Antiarrhythmic drugs, clofilium and cibenzoline are potent inhibitors of glibenclamide-sensitive K^+ currents in *Xenopus* oocytes

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1 The novel K^+ channel opener, Y-26763 induced outward K^+ currents in voltage-clamped follicle-enclosed *Xenopus* oocytes in a concentration-dependent manner with an EC_{50} value of $58 \mu M$.

2 The Y-26763-induced K^+ current was completely and reversibly blocked by glibenclamide (an ATP-sensitive K^+ channel blocker) in a concentration-dependent manner (IC_{50} $140 nM$). Effects of several antiarrhythmic drugs on Y-26763-induced glibenclamide-sensitive K^+ currents were investigated.

3 ($\pm$)-Cibenzoline, RS-2135, pirlmenol, lorcaïnide and KW-3407 (class I antiarrhythmic drugs, Na^+ channel blockers) suppressed Y-26763 responses in a concentration-dependent manner with IC_{50} values (in μM) of 6.6, 54, 68, 71 and 370, respectively.

4 Clofilium, E-4031, MS-551 and bretylium (class III antiarrhythmic drugs which increase the action potential duration) also suppressed Y-26763 responses concentration-dependently, IC_{50} values (in μM) were 3.3, 660, 980 and ≥ 2000 , respectively. N-acetylprocainamide (class III antiarrhythmic drug) scarcely suppressed Y-26763 responses.

5 The glibenclamide-sensitive K^+ currents elicited by KRN2391 were also suppressed by all these antiarrhythmic drugs.

6 The antiarrhythmic drugs, clofilium and ($\pm$)-cibenzoline block glibenclamide-sensitive K^+ channels in *Xenopus* oocytes at concentrations comparable to their therapeutic plasma levels.

Keywords: Antiarrhythmic drug; cibenzoline; clofilium; Y-26763; KRN2391; glibenclamide; K^+ channel; *Xenopus laevis* oocyte; calmodulin antagonist

Introduction

The ATP-sensitive K^+ (K_{ATP}) channel was first described in mammalian myocardial cells (Noma, 1983), and has also been observed in the pancreatic β -cell (Ashcroft *et al.*, 1984; Cook & Hales, 1984), the smooth muscle (Noack *et al.*, 1992), the skeletal muscle (Spruce *et al.*, 1985) and the brain (Ashford *et al.*, 1988). This K^+ channel is activated in the presence of ATP by a new class of K^+ channel openers such as cromakalim, pinacidil or diazoxide (Escande *et al.*, 1988), and is inactivated by sulphonylureas such as glibenclamide (Sturgess *et al.*, 1985), by imidazoline derivatives such as phentolamine (Plant & Henquin, 1990; Dunne, 1991), by barbiturates such as pentobarbitone (Kozłowski & Ashford, 1991), by phenothiazines such as chlorpromazine (Müller *et al.*, 1991) and by the inhibitor of Ca^{2+} mobilization, TMB-8 (Szewczyk *et al.*, 1992).

The oocyte of *Xenopus laevis* has an endogenous K^+ channel which is activated by K^+ channel openers such as cromakalim and pinacidil (Honoré & Lazdunski, 1991) and KRN2391 (Sakuta *et al.*, 1992a). Since this oocyte K^+ channel is inactivated by glibenclamide (Honoré & Lazdunski, 1991) but not by charybdotoxin (an inhibitor of Ca^{2+} -activated K^+ channel) or dendrotoxin (an inhibitor of voltage-dependent K^+ channel) (Honoré & Lazdunski, 1991), the K^+ channel appears to belong to the family of ATP-sensitive K^+ channels, though its sensitivity to ATP is not yet proven by single channel studies.

An agent which acts to suppress the ATP/glibenclamide-sensitive K^+ channel has potential as an antiarrhythmic drug (Kantor *et al.*, 1990; Notsu *et al.*, 1992). Among conventional antiarrhythmic drugs, some, such as quinidine and verapamil, do suppress ATP/glibenclamide-sensitive K^+ channels in rat cardiac cells (Haworth *et al.*, 1989). Antiarrhythmic drugs, disopyramide, aprindine, bepridil and pre-

nylamine potently block glibenclamide-sensitive K^+ channels in *Xenopus* oocytes with IC_{50} values of less than $50 \mu M$ (Sakuta *et al.*, 1992a). The suppression of ATP/glibenclamide-sensitive K^+ channels may be involved in either favourable or unfavourable clinical effects of some antiarrhythmic drugs (see Discussion).

