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Short communication

Imidazoline compounds inhibit K_{ATP} channels in guinea pig ventricular myocytes

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Abstract

Phentolamine and related imidazolines inhibit K_{ATP} channel activity in the pancreatic β cell. In the present study, the effects of several imidazoline-based compounds were examined upon K_{ATP} channel activity in guinea pig ventricular myocytes. Phentolamine produced a potent inhibition of K_{ATP} channel activity when examined in either excised inside-out patches or in the whole-cell configuration. This effect was unrelated to phentolamine's ability to antagonise α-adrenoceptors since the non-selective α-adrenoceptor antagonists, benextramine and phenoxybenzamine, failed to affect channel activity. Furthermore, the α-adrenoceptor agonist clonidine together with several related imidazolines inhibited channel activity. This suggests that imidazoline compounds modulate K_{ATP} channel activity in guinea pig ventricular myocytes and this may have clinical implications for the use of such agents as hypoglycemic drugs.

Keywords: K_{ATP} channel; Imidazoline; Cardiac myocyte; (Guinea-pig)

1. Introduction

The ATP-sensitive K^+ (K_{ATP}) channel is present in a wide range of tissues and is believed to play a role in the coupling between cellular metabolic status and excitability (Ashcroft and Ashcroft, 1990). In the heart, the K_{ATP} channel is normally quiescent but opens during ischemic episodes and may protect the compromised myocardium (Cole et al., 1991; Galiñanes et al., 1992).

The non-selective α-adrenoceptor antagonist, phentolamine, and structurally related imidazoline compounds, block K_{ATP} channel activity in the pancreatic β cell (Shulz and Hasselblatt, 1989). Though such compounds may have therapeutic potential as hypoglycemic agents (Dunne, 1990), their specificity for the β cell K_{ATP} channel has not been established.

In a recent study, phentolamine was shown to inhibit K_{ATP} channel activity in ventricular myocytes (Wilde et al., 1994). Therefore, we investigated whether this effect is mediated by a putative imidazoline receptor and is unrelated to the compound's ability to act as an α-adrenoceptor antagonist. The results are consistent with channel inhibition independent of α-adrenoceptor interaction. Moreover, other compounds which contain an imidazoline ring within their structure are shown to inhibit K_{ATP} channel activity in this tissue.

2. Materials and methods

2.1. Ventricular myocyte isolation

Guinea pig ventricular myocytes were isolated as previously described (Zygmunt and Maylie, 1990). Hearts were perfused retrograde with 100% O_2-Ca^{2+}-free-Tyrode solution which contained (in mM): NaCl 140.0, KCl 5.4, $MgCl_2$ 1.0, glucose 10.0, Hepes 10.0, (adjusted with NaOH to pH 7.35) for 5 min. The solution was changed to one containing collagenase

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(type II, 275 units/ml; Worthington Biochemical Corp., Freehold, NJ, USA) and protease (type XIV, 0.8 units/ml; Sigma Chemical Co., St. Louis, MO, USA) for 7 min followed by 0.1 mM Ca^{2+}-Tyrode solution for 10 min. Ventricles were dissected, minced and placed in 0.1 mM Ca^{2+}-Tyrode solution. Cells were studied within 8 h after dissociation.

2.2. Single channel studies

For inside-out patch recordings, the patch pipette contained (in mM): KCl 140.0, $MgCl_2$ 1.0, $CaCl_2$ 2.0, Hepes 10.0 (pH 7.2 with KOH) while the bath (intracellular) solution contained (in mM); KCl 140.0, $MgCl_2$ 1.0, $CaCl_2$ 2.0, EGTA 10.0, Hepes 10.0 (pH 7.2 with KOH). Single channel events were detected using an Axopatch 1B patch-clamp amplifier with storage on video tape. Single channel current analysis was performed offline using the analysis program PAT 6.2 (Dempster, 1993). Data segments between 30 s and 90 s in duration were replayed at recorded speed, filtered at 1 kHz with a Bessel filter and digitised at 5 kHz with a Labmaster DMA A/D converter. The average channel activity, $N_f \cdot P_o$, where N_f is the number of functional channels and P_o is the open state probability, was determined by measuring the total time spent at each unitary current level and expressed as a proportion of the total segment time (Lee et al., 1994).

