Eur Biophys J (1992) 21, 299–302

European Biophysics Journal

Differential sensitivity of cardiac $K^+_{(ATP)}$ channels to guanine nucleotides – evidence for a heterogeneous channel population

I. Benz and M. Kohlhardt*

Physiological Institute of the University Freiburg, Hermann-Herder-Str. 7, W-7800 Freiburg/Br., Federal Republic of Germany

Received March 7, 1992/Accepted in revised form May 29, 1992

Abstract. In cell-free patches from cultured neonatal rat cardiocytes, the cytosolic presence of GTP-γ-S (100 μmol/l) or GDP-β-S (100 μmol/l) activated $K^+_{(ATP)}$ channels. GTP-γ-S required cytosolic Mg^{++}, suggesting that an activated G-protein causes the increase in open probability. The great variations of the channel response to GTP-γ-S and GDP-β-S indicates that cardiac $K^+_{(ATP)}$ channels represent a heterogeneous family.

Key words: Cardiac $K^+_{(ATP)}$ channels – GTP – GDP – Heart muscle

Introduction

ATP-sensitive K^+ channels initially detected in heart muscle (Noma 1983) are distributed in a great variety of tissues including brain, pancreatic insulin-secreting B cells, skeletal and smooth muscle. Although ATP depletion opens $K^+_{(ATP)}$ channels, thus coupling membrane potential to cellular energy metabolism, ATP fluctuations do not act alone to modulate channel gating since channel activation requires a phosphorylation reaction of the channel protein, most likely at its cytoplasmic surface (for review see Ashcroft 1988; Noma and Takano 1991). GTP and other guanine nucleotides represent another group of modulators (Parent and Coronado 1989; Dunne and Petersen 1986), but, in heart muscle, the GTP influence seems controversial and has been reported to be inhibitory (Kakei et al. 1985; Lederer and Nichols 1989) or activating (Kirsch et al. 1990) even under well-controlled cell-free and comparable biochemical conditions.

In re-examining the channel responsiveness to GTP-γ-S and GDP-β-S in cell-free conditions, the present patch clamp experiments provide evidence that neonatal cardiac $K^+_{(ATP)}$ channels represent a heterogeneous population with distinctly different guanine nucleotide sensitivity.

* *Correspondence to:* M. Kohlhardt

Methods

Elementary K^+ currents through single $K^+_{(ATP)}$ channels were recorded in inside-out patches from cultured neonatal rat cardiocytes. Cultivating and handling of the short-time (18–24 hours) cultured cardiocytes were essentially the same as already described in detail (Kohlhardt et al. 1986). The patch clamp recordings were filtered at 1 kHz, stored on tape, and digitized with a sampling rate of 5 kHz. Single channel analysis concentrated on open probability (P_0; analysed for periods of 30 s over the whole experiment to give a P_0 profile), unitary current size, open state and closed state kinetics. The latter were obtained from open and closed time histograms constructed from non-overlapping single events and fitted by the least squares method.

Solutions (composition in mmol/l): A. Isotonic K^+ solution (facing the cytosolic membrane surface): KCl 140; $MgCl_2$ 2; glucose 20; Hepes 10; EGTA 2; pH 7.4; temperature 19 ± 0.5 °C. B. Pipette solution (facing the external side of the membrane): KCl 5; NaCl 135; $MgCl_2$ 2; Hepes 10; pH 7.4. Compounds: GTP-γ-S and GDP-β-S were purchased from Sigma Chemie, München. A microinjection device was used to change the cytosolic solution facing the inside-out patches in a jump-like fashion.