In the present study, we have examined the effects of five class I antiarrhythmic drugs (Na^+ channel blockers) and five class III antiarrhythmic drugs (agents prolonging the action potential duration (APD)) on glibenclamide-sensitive K^+ channels in *Xenopus* oocytes. As a result, we have found that ($\pm$)-cibenzoline (a class I antiarrhythmic drug) and clofilium (a class III antiarrhythmic drug) potently suppress glibenclamide-sensitive K^+ channels in *Xenopus* oocytes.

Methods

Voltage-clamped recording

As described previously (Sakuta *et al.*, 1992a,b), oocytes at stage V–VI were collected from female frogs (*Xenopus laevis*) anaesthetized in ice, each oocyte was placed in a recording well (0.2 ml in capacity) and superfused with frog Ringer solution consisting of (in mM) NaCl 120, KCl 2, $CaCl_2$ 1.8 and HEPES 5 (pH 7.4). The membrane potential of the oocyte was then voltage-clamped at a holding potential of $-20 mV$ with two glass microelectrodes each filled with 3 M KCl (1–2 Mohm) using the voltage-clamp amplifier, CEA-1200 (Nihon Kohden, Tokyo, Japan). This holding potential of $-20 mV$ is close to the predicted Cl^- equilibrium potential of the oocyte and was chosen to minimize the Ca^{2+} -activated Cl^- current (Honoré & Lazdunski, 1991; Sakuta *et al.*, 1992a,b). All current responses of the oocyte were recorded at room temperature ($19-23^\circ C$) with a Thermalarray Recorder, RTA-1100 (Nihon Kohden). Only follicle-

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enclosed oocytes (not treated with collagenase) were used for all experiments in the present study.

Application of drugs and agents

Y-26763 or KRN2391 (K⁺ channel openers) dissolved in frog Ringer solution were applied for 20 s by constant flow superfusion (3 ml min⁻¹) to evoke a K⁺ current. Y-26763 was applied at 50 μ M routinely and at 10–2000 μ M to obtain a concentration-response curve, and KRN2391 was routinely applied at 100 μ M. Each test drug or agent, except for charybdotoxin and dendrotoxin, was applied by superfusion for 2 min, and the oocyte was washed for 15 s by superfusion of frog Ringer solution; then the K⁺ channel opener was applied again to observe the effect of the test drug. As described previously (Sakuta *et al.*, 1992a), this 15 s washing period did not reduce the suppressive effect of the test drug, indicating little wash-off. TMB-8, W-7, W-13, W-5 or antiarrhythmic drugs were dissolved in frog Ringer solution to appropriate concentrations and applied by superfusion (3 ml min⁻¹) as described above. Glibenclamide was dissolved in ethanol to be 2.5 mM, then diluted with frog Ringer solution to a final concentration of 5 nM–10 μ M (pH 7.4) and applied to each oocyte by superfusion as described previously (Sakuta *et al.*, 1992a).

Drugs and agents used

The following drugs and agents were used (full name and source): Y-26763 ((+)-(3S,4R)-4-(N-acetyl-N-benzyloxy-amino)-6-cyano-3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-3-ol, an active desbenzoyl form of Y27152, gift from Yoshitomi Pharmaceutical Industry Ltd., Osaka, Japan); KRN2391 (N-cyano-N'-(2-nitroxyethyl)-3-pyridinecarboximidamide methanesulphonate, gift from Kirin Brewery Co., Takasaki, Japan); E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-(4-methanesulphonamidobenzoyl) piperidine, gift from Eisai Pharmaceutical Co., Tokyo, Japan); RS-2135, (3aR,12R,12aR,12bS)-12-amino-2,3,3a,4,11,12,12a,12b-octahydro-10-hydroxyisoquinoline [2,1,8-1ma]carbazole-5(1H)-one hydrochloride 1.5-hydrate, gift from Sankyo Pharmaceutical Co., Tokyo); MS-551 (1,3-dimethyl-6-{2-[N-(hydroxyethyl)-3-(4-nitrophenyl)propylamino]ethylamino}-2,4 (1H,3H)-pyrimidinedione hydrochloride, gift from Mitsui Pharmaceutical Co., Osaka, Japan); KW-3407 (5-[[2-(diethylamino)ethyl]amino]-7-methoxy-5,11-dihydro[1]-benzoxepino [3,4-b]pyridine 1.5-fumarate, gift from Kyowa Hakko Co., Tokyo, Japan); ($\pm$)-cibenzoline succinate (gift from Fujisawa Pharmaceutical Co., Osaka, Japan), pirlmenol hydrochloride (gift from Warner-Lambert Inc., Tokyo, Japan); clofilium tosylate, bretylium tosylate, lorcanide hydrochloride, glibenclamide, charybdotoxin and dendrotoxin (purchased from Funakoshi Co., Tokyo, Japan); W-5 (N-(6-aminoethyl)-1-naphthalenesulphonamide), W-7 (N-(6-aminoethyl)-5-chloro-1-naphthalenesulphonamide), W-13 (N-(4-aminobutyl)-5-chloro-2-naphthalenesulphonamide), N-acetylprocainamide hydrochloride, TMB-8 (3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester), H-7 (1-(5-isoquinolinylnsulphonyl)-2-methylpiperazine) and staurosporine (purchased from Sigma Chemical Co., St. Louis, USA), and collagenase and TEA (tetraethylammonium chloride, purchased from Wako Pure Chemical Co., Osaka, Japan).

