Single cardiac outwardly rectifying K⁺ channels modulated by protein kinase A and a G-protein

I. Benz, U. Fröbe, and M. Kohlhardt

Physiologisches Institut, Universität Freiburg, Hermann-Herder-Strasse 7, W-7800 Freiburg, Federal Republic of Germany

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Abstract. Elementary K⁺ currents were recorded at 19 °C in cell-attached and in inside-out patches excised from neonatal rat heart myocytes. An outwardly rectifying K⁺ channel which prevented Na⁺ ions from permeating could be detected in about 10% of the patches attaining (at 5 mmol/l external K⁺ and between -20 mV and +20 mV) a unitary conductance of 66 ± 3.9 pS. K⁺ channels have one open and at least two closed states. Open probability and t̄open rose steeply on shifting the membrane potential in the positive direction, thereby tending to saturate. Open probability (at -7 mV) was as low as 3 ± 1% but increased several-fold on exposing the cytoplasmic surface to Mg-ATP (100 μmol/l) without a concomitant change of t̄open. No channel activation occurred in response to ATP in the absence of cytoplasmic Mg⁺⁺. The cytoplasmic administration of the catalytic subunit of protein kinase A (120-150 μg/ml) or GTP-γ-S (100 μmol/l) caused a similar channel activation. GDP-β-S (100 μmol/l) was also tested and found to be ineffective in this respect. This suggests that cardiac K⁺ channels are metabolically modulated by both cAMP-dependent phosphorylation and a G-protein.

Key words: Cardiac K⁺ channels – Phosphorylation – GTP – GDP – Neonatal rat heart myocytes

Introduction

Cardiac K⁺ channels represent a family of ionic channels with distinctly heterogeneous elementary properties that are intimately involved in controlling heart muscle function by setting the resting potential and by influencing the shape of the cardiac action potential. Besides acetylcholine and ATP (Noma 1983), β-adrenergic catecholamines are another physiological modulator of K⁺ channel activity and exert their activating effect via the formation of cAMP (for review see Szabo and Otero 1990). As shown in voltage-clamped cardiac Purkinje fibres and isolated myocytes, K⁺ conductances sensitive to β-adrenergic stimulation comprise the inwardly rectifying K⁺ current (Gadsby 1983), the transient outward current (Nakayama and Fozzard 1988), and the delayed rectifier K⁺ current (Walsh et al. 1988). Since, surprisingly, the stimulatory influence of β-adrenergic catecholamines and protein kinase A have been reported to be temperature-dependent (Walsh et al. 1988; Walsh and Kass 1988), at least the delayed rectifier K⁺ current seems to be controlled by a cascade of molecular events which are not identical in all respects with the cAMP-dependent phosphorylation in other ionic channels.

Based on permeation properties, two classes of cardiac K⁺ channels can be discriminated, an inwardly rectifying K⁺ channel with a conductance of 3.6 pS at 5.4 mmol/l external K⁺ (Sakmann and Trube 1984) and a non-rectifying K⁺ channel with a conductance of about 15 pS at a physiological external K⁺ concentration reported to exist in embryonic avian (Clapham and Logothetis 1988) and adult mammalian (Yue and Marban 1988) heart muscle. The latter type represents the candidate which mediates the K⁺ conductance during the repolarization phase of the cardiac action potential.

The present patch clamp experiments with neonatal rat heart myocytes newly identified a cardiac K⁺ channel with outwardly rectifying properties. Evidence will be represented that its activity is metabolically regulated in that both cAMP-dependent phosphorylation and a G-protein open this K⁺ channel.

Methods

Elementary K⁺ currents were recorded in cell-attached and in inside-out patches excised from cultured neonatal rat heart myocytes by employing the standard patch clamp technique (Hamill et al. 1981). Cell disaggregation and the handling of the short-time (18–24 h) cultured myocytes have been already described in detail (Kohlhardt et al. 1986). To avoid spontaneous activity, the
myocytes were kept in an isotonic K⁺ solution buffered with EGTA so that nominally Ca²⁺-free conditions were achieved. Before excision, the patches were kept for at least 5 min in the cell-attached configuration. Patch pipettes were fabricated from borosilicate glass and had resistances (after filling with pipette solution) between 5 and 8 MΩ. This glass type is of advantage since it has been reported to minimize artifacts in the shape of the iv-relationship (Cota and Armstrong 1988).