2.3. Whole-cell studies

Whole-cell currents were recorded from Ca^{2+}-tolerant myocytes using patch electrodes (A-M Systems, Everett, WA, USA) with resistances of 2.0–2.5 MΩ when filled with an ATP-free internal solution (in mM): Kaspartate 90.0, KCl 40.0, NaCl 10.0, $MgCl_2$ 1.0, $CaCl_2$ 1.0, EGTA 10.0, Hepes 10.0 (adjusted with KOH to pH 7.1). Whole-cell currents were recorded with an EPC-9 amplifier using Pulse (Heka Electronics) with data storage on a Macintosh Quadra 800 (Apple Computer). Capacitive transients and series resistance were electronically compensated prior to data storage.

Cells were perfused at a constant rate of 1.5 ml/min with 1.8 mM Ca^{2+}-Tyrode solution at room temperature. Myocytes were held at a membrane potential of −75 mV and voltage ramps from −100 mV to 0 mV at 100 mV/s recorded every minute to assess onset of K_{ATP} channel activity. Current-voltage relationships were measured between −100 and 0 mV at 10 mV increments with 100 ms pulses.

With the ATP-free internal solution, there was run-down of Ca^{2+} channel activity within 5 min of whole-cell formation. Phentolamine effects were thus quantified at 0 mV before (I) and during drug exposure (I_p). The concentration-inhibition curve was fitted by non-linear regression to: $I/I_p = 1/(1 + ([P]/IC_{50})^n)$, where [P] is phentolamine concentration, IC_{50} is half-maximal inhibitory concentration, and n is the Hill coefficient.

All drugs were obtained from Sigma with the exception of phenoxybenzamine which was obtained from Research Biochemicals International (Natick, MA, USA). Drugs were made freshly on the day of the experiment. Phentolamine, idazoxan and clonidine (100 mM) in dimethyl sulfoxide (DMSO); antazoline (50 mM) in distilled water; benextramine and phenoxybenzamine (100 mM) in 0.1 M KOH.

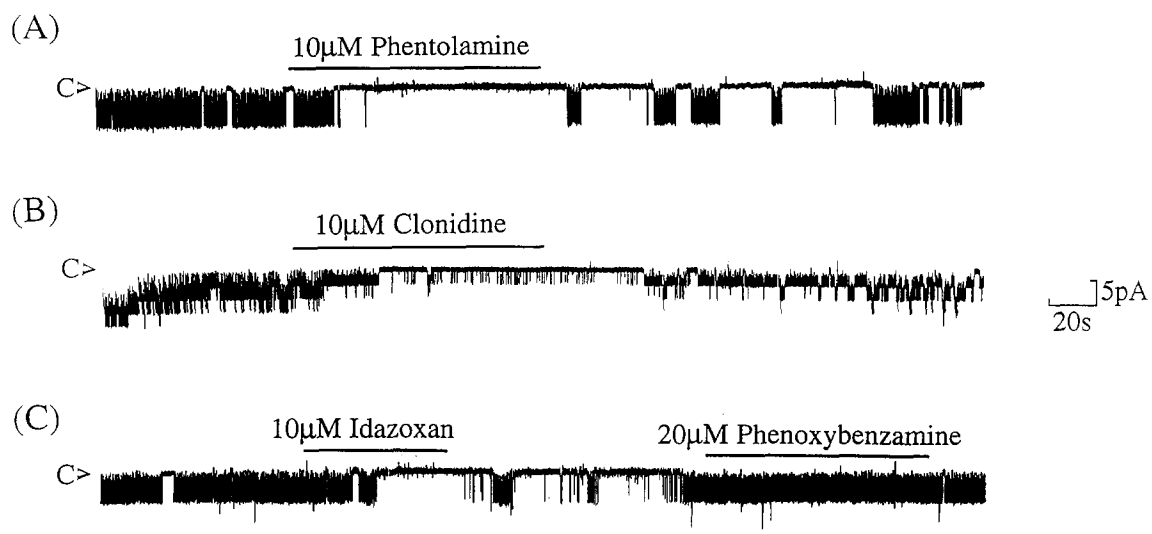

Fig. 1. Continuous single channel recordings from three separate inside-out patches excised from guinea-pig ventricular myocytes. (A) The application of 10 μM phentolamine to a patch held at −80 mV was associated with a reversible inhibition of K_{ATP} channel activity. Similarly 10 μM clonidine (B, holding potential −40 mV) or 10 μM idazoxan (C, holding potential −60 mV) was also found to reversibly inhibit K_{ATP} channel activity. In contrast, 20 μM phenoxybenzamine had no effect on K_{ATP} channel activity (C).