Estimation of IC₅₀ and EC₅₀ values

The IC₅₀ value for the inhibition of Y-26763 responses by each test drug was estimated from the log concentration-inhibition curve drawn by linearly connecting observed mean values (cf. Figures 3–7). The EC₅₀ value for Y-26763 was also estimated from the log concentration-response curve in the same manner (cf. Figure 1).

Results

The current response induced by Y-26763 in follicle-enclosed *Xenopus* oocytes

In all follicle-enclosed oocytes, Y-26763 (a novel K⁺ channel opener, Nakajima *et al.*, 1992) at 10–2000 μ M induced a non-oscillating and slowly rising outward current at a voltage-clamped membrane potential of –20 mV (Figure 1a). Y-26763 showed a bell-shaped concentration-response curve, that is, the amplitude of Y-26763 responses increased

Figure 1 Y-26763-induced outward currents in follicle-enclosed *Xenopus* oocytes. (a) Responses to 5–2000 μ M Y-26763 applied for 20 s (at bars) in one oocyte. (b) A log concentration-response curve for Y-26763 (mean $\pm$ s.d., $n = 8$) showing a bell shape. All oocytes used were voltage-clamped at –20 mV.

Figure 2 The reversal potential of Y-26763 responses, and the effects thereon of K⁺ channel blockers. (a) An *I-V* relation for responses to Y-26763 (50 μ M, 20 s) in one oocyte showing the reversal at about –107 mV. (b)–(d) The effect of tetraethylammonium (TEA, 30 mM, 2 min), charybdotoxin (50 nM, 2 min) and dendrotoxin (100 nM, 2 min) on Y-26763 (50 μ M) responses in 3 different oocytes voltage clamped at –20 mV.

as the concentration of Y-26763 was raised from 10 μM to 200 μM (apparent $\text{EC}_{50} = 57.5 \mu\text{M}$) and decreased when the concentration was increased from 500 μM to 2 mM as shown in Figure 1a and b. This apparent EC_{50} value of 58 μM was smaller than EC_{50} values for cromakalim (280 μM) and KRN2391 (400 μM) all studied under similar conditions (Sakuta *et al.*, 1992a,b).

The Y-26763-induced current response was carried by the efflux of K^+ , since it was reversed at a holding potential of about -100 mV (Figure 2a) which is close to the K^+ equilibrium potential of the oocyte in frog Ringer solution (Kusano *et al.*, 1982), and was suppressed to $55 \pm 6\%$ (mean $\pm$ s.d., $n = 5$) by 30 mM TEA (Figure 2b). In five oocytes tested, Y-26763-induced K^+ currents were not affected either by 50 nM charybdotoxin (an inhibitor of Ca^{2+} -activated K^+ channel) or by 100 nM dendrotoxin (an inhibitor of voltage-dependent K^+ channel) (Figure 2c and d).

The relatively selective inhibitor of ATP-sensitive K^+ channels, glibenclamide suppressed the K^+ currents induced by 50 μM Y-26763 in a concentration-dependent fashion with an IC_{50} of 140 μM (Figure 3a and c). TMB-8 also blocked responses to 50 μM Y-26763 in a concentration-dependent manner with an IC_{50} value of 48 μM (Figure 3b and c). TMB-8 is known to block not only the ATP-sensitive K^+ channel in insulin-secreting cells (Szewczyk *et al.*, 1992) but also the voltage-dependent Na^+ channel and the voltage-dependent Ca^{2+} channel (Himmel & Raven, 1990).

