) and macroscopic background currents (B) in an isolated ventricular myocyte. Currents were recorded at a holding potential of 0 mV and approximately 5 s separates the end of each current trace and the corresponding action potential. Current traces and action potentials labelled 1, 2, 3, 4 and 5 were recorded before and 0.8, 2.0, 5.5, and 7.2 min after SR 44866 was applied to the myocyte.

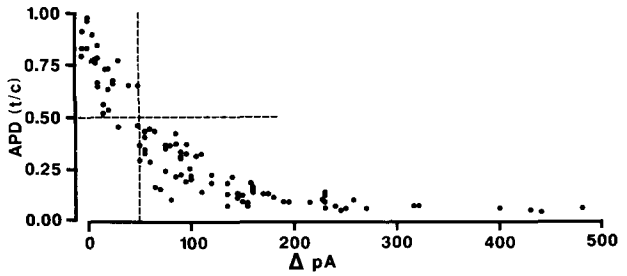

Fig. 2. The relationship between action potential duration (APD) and the membrane current evoked by SR 44866. Both APD and membrane currents were measured at 0 mV. The horizontal scale represents the membrane current increase (ΔpA) induced by 2, 5 or 10 μM SR 44866 applied to different cells. The vertical scale represents APD in test conditions divided by APD in control solution (APD (t/c)). The vertical dashed line marks an increase of 50 pA.

Fig. 2 shows the results obtained from 20 similar experiments conducted with 2, 5 or 10 μM SR 44866. The number of points that could be recorded in each experiment depended upon how quickly SR 44866 induced inexcitability. The *I*-0mV associated with inexcitability varied between 200 and 800 pA in the different myocytes (not shown). Disregarding this variability it is clear that when *I*-0mV was increased by 50 pA the APD-0mV was reduced by more than 50%.

Similar results were obtained from experiments where DNP was used to activate the K-ATP current. These results are not included here since the initial effect of DNP was to increase action potential duration [5,11] before inducing its reduction and often to reduce *I*-0mV before increasing it. This considerably increased variability in the relationship and precluded a clear estimation of the contribution of the K-ATP current. Nevertheless we can report that in quantitative terms, after these initial variations, DNP placed the *I*-0mV : APD-0mV relationship slightly to the right of that shown in Fig. 2 such that APD-0mV was reduced by 50% with a 100 pA increase in *I*-0mV. Clearly this indicates that, in the whole cell, DNP affects more than one current type.

Effect of metabolic inhibitors upon channel activity in intact isolated ventricular myocytes

In this report we confine our attention to 18 cell-attached membrane patches where we were able to record membrane channel activity under control conditions, then in the presence of metabolic inhibitors until the cell went into rigor contracture, followed by excision as inside-out membrane patches. Not surprisingly this was but a small proportion of the attempted experiments. In these experiments no attempt was made to see whether rigor contracture induced by the metabolic poisons was reversible. That we recorded openings of K-ATP channels was confirmed in each experiment by their inhibition upon the application of ATP to the excised patch; the superfusion of ATP had no effect on the ion channels recorded from intact myocytes. Although individ-