Results and discussion

$K^+_{(ATP)}$ channels from neonatal cardiocytes were found to have an unitary conductance of 23 ± 2.4 pS (at 5 mmol/l external K^+; $n = 5$). Both open and closed time histograms could be best fitted by the sum of two exponentials, indicating the existence of two open and two closed states. $K^+_{(ATP)}$ channels became activated on patch excision in an ATP-free environment but were blocked in the cytosolic presence of 0.5–1 mmol/l ATP. Run-down develops in cell-free conditions (Nichols and Lederer 1991) within a few minutes and significantly suppresses or, in the present inside-out experiments, mostly abolish-

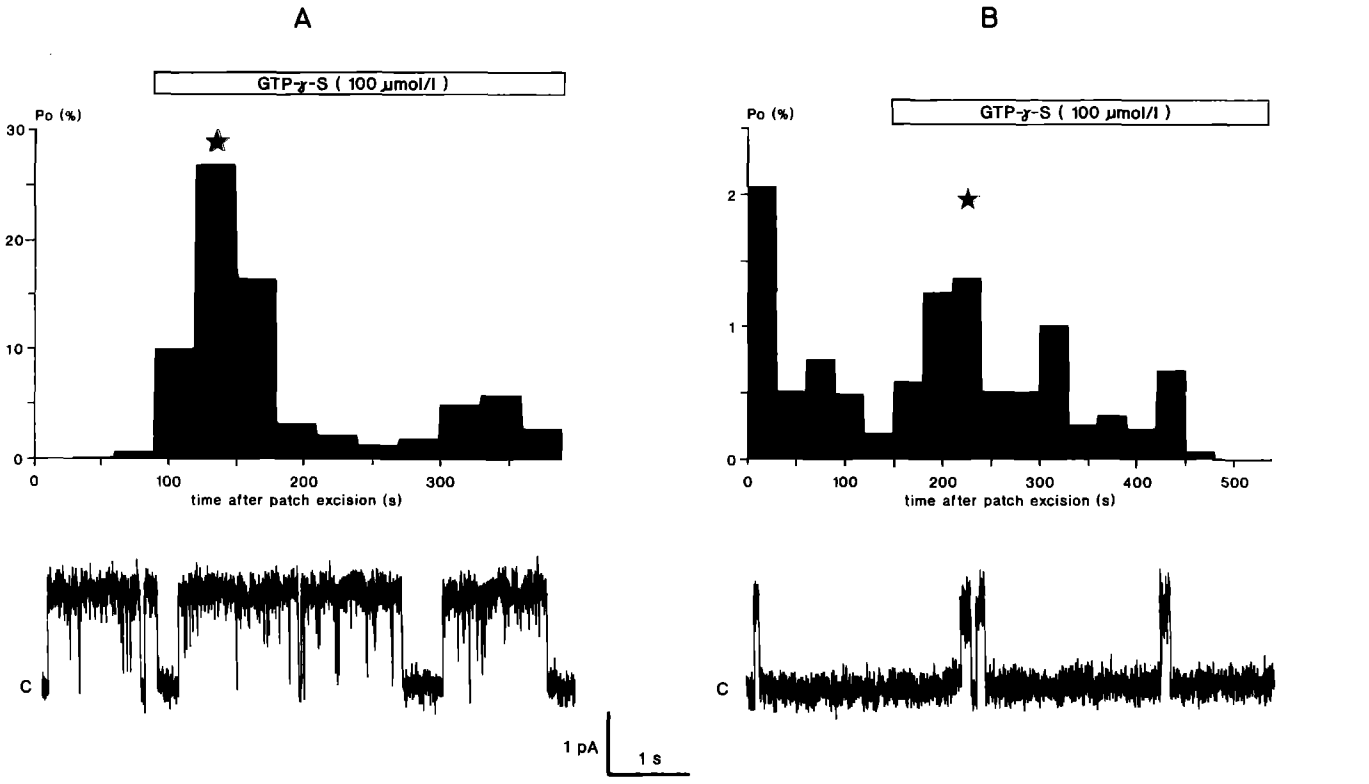

Fig. 1 A, B. Differential activation of isolated cardiac $K^+_{(ATP)}$ channels by cytosolic GTP-γ-S (100 µmol/l). P_0 profiles (upper part in **A** and **B**) and records (lower part in **A** and **B**) of elementary K^+ currents through $K^+_{(ATP)}$ channels (upward deflections mean outward current and channel opening, respectively; *c* indicates the closed channel state). The records are representative for the time when the maximal P_0 was attained (indicated by an asterisk in the P_0 profiles). Parts **A** and **B** illustrate two individual experiments (exp 327IO and exp 328IO). Membrane potential −7 mV

es channel activity. The effects of cytoplasmically applied guanine nucleotides were studied at a test potential of −7 mV.