K^+ channels which were activated by Y-26763 appear to be present in the follicle cells surrounding the oocyte rather than the plasma membrane of the oocyte itself, because oocytes defolliculated by incubation with collagenase (1 mg ml^{-1} for 4 h) scarcely responded to Y-26763 (data not shown).

Effects of class I and class III antiarrhythmic drugs on Y-26763-induced K^+ current

Class I antiarrhythmic drugs, ($\pm$)-cibenzoline, RS-2135 (Yorikane *et al.*, 1991), pirmenol, lorcainide and KW-3407

Figure 3 Effects of ATP-sensitive K^+ channel blockers on Y-26763 responses. (a) and (b) Suppression of responses of Y-26763 (50 μM , 20 s, short bars) by glibenclamide (0.4 μM , 2 min, long bar in a) and TMB-8 (50 μM , 2 min, long bar in b) in different oocytes voltage clamped at -20 mV . Calibrations: 200 nA and 1 min. (c) Log concentration-inhibition curves (mean $\pm$ s.d., $n = 5$) for the inhibition of responses to Y-26763 (50 μM , 20 s) by glibenclamide (○) and TMB-8 (●). The ordinate scale shows the peak amplitude of the Y-26763 response (as a % of the control, i.e. in the absence of the test drug), and the abscissa scale is the log molar concentration of test drugs.

Figure 4 Effects of class I antiarrhythmic drugs on Y-26763 responses. (a)–(e) Suppression of responses to Y-26763 (50 μM , 20 s, short bars) by the class I drugs indicated (applied for 2 min, long bars) at concentrations shown in μM in different oocytes voltage clamped at -20 mV . Calibrations: 200 nA and 1 min. (f) Log concentration-inhibition curves (mean $\pm$ s.d., $n = 5$) for the inhibition of responses to Y-26763 (50 μM , 20 s) by ($\pm$)-cibenzoline (○), RS-2135 (●), pirmenol (□), lorcainide (■) and KW-3407 (Δ). The ordinate scale is the peak amplitude of the Y-26763 response (as a % of the control, i.e. in the absence of an antiarrhythmic drug), and the abscissa scale is the log molar concentration of antiarrhythmic drugs.

(Kumazawa *et al.*, 1990) reversibly suppressed the K^+ current induced by 50 μM Y-26763 in a concentration-dependent manner with IC_{50} values (in μM) of 6.6, 54, 68, 71, and 370 as shown in Figure 4a–f. ($\pm$)-Cibenzoline was the most potent in this class of drugs tested and its suppressive effect was observed even at concentrations lower than 1 μM .

The class III antiarrhythmic drug, clofilium, also potently blocked Y-26763 responses with an IC_{50} value of 3.3 μM (Figure 5a and f). Other class III antiarrhythmic drugs tested, E-4031 (Adaniya & Hiraoka, 1990), MS-551 (Kamiya *et al.*, 1992) and bretylium, weakly suppressed Y-26763 responses with IC_{50} values (in μM) of 660, 980 and ≥ 2000 , respectively (Figure 5b–d and f), whilst N-acetylprocainamide scarcely suppressed Y-26763 responses (Figure 5e and f).

Effects of class I and class III antiarrhythmic drugs on KRN2391-induced K^+ current

Another K^+ channel opener, KRN2391 also induces a glibenclamide-sensitive K^+ current in *Xenopus* oocytes (Sakuta *et al.*, 1992a). The effect of the above antiarrhythmic drugs on the KRN2391-induced current was examined. As shown in Figure 6a, all class I antiarrhythmic drugs tested, namely ($\pm$)-cibenzoline, RS-2135, pirmenol, lorcainide and KW-3407 suppressed current responses to KRN2391 (100 μM) in a concentration-dependent manner with IC_{50} values

Figure 5 Effects of class III antiarrhythmic drugs on Y-26763 responses. (a)–(e) Suppression of responses to Y-26763 (50 μ M, 20 s, short bars) by the class III drugs indicated (applied for 2 min (long bars) at concentrations shown in μ M) in different oocytes voltage clamped at -20 mV. Calibrations: 200 nA and 1 min. (f) Log concentration-inhibition curves (mean $\pm$ s.d., $n = 5$) for the inhibition of responses to Y-26763 (50 μ M, 20 s) by clofilium ($\circ$), E-4031 ($\bullet$), bretylium ($\square$), MS-551 ($\blacksquare$) and N-acetylprocainamide (Δ). The ordinate scale is the peak amplitude of the Y-26763 response (as a % of the control, i.e. in the absence of an antiarrhythmic drug), and the abscissa scale is the log molar concentration of antiarrhythmic drugs.