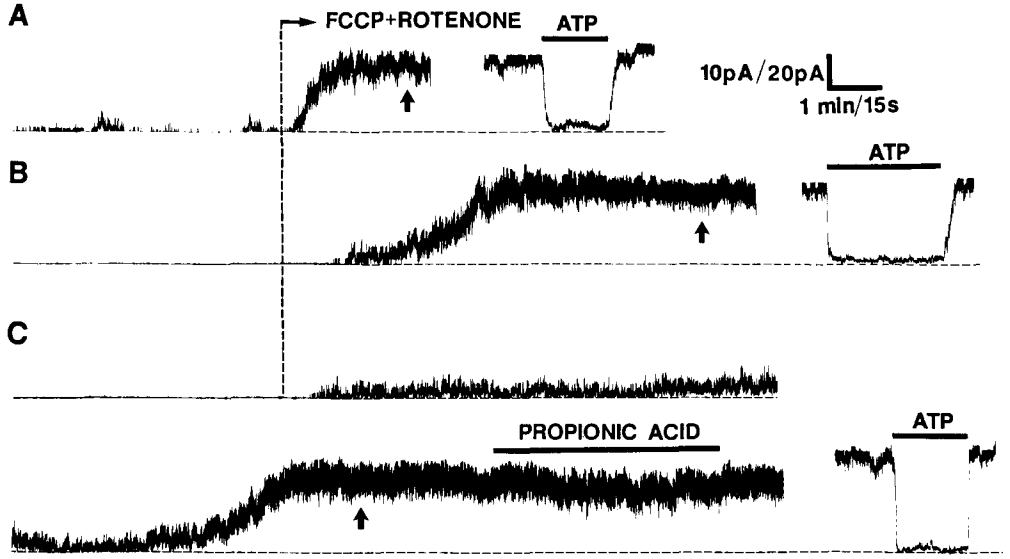

Fig. 3. In situ activation of K-ATP channels by metabolic inhibitors. A, B and C (upper and lower traces) represent continuous recordings of membrane currents recorded at 0 mV obtained from cell-attached membrane patches in three different ventricular myocytes. The vertical dashed line marks the onset of superfusion of these cells with solution which contained 0.5 μM FCCP and 5 μM rotenone. The arrows indicate the time of onset of contracture of each cell observed under the microscope. In C, after cell contracture, the cell was superfused with solution which also contained 20 mM propionic acid (bar above trace). The current records to the right of each cell-attached patch recording were obtained from the same membrane patches after they had been excised from the myocytes into divalent cation free solution where single-channel currents are larger and show the inhibition of channel opening evoked by the application of 2 mM ATP (bars above traces). The horizontal dashed lines indicate patch current levels recorded when all channels were closed. The values associated with the scale bars correspond to the cell-attached (left) and excised patch (right) records, respectively.

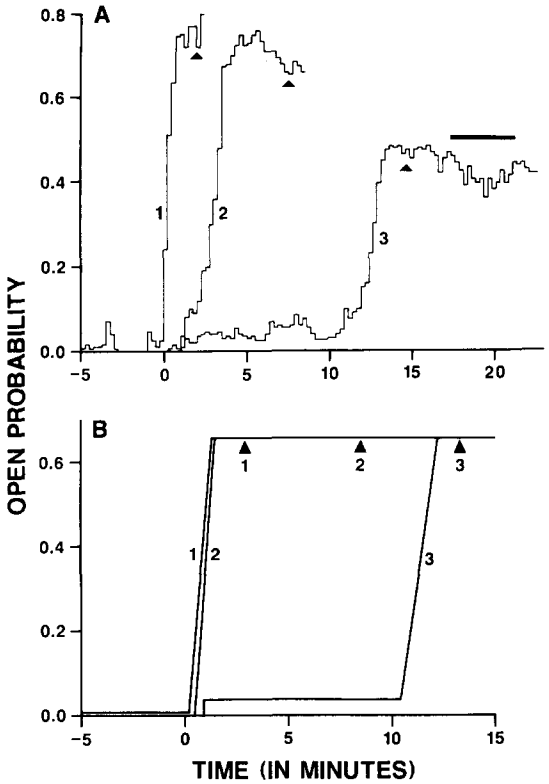

Fig. 4. Analysis of the effects of metabolic inhibition upon K-ATP channels in intact ventricular myocytes. (A) K-ATP channel open-probability calculated over 15-s segments of the channel current records shown in Fig. 3. Histograms 1, 2 and 3 correspond to Figs. 3A, B and C, respectively. FCCP and rotenone were applied at time 0. The arrow heads mark the onset of cell contracture and the horizontal bar marks the superfusion of propionic acid in Fig. 3C. (B) The averaged open probability and average duration of the different phases of the response to metabolic inhibitors. Lines marked 1, 2 and 3 were calculated from 4, 5 and 9 different experiments, respectively, the plateau and contracture (marked by arrow heads) level of channel open probability was calculated from all 18 experiments.

ual experiments showed similar responses to metabolic inhibition, i.e., activation of K-ATP channels [1,2], there were sufficiently marked differences in both the time scale of events and the quantitative nature of the results for us to be able to divide them into three groups.

The first of these we consider to represent 'bad' cells since its principal characteristic was that channel openings were recorded from the myocytes under control conditions (Figs. 3A and 4A1). In these cases the application of metabolic inhibitors evoked, after a very short latency (10–15 s), marked activation of channel openings which rapidly reached a plateau level quickly followed by the onset of cell contracture.

The second type of response, or 'intermediate' cells, did not show channel openings in control solution but the application of metabolic inhibitors also markedly activated channel openings which rapidly reached a plateau level (Figs. 3B and 4A2). These cells were distinguished by the fact that this high level of channel activity was not rapidly followed by cell contracture but it could be sustained for 4–7 min before cell contracture began.