The non-hydrolyzable GTP analogue GTP-γ-S (100 µmol/l) restored, within 30–40 seconds, the activity of cardiac $K^+_{(ATP)}$ channels (Fig. 1). This channel activation developed in the absence of phosphorylating conditions but required the cytosolic presence of Mg^{++}: when administered in the absence of Mg^{++}, 100 µmol/l GTP-γ-S was found in 3 experiments to be unable to reactivate $K^+_{(ATP)}$ channels. This suggests that an activated G-protein is involved in channel activation. Likewise in neonatal rat cardiocytes, Kirsch et al. (1990) identified a G_i-protein which might couple adenosine receptors to cardiac $K^+_{(ATP)}$ channels. Surprisingly, $K^+_{(ATP)}$ channels in adult myocytes have been reported not to respond with an increase in open probability when exposed to these guanine nucleotides (Kakei et al. 1985; Lederer and Nichols 1989). Despite the continuous cytosolic GTP-γ-S presence, however, the $K^+_{(ATP)}$ channels could not maintain a stable activity since P_0 significantly declined with time after attaining a maximum (see Fig. 1). In all 8 experiments with GTP-γ-S, P_0 finally approached a level of 0%.

Although studied under carefully controlled cell-free conditions, GTP-γ-S (100 µmol/l) exerted distinctly different activating effects since the maximal P_0 was found to vary in a broad range, between 0.6% and 44%. Two types of responses could be distinguished, one of them showing a $P_{0\,(max)}$ of $1.2 \pm 0.3\%$ ($n=4$) and the other type with a $P_{0\,(max)}$ of $25 \pm 7.3\%$ ($n=4$). A heterogeneous developmental stage of the cultured cardiocytes is unlikely to be the reason for this differential GTP-γ-S sensitivity of the $K^+_{(ATP)}$ channels since from the two cell populations in culture, spherocytes and rod-shaped cardiocytes, only the latter were used for the present experiments. Even cardiocytes from the same culture contain $K^+_{(ATP)}$ channels with a high ($P_{0\,(max)}$ 27%; Fig. 1 A) or a low ($P_{0\,(max)}$ 1.4%; Fig. 1 B) sensitivity to GTP-γ-S. This is mirrored in open state kinetics: whilst $\tau_{open\,(1)}$ was almost identical (0.43 ms and 0.74 ms, respectively), $\tau_{open\,(2)}$ was several-fold larger in highly GTP-γ-S sensitive channels (16.3 ms) when compared with the less GTP-γ-S sensitive type (5.3 ms).

Although neonatal rat heart cells are capable of expressing a G_i-protein and incorporating it in the sarcolemma, this G_i-protein has been reported to be non-functional (Allen et al. 1988). To exclude the possibility that the differential GTP-γ-S sensitivity of the $K^+_{(ATP)}$ channel could be related to a biochemical peculiarity, the responsiveness to the non-hydrolyzable GDP-β-S was examined. GDP-β-S activates $K^+_{(ATP)}$ channels in adult myocytes (Lederer and Nichols 1989; Tung and Kurachi 1991), frog atrial cells (Pilsudski et al. 1990) and in pancreatic insulin-secreting B cells (Dunne and Petersen 1986) but the significance of cytosolic Mg^{++} ions for this effect is still controversial. In the simultaneous presence of 2 mmol/l Mg^{++}, the cytosolic administration of