(in μ M) of 4.5, 71, 96, 120 and 320, respectively). Their relative suppressive potencies on the KRN2391-evoked response were comparable to those on the Y-26763 response.

The K⁺ current induced by KRN2391 was also suppressed by class III antiarrhythmic drugs, clofilium, MS-551, bretylium and E-4031 with IC₅₀ values (in μ M) of 5.8, 1200, 1700 and >2000 (Figure 6b), respectively. The IC₅₀ values for clofilium and bretylium were comparable to those on Y-26763-induced currents, but the KRN2391-induced K⁺ current was less sensitive to E-4031 than the Y-26763-induced K⁺ current either in different oocytes as shown Figure 6b or in a single oocyte (data not shown). N-acetylprocainamide scarcely suppressed KRN2391 responses.

Effects of calmodulin antagonists on Y-26763 responses

Of the antiarrhythmic drugs tested in the present study, clofilium and lorcinide possess calmodulin antagonist activity (Silver *et al.*, 1989; Mannhold *et al.*, 1990). Calmodulin antagonists, W-7, W-13 and W-5 (Hidaka & Tanaka, 1983), suppressed the K⁺ current induced by 50 μ M Y-26763 in a concentration-dependent manner with IC₅₀ values (in μ M) of 18, 35 and 54, respectively (Figure 7a–d). This rank order of potency agrees with that of their calmodulin antagonist activities (Hidaka & Tanaka, 1983).

Figure 6 Log concentration-inhibition curves for the inhibition by class I and III antiarrhythmic drugs of the outward K⁺ currents induced by KRN2391 (100 μ M, 20 s) responses. All values plotted were mean $\pm$ s.d. ($n = 5$). (a) The curves for class I drugs, ($\pm$)-cibenzoline ($\circ$), RS-2135 ($\bullet$), pirmenol ($\square$), lorcinide ($\blacksquare$) and KW-3407 (Δ); (b) the curves for class III drugs, clofilium ($\circ$), E-4031 ($\bullet$), bretylium ($\square$), MS-551 ($\blacksquare$) and N-acetylprocainamide (Δ). Ordinates are the peak amplitude of the KRN2391 response (as a % of the control, i.e. in the absence of an antiarrhythmic drug), and abscissae are the log molar concentration of antiarrhythmic drugs.

Discussion

Properties of Y-26763-induced outward currents

It is suggested that Y-26763 activates ATP-sensitive K⁺ channels in ventricular cardiomyocytes (Ishiguro *et al.*, 1992), and it was demonstrated in the present study that this K⁺ channel opener evokes glibenclamide-sensitive K⁺ currents in follicle-enclosed *Xenopus* oocytes (cf. Figures 2 and 3). The Y-26763-induced K⁺ current was completely blocked by glibenclamide but was insensitive to both charybdotoxin and dendrotoxin (cf. Figure 2). Therefore, Y-26763-induced K⁺ current may not contain either a Ca²⁺-activated K⁺ or a voltage-dependent K⁺ current.

TEA at a very high concentration (30 mM) suppressed Y-26763-induced currents by about 55% (cf. Figure 2). This TEA effect is not necessarily contradictory to the property of ATP-sensitive K⁺ currents, because the ATP-sensitive K⁺ current in mouse pancreatic β -cells also shows a weak sensitivity to TEA (Bokvist *et al.*, 1990).

Blockade of glibenclamide-sensitive K⁺ channels by antiarrhythmic drugs and possible mechanism

Glibenclamide-sensitive K⁺ channels in *Xenopus* oocytes were potentially blocked by clofilium and ($\pm$)-cibenzoline in the present study. We have previously reported that various antiarrhythmic drugs block the glibenclamide-sensitive K⁺ currents induced by KRN2391 in oocytes with potencies in terms of IC₅₀ of 6.3 μ M for bepridil, 18 μ M for disopyramide, 30 μ M for aprindine and 38 μ M for prenylamine (Sakuta *et*