The final, and most frequent form of response is shown in Figs. 3C and 4A3 and we consider it to represent 'good' cells. No channel openings were observed in control solution. The application of metabolic inhibitors evoked channel openings after a latency of approx. 30 s. In contrast to the first two groups these openings were brief and comparatively infrequent. This low level of channel activity was sustained for between 7 and 25 min in different experiments (approx. 12 min in the example shown in Fig. 3C). There was no gradual increase in channel-open probability over this period and it was followed by an abrupt increase in channel openings to a plateau level which was rapidly followed by the onset of cell contracture. In this particular experiment (Fig. 3C) propionic acid was applied to the contracted cell to test whether or not intracellular acidification might inhibit channel opening [17]. It had no obvious effect.

It is obvious that each of these results shares a number of common features. Silence in control conditions except for the 'bad' cells. High level channel open probability before and during contracture in all cells. The surprisingly abrupt transition from silence or low level channel activity to high level channel activity when one might have expected a more gradual transition. We were thus able to divide the 18 recordings into obviously distinct phases such as silent, low level open probability (< 0.1), the plateau high level open probability which included the period of cell contracture, and the time of transition from low to high level channel opening (Fig. 4B). The most important observation is that in the majority of cells channel openings corresponding to an open probability of 1–2% began almost immediately after the application of metabolic poisons and were maintained for a long period in the absence of cell contracture.

Discussion

The results we present here form a quantitative analysis of the reduction of the duration of the ventricular action potential by stimulation of the K-ATP cell current and the activation of K-ATP channels by metabolic inhibition in intact ventricular myocytes. We are thus able to provide precise information about the role of this current in changes in cardiac electrophysiology which may be associated with hypoxic/ischemic conditions.

We demonstrate that an increase of 50 pA of the background current at 0 mV is associated with at least a 50% reduction of action potential duration. We have previously proved that SR 44866 acts by opening K-ATP channels in ventricular myocytes at both single-channel

and whole-cell ion current levels [13], there thus can be no doubting that the current evoked in these experiments corresponds to that flowing through K-ATP channels. When one considers that ventricular myocytes each contain 2000–3000 K-ATP channels [3,18] and that the single-channel current flowing through an individual open K-ATP channel in an intact guinea-pig ventricular myocyte at 0 mV is approx. 1.5 pA [13], 50 pA of whole-cell current corresponds to the simultaneous opening of approx. 30 channels or 1% of the cell population. That the opening of only 30 K-ATP channels results in at least a 50% reduction of action potential duration is explained by the time- and voltage-independence of the K-ATP current [3] which notwithstanding its slight 'inward rectification' [19,20] would provide a significant outward membrane conductance to promote repolarisation from the low conductance of the cardiac action potential plateau [21].

Elliott et al. [11] concluded that the K-ATP channel was not responsible for action potential shortening during hypoxia: (1) because this occurred before they could measure a significant reduction of cellular ATP and (2) because published values for the inhibition of channel opening by 50% required only tens of μM ATP. Our present results clearly show that the cardiac action potential can be regulated by a K-ATP channel open probability of approximately 1% which is not surprising when the opening of less than 10% of their K-ATP current renders isolated myocytes inexcitable [13]. It is thus clear that the current which would flow through 50% of the 3000 K-ATP channels in each ventricular myocyte is unlikely to be physiologically relevant.

Even measurements of the cell content of ATP may not accurately reflect the regulation of the K-ATP channel [22,23]. In this context our quantitative analysis of the activation of K-ATP channels in intact isolated myocytes by metabolic inhibitors has two important points to contribute. First, in each cell, notwithstanding their subsequent response, K-ATP channel activity was recorded within one minute of the application of the metabolic inhibitors which is well in advance of cellular ATP content reaching levels low enough [24] for the published dose-response curves. Second, our most frequent observation (Fig. 3C) was a long period of stable, low open probability channel activity which corresponded exactly with the channel-open probability required for significant action potential shortening. This phase was followed by the abrupt onset of high open probability channel activity and cell contracture. This result is difficult to reconcile with the progressive decline in ATP content measured in intact cells [24]. It seems probable that the final high open probability phase of K-ATP channel activity is associated with the 'whole cell' depletion of ATP since this is recorded during cell rigor contraction (Fig. 3. and [10]). What is happening before this final stage remains to be determined but it is clear from the work of Weiss and Lamp [22,23] that metabolic processes closely associated with the cell membrane are likely to be involved.

Our subdivision of the results obtained from cell-attached recordings of individual myocytes and our designation of those cells as 'good' or 'bad' is of course entirely subjective. It is based upon the assumption that a 'good' cell would be one whose metabolism is intact. A 'bad' cell would be one with a damaged or impaired metabolism. We cannot say whether this variability might be reflected in the intact heart but we consider it more likely that it reflects variation in the extent of cell viability after the isolation procedure and maintenance in the absence of metabolic substrates. A similar heterogeneity, subdivided into three groups, has been described for isolated rabbit ventricular myocytes [25]. We did not observe such distinct grouping in whole-cell recording experiments, presumably because we flood the cell with ATP from the pipette solution and thus counteract any significant variation in cellular ATP content.