3. Results

K_{ATP} channel currents were studied at the single channel level using the inside-out patch clamp configuration under conditions of symmetrical 140 mM KCl. The channel was identified on the basis of single channel conductance between 0 and −80 mV (82.3 ± 3.2 pS, $n = 6$), sensitivity to ATP (78.4 ± 3.5% inhibition by 100 μM MgATP, $n = 5$), voltage independence of P_o, and inward rectification. Under these conditions, application of 10 μM phentolamine reversibly inhibited K_{ATP} channel activity independent of membrane holding potential between ±80 mV (10 μM phentolamine produced 84.5 ± 3.4% ($n = 6$) inhibition, Fig. 1A). In the pancreatic β cell, the inhibitory effects of phentolamine on K_{ATP} channel activity are related to the imidazoline moiety rather than interactions with adrenoceptors (Shulz and Hasselblatt, 1989). To determine if the same is true for cardiac K_{ATP} channels in guinea pig ventricular myocytes, the inhibitory effects of several related imidazoline compounds were examined. As illustrated in Fig. 1B, the imidazoline clonidine, an α-adrenoceptor agonist, also inhibited K_{ATP} channel activity. For example 10 μM clonidine produced 85.2 ± 5.9% ($n = 7$) inhibition of K_{ATP} channel activity. Furthermore, the stucturally related imidazolines, antazoline (H_1 antihistamine) and idazoxan (weak α-adrenoceptor antagonist, Fig. 1C), produced a similarly potent inhibition of K_{ATP} channel activity (68.4 ± 7.3% ($n = 5$) and 58.1 ± 13.1% ($n = 5$) inhibition produced by 10 μM antazoline and 10 μM idazoxan respectively). In contrast, non-selective α-adrenoceptor antagonists lacking the imidazoline moiety, benextramine (20 μM, $n = 4$) and phenoxybenzamine (20 μM, $n = 3$), failed to affect K_{ATP} channel activity at either positive or negative potentials (Fig. 1C).

In whole-cell patch clamp experiments, activation of a large time-independent current with properties consistent with the K_{ATP} channel occurred over 20 min following whole-cell formation. The current had a reversal potential at −75 mV (not corrected for junction potential), was completely inhibited by 10 μM glibenclamide ($n = 4$), and was not observed with 5 mM ATP present in the internal (pipette) solution. The K_{ATP} channel current maximally activated in 18 ± 2 min (160 ± 30 pA/pF measured at 0 mV ($n = 15$, Fig. 2)) and remained relatively constant for approximately 15–20 min. Subsequently the K_{ATP} channel current underwent a rapid decrease that was associated with myocyte death. To compare the sensitivity of the cardiac K_{ATP} channel to that observed in the pancreatic β cell, the effect of phentolamine upon whole-cell K_{ATP} channel currents was examined (Fig. 2C). Phentolamine (0.2–20 μM) significantly inhibited the outward current at 0 mV in a reversible manner. The concentration-inhibition curve for phentolamine is shown in Fig. 2E; phentolamine inhibited K_{ATP} channel currents with an IC_{50} value of 1.0 ± 0.10 μM ($n = 24$) and a Hill coefficient of 1.0.