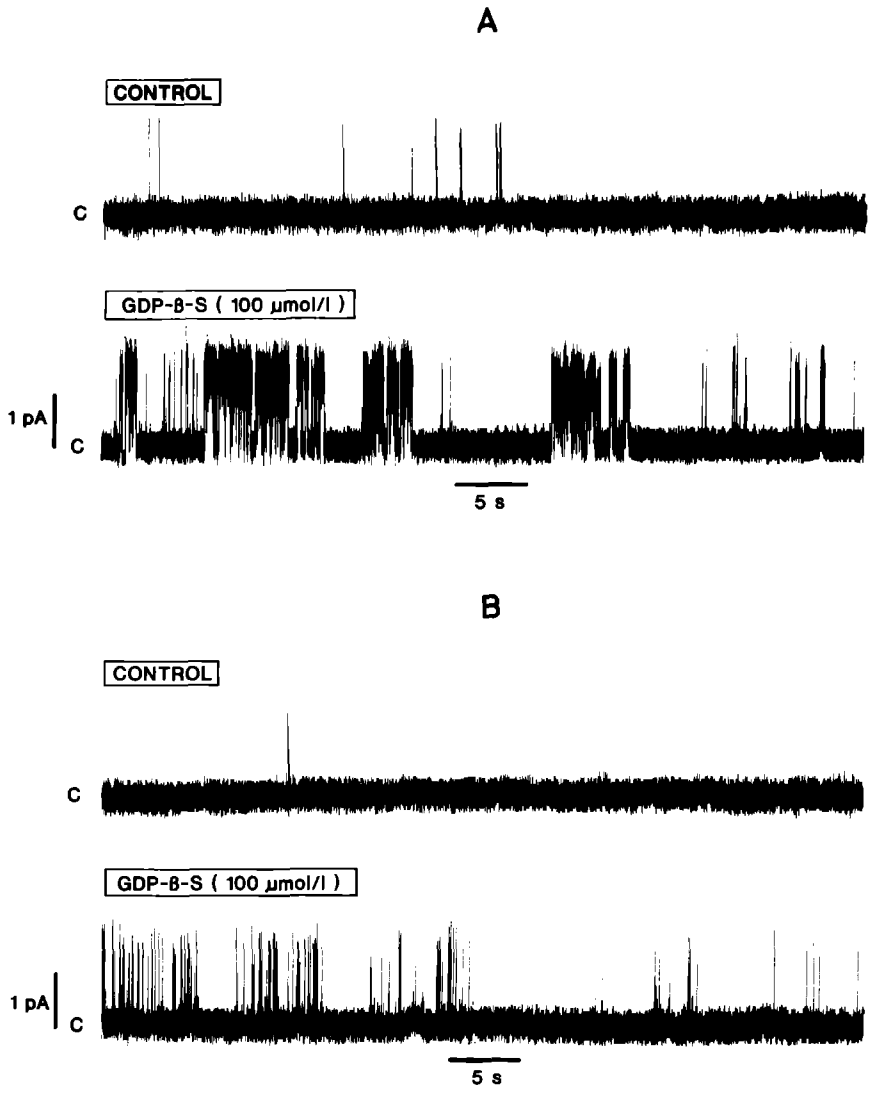

Fig. 2A, B. Differential activation of isolated cardiac $K^+_{(ATP)}$ channels by cytosolic GDP-β-S (100 µmol/l) in two individual inside-out patches (part **A**: exp 174IO; part **B** exp 175IO). The upper record of elementary K^+ currents in part **A** and **B** was taken under control conditions, after development of the run-down, and the lower part in **A** and **B** in the cytosolic presence of GDP-β-S. Upward deflections: channel opening, *c* indicates the closed channel state. Membrane potential −7 mV

100 µmol/l GDP-β-S was found to restore channel activity, likewise under conditions where $K^+_{(ATP)}$ channels may be supposed to be dephosphorylated (Fig. 2). GDP-β-S failed to induce sustained channel activity since P_0 decreased with time. More important is the result that $P_{0\,(max)}$ differed greatly from one experiment to another: in the experiments illustrated in Fig. 2A and 2B, for example, $P_{0\,(max)}$ was 16% and 3%, respectively. The mean values for $P_{0\,(max)}$ were $16 \pm 2\%$ ($n=2$) and $3.0 \pm 0.5\%$ ($n=3$), respectively.