In conclusion let us emphasise the points that we make in this report. We are not suggesting that the K-ATP channel controls the duration of the cardiac action potential, we are not even suggesting an active role for this channel in cardiac muscle under the best of physiological conditions though this may occur in less than optimal conditions [26]. We are indicating, with quantitative evidence, that when this current does intervene it does so either with a very low open probability evenly distributed over the cell population of channels or the simultaneous opening of a small number of channels. This is not an original proposition. Cook, Satin, Ashford and Hales [27] proposed the 'spare channel hypothesis' for the K-ATP channel of insulin-secreting cells where there was also an apparent discrepancy between K-ATP channel sensitivity to ATP, B-cell ATP content and the low open probability channel activity recorded in intact cells. Their hypothesis may also be applicable to the K-ATP channel in cardiac muscle. The application of the parameters of the K-ATP channel current to a computer model of the rat ventricular action potential indicated that significant shortening would result from changes in ATP content in the millimolar range [14]. This supports our data showing that it is either very low K-ATP channel open probabilities or the opening of only a small number of the cells K-ATP channels that is of physiological importance.

Acknowledgements

We thank Prof. E. Coraboeuf for critical reading of this text, Dr. P. Gautier of Sanofi Recherche, Montpellier, France for supplying us with SR 44866, Gerard Sadoc for developing some of the software used in this

study and Paulette Richer for preparing the isolated cells.

References

1 Noma, A. (1983) Nature 305, 147–148.
2 Trube, G. and Hescheler, J. (1984) Pflügers Arch. 401. 178–184.
3 Noma, A. and Shibasaki, T. (1985) J. Physiol. 363, 463–480.
4 Vleugels, A., Vereecke, J. and Carmeliet, E. (1980) Circ. Res. 47, 501–508.
5 Isenberg, G., Vereecke, J., Van der Heyden, G. and Carmeliet, E. (1983) Pflügers Arch. 397, 251–259.
6 Tanigushi, J., Noma, A. and Irisawa, H. (1983) Circ. Res. 53, 131–139.
7 Kakei, M., Noma, A. and Shibasaki, T. (1985) J. Physiol. 363, 441–462.
8 Findlay, I. (1988) Pflügers Arch. 412, 37–41.
9 Lederer, W.J. and Nichols, C.G. (1989) J. Physiol. 419, 193–211.
10 Lederer, W.J. and Nichols, C.G. (1988) J. Physiol. 407, 98P.
11 Elliott, A.C., Smith, G.L. and Allen, D.G. (1989) Circ. Res. 64, 583–591.
12 Quast, U. and Cook, N.S. (1989) Trends Pharmacol. Sci. 10, 431–435.
13 Findlay, I., Deroubaix, E., Guiraudou, P. and Coraboeuf, E. (1989) Am. J. Physiol. 257, H1551–H1559.
14 Nichols, C.G. and Lederer, W.J. (1990) J. Physiol. 423, 91–110.
15 Mitra, R. and Morad, M. (1985) Am. J. Physiol. 249, H1056–H1060.
16 Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflügers Arch. 391, 85–100.
17 Misler, S., Gillis, K. and Tabcharani, J. (1989) J. Membr. Biol. 109, 135–143.
18 Belles, B., Hescheler, J. and Trube, G. (1987) Pflügers Arch. 409, 582–588.
19 Horie, M., Irisawa, H. and Noma, A. (1987) J. Physiol. 387, 251–272.
20 Findlay, I. (1987) Pflügers Arch. 410, 313–320.
21 Weidmann, S. (1951) J. Physiol. 115, 227–236.
22 Weiss, J.N. and Lamp, S.T. (1987) Science 238, 67–69.
23 Weiss, J.N. and Lamp, S.T. (1989) J. Gen. Physiol. 94, 911–935.
24 Haworth, R.A., Nicolaus, A., Goknur, A.B. and Berkoff, H.A. (1988) J. Mol. Cell. Cardiol. 20, 837–846.
25 Masuda, M.O., de Magalhaes Engel, G. and Barbosa Moreira, A.P. (1987) J. Mol. Cell. Cardiol. 19, 831–839.
26 Faivre, J.-F. and Findlay, I. (1989) Biochim. Biophys. Acta 984, 1–5.
27 Cook, D.L., Satin, L.S., Ashford, M.L.J. and Hales, C.N. (1988) Diabetes 37, 495–498.