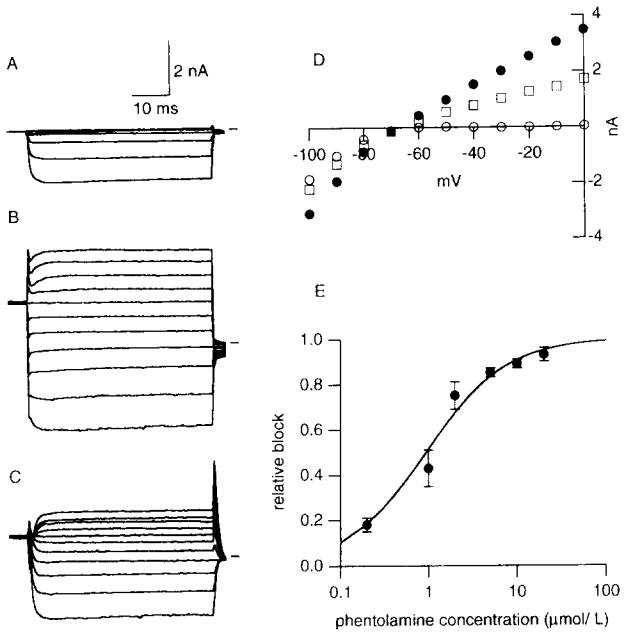

Fig. 2. Effect of phentolamine on whole-cell K_{ATP} channel currents. Whole-cell current traces for voltages from −100 to 0 mV (A, B, C) and composite current-voltage relationships (D) immediately after whole-cell formation (A and open circles), after maximal K_{ATP} channel activation (B and closed circles), and with application of 1 μM phentolamine (C and open squares). (E) Concentration-inhibition curve for phentolamine on whole-cell K_{ATP} channel current measured at 0 mV.

4. Discussion

The major finding from this study is that imidazoline compounds inhibit K_{ATP} channel activity in guinea pig ventricular myocytes independent of α-adrenoceptors, and with similar potency as in the pancreatic β cell. Phentolamine has been previously shown to inhibit K_{ATP} channel activity in rabbit ventricular myocytes but the mechanism by which this occurred was not established (Wilde et al., 1994). The results presented here demonstrate that the inhibition of K_{ATP} channel activity is unrelated to the compound's ability to interact with adrenoceptors since the non-imidazoline adrenoceptor antagonists, benextramine and phenoxybenzamine, fail to affect K_{ATP} channel activity. Furthermore, clonidine, an α_2-adrenoceptor agonist, is a potent K_{ATP} channel inhibitor. These results indicate that the K_{ATP} channel in cardiac ventricular myocytes is modulated by compounds containing an imidazoline ring regardless of their ability to interact with α-adrenoceptors.

Whole-cell patch-clamp experiments demonstrate that phentolamine inhibits K_{ATP} channel currents with

a potency similar to that reported for inhibition of whole-cell K_{ATP} channel currents in the CRI-G1 insulin secreting cell line (IC_{50} 0.8 ± 0.1 μM, Lee et al., 1994). This suggests that the K_{ATP} channel present in ventricular myocytes exhibits a similar sensitivity to phentolamine as the β cell K_{ATP} channel. It has been suggested that imidazolines inhibit K_{ATP} channel activity in the pancreatic β cell via a specific receptor, although the nature of this molecule remains to be established (Chan et al., 1994). On the basis of the present findings, it appears that a similar situation pertains to cardiac myocytes and may be of even more widespread physiological and pharmacological significance.

However, it is also noteworthy that clonidine appears to be a more effective inhibitor of K_{ATP} channel activity in ventricular myocytes than in the pancreatic β cell (Chan et al., 1994). This finding suggests that although K_{ATP} channel activity can be regulated by imidazoline compounds in both tissues, there appears to be some pharmacological diversity. This situation is therefore somewhat similar to the variable effects of other K_{ATP} channel modulators between tissues (Ashcroft and Ashcroft, 1990).

In view of these observations, it may be necessary to review the therapeutic potential of imidazoline-based hypoglycemic agents. Furthermore, many agents currently in clinical usage contain an imidazoline moiety and may therefore affect K_{ATP} channel activity. Activation of the K_{ATP} channel during myocardial ischemia may reduce membrane excitability protecting the affected site from further energy depletion. Thus, agents which block the cardiac K_{ATP} channel opening may worsen prognosis in ischemic heart disease (Hofman and Opie, 1993; Rytter et al., 1985).

In conclusion, we have demonstrated that the class of drugs known as imidazolines possesses the ability to interact with the cardiac K_{ATP} channel with a potency similar to that observed in the pancreatic β cell. These findings suggest that an imidazoline receptor may be functionally linked to the K_{ATP} channel in these two tissue types and this may be important clinically due to the widespread use of compounds containing this moiety.

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