Two protocols were applied to trigger elementary K⁺ currents: (i) setting the membrane potential to values positive or negative to \( E_{\text{rev}} \); (ii) stepping the membrane from \( E_{\text{rev}} \) to -10 mV or +33 mV for 370 ms at a rate of 0.2 Hz. In the latter case, the records were idealized by subtracting capacity and leakage currents. The patch clamp recordings were filtered at 1 kHz with an 8-pole Bessel filter, stored on tape and subsequently digitized with a sampling rate of 5 kHz in order to be analyzed. The dead time was 0.2 ms. The single channel analysis concentrated on the open probability \( (P_0) \) and on the open and closed time. In analyzing the channel kinetics, the 50% unitary current method (Colquhoun and Sigworth 1983) was used. Histograms were constructed from non-overlapping single channel events and fitted by the least square \( (x^2) \) method to yield \( \tau_{\text{open}} \) and \( \tau_{\text{closed}} \), respectively. \( P_0 \) was determined for periods of 2 s in duration in order to give a \( P_0 \) profile over the whole life time of an individual patch. The unitary current size was determined from opening events with a minimum life time of 2 ms.

Whenever possible, the data are expressed as mean ± SEM.

### Solutions (composition in mmol/l)

**A.** Isotonic K⁺ solution (facing the cytoplasmic membrane surface of the inside-out patches): KCl 140; MgCl₂ 2; glucose 20; Hepes 10; EGTA 2; pH 7.4; temperature 19 ± 0.5 °C. **B.** Pipette solution (facing the external membrane surface): KCl 5; NaCl 135; MgCl₂ 2; Hepes 10; pH 7.4.

### Compounds

All compounds (ATP, catalytic subunit of protein kinase A, GTP-γ-S, GDP-β-S) were purchased from Sigma Chemie, Munich, and freshly dissolved in isotonic K⁺ solution just before use. A microinjection device was employed in order to change the internal solution facing the cytoplasmic membrane surface in a jump-like fashion.

### Results and discussion

Under quasi-physiological conditions, i.e. in the presence of an asymmetrical K⁺ concentration across the membrane with 5 mmol/l at the external side, single K⁺ channel events could be detected in the cell-attached as well as in the inside-out mode, which differed in elementary current size and in open time from ATP-sensitive K⁺ channels, the latter being the most abundant K⁺ channel observed in the present recording conditions. At -7 mV, for example, \( i_{\text{out}} \) of the K⁺ channel was 1.6 ± 0.08 pA, in contrast to 2.5 ± 0.11 pA of these particular openings. They reflect a K⁺ channel with outward-rectifying properties and were seen in both spherocytes and cardiocytes with a rod-shaped morphology, the latter reflecting a more advanced developmental stage during cell culture. This particular K⁺ channel was present in only 10% of a total of about 200 patches. Whether this low incidence is related to the special cell type, neonatal cardiocytes, remains still to be clarified.

Figure 1 demonstrates that outward rectification becomes prominent in a potential range of about 40 mV more positive that the reversal potential, \( E_{\text{rev}} \). In this inside-out experiment, \( E_{\text{rev}} \) was determined to be -85 mV which coincides with the value for \( E_{\text{rev}} \) calculated from the

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**Fig. 1.** Elementary K⁺ currents through cardiac K⁺ (outw.-rect.) channels. Left part: Records of elementary K⁺ currents at different membrane potentials; upward deflections indicate channel openings. C indicates the closed channel state. Right part: Current-voltage relationship obtained under asymmetric cationic conditions: 5 mmol/l K⁺/140 mmol/l K⁺; 0 mmol/l Na⁺/135 mmol/l Na⁺. The curve was drawn by eye. Patch 184. Inside-out recording conditions.