The different sensitivities to stimulating guanine nucleotides indicates that cardiac $K^+_{(ATP)}$ channels represent a heterogeneous family. Further support for this conclusion arises from the considerable variation in their ATP sensitivity recently reported in adult myocardial cells from rats (Findlay and Faivre 1991). A heterogeneous $K^+_{(ATP)}$ channel family is consistent with observations in metabolically exhausted cardiocytes where only a small percentage of the $K^+_{(ATP)}$ channels installed in the sarcolemma is involved in the shortening of the action potential (Faivre and Findlay 1990) and also provides the basis of the spare channel hypothesis (Cook et al. 1988). Since guanine nucleotides and also ATP evoke different molecular reactions to modulate $K^+_{(ATP)}$ channels, the non-uniform channel sensitivity to all of these cytosolic influences is difficult to explain with a structural variation in one and the same regulatory site of the $K^+_{(ATP)}$ channel.

References

Allen IS, Gaa ST, Rogers TB (1988) Changes in expression of a functional G_i protein in cultured rat heart cells. Am J Physiol 255:C51–C59

Ashcroft FM (1988) Adenosine 5′-triphosphate-sensitive potassium channels. Annu Rev Neurosci 11:97–118

Cook DL, Satin LS, Ashford MLJ, Hales CN (1988) ATP-sensitive K^+ channels in pancreatic β-cells. Diabetes 37:495–498

Dunne MJ, Petersen OH (1986) GTP and GDP activation of K^+ channels that can be inhibited by ATP. Pflügers Arch 407:564–565

Faivre JF, Findlay I (1990) Action potential duration and activation of ATP-sensitive potassium current in isolated guinea-pig ventricular myocytes. Biochim Biophys Acta 1029:167–172

Findlay I, Faivre JF (1991) ATP-sensitive K channels in heart muscle. Spare channels. FEBS Letters 279:95–97

Kakei M, Noma A, Shibasaki T (1985) Properties of adenosine-triphosphate regulated potassium channels in guinea-pig ventricular cells. J Physiol 363:441–462

Kirsch GE, Codina J, Birnbaumer L, Brown AM (1990) Coupling of ATP-sensitive K^+ channels to A_1 receptors by G proteins in rat ventricular myocytes. Am J Physiol 259:H820–H826

Kohlhardt M, Fröbe U, Herzig JW (1986) Modification of single cardiac Na^+ channels by DPI 201–206. J Membrane Biol 89:163–172

Lederer WJ, Nichols CG (1989) Nucleotide modulation of the activity of rat heart ATP-sensitive K^+ channels in isolated membrane patches. J Physiol 419:193–211

Nichols CG, Lederer WJ (1991) Adenosine triphosphate-sensitive potassium channels in the cardiovascular system. Am J Physiol 261:H1675–H1686

Noma A (1983) ATP-regulated K^+ channels in cardiac muscle. Nature 305:147–148

Noma A, Takano M (1991) The ATP-sensitive K^+ channel. Jpn Physiol 41:177–187

Parent L, Coronado R (1989) Reconstitution of the ATP-sensitive potassium channel of skeletal muscle. J Gen Physiol 94:445–463

Pilsudski R, Rougier O, Tourneur Y (1990) Reversible activation of the ATP-dependent potassium current with dialysis of frog atrial cell by micromolar concentrations of GDP. J Membrane Biol 117:223–231

Tung RT, Kurachi Y (1991) On the mechanism of nucleotide diphosphate activation of the ATP-sensitive K^+ channel in ventricular cell of guinea pig. J Physiol 437:239–256