Figure 7 Effects of calmodulin antagonist naphthalenesulphonamides on Y-26763 responses. (a)–(c) Suppression by W-7 (50 μ M, 2 min, long bar in a), W-13 (50 μ M, 2 min, long bar in b) and W-5 (50 μ M, 2 min, long bar in c) of responses to Y-26763 (50 μ M, 20 s, short bars) in 3 different oocytes voltage clamped at -20 mV. Note slow recovery from inhibition by each drug. Calibrations: 200 nA and 90 s. (d) Log concentration-inhibition curves (mean $\pm$ s.d., $n = 5$) for the inhibition of responses to Y-26763 (50 μ M, 20 s) by W-7 ($\circ$), W-13 ($\bullet$) and W-5 (Δ). The ordinate scale is the peak amplitude of the Y-26763 response (as a % of the control, i.e., in the absence of a naphthalenesulphonamide), and the abscissa scale is the log molar concentration of naphthalenesulphonamides.

et al., 1992a). Thus, the potencies of clofilium (IC_{50} 5.8 μ M) and ($\pm$)-cibenzoline (4.5 μ M) in blocking KRN2391-induced glibenclamide-sensitive K^+ currents are comparable to the potency of bepridil.

W-7, W-13 and W-5 (calmodulin antagonists), clofilium and lorcanide blocked the K^+ currents induced by Y-26763 in oocytes with the rank order of clofilium $>$ W-7 $>$ W-13 $>$ W-5 $\geq$ lorcanide. This rank order agreed with their reported potencies as calmodulin antagonists (potencies to inhibit Ca^{2+} /calmodulin-dependent phosphodiesterase), that is clofilium $>$ W-7 $>$ W-13 $>$ lorcanide $\geq$ W-5 (Silver *et al.*, 1989; Mannhold *et al.*, 1990; Hidaka & Tanaka, 1983). In addition, several antiarrhythmic drugs and agents having a calmodulin antagonizing activity also block the glibenclamide-sensitive K^+ current induced by KRN2391 in *Xenopus* oocytes (Sakuta *et al.*, 1992a). These previous and present findings suggest that the calmodulin antagonist activity of clofilium and lorcanide contributes at least partly to their K^+ channel-blocking actions.

The blocking action of KW-3407 and RS-2135 on glibenclamide-sensitive K^+ currents appears to be related to phenothiazine-like aromatic rings in their structures. Phenothiazine derivatives such as trifluoperazine and chlorpromazine block ATP/glibenclamide-sensitive K^+ channels (Müller *et al.*, 1991). It is suggested that the calmodulin antagonizing activity of phenothiazine derivatives (Weiss & Levin, 1978) contributes to their blocking effect on ATP/glibenclamide-sensitive K^+ channels (Sakuta *et al.*, 1992b).

The suppressive effect of ($\pm$)-cibenzoline on glibenclamide-sensitive K^+ channels in oocytes seems to be related to its imidazoline structure, since several imidazoline

derivatives such as phentolamine, tolazoline and antazoline have been reported to block ATP/glibenclamide-sensitive K^+ channels in insulin-secreting cells (Plant & Henquin, 1990; Dunne, 1991). For imidazolines, however, a mechanism other than calmodulin antagonism appears to be involved in the blockade of glibenclamide-sensitive K^+ channels in *Xenopus* oocytes, because phentolamine and tolazoline are very weak calmodulin antagonists, their IC_{50} values being ≥ 500 μ M (Earl *et al.*, 1984).

The glibenclamide-sensitive K^+ current in *Xenopus* oocytes is enhanced by activation of adenylate cyclase and is decreased by activation of protein kinase C (Honore & Lazdunski, 1991). Except for clofilium, however, all drugs tested are known to affect neither the intracellular level of cyclic-AMP nor the activity of protein kinase C. Only clofilium was reported to inhibit protein kinase C, but it requires a concentration of 100 μ M to do so (Silver *et al.*, 1989), which is about 30 times higher than its IC_{50} value (3.3 μ M) for the blockade of Y-26763-induced currents. In addition, protein kinase C inhibitors, staurosporine (1 μ M) and H-7 (100 μ M) do not affect Y-26763-induced K^+ currents (our unpublished observation). Thus, it seems unlikely that the suppressive effect of all 10 antiarrhythmic drugs tested on Y-26763-induced K^+ currents is mediated by drug-induced changes in second messengers, cyclic-AMP and protein kinase C.

Class I antiarrhythmic drugs, cibenzoline (Matsuoka *et al.*, 1991) and pirmenol (Reder *et al.*, 1980), also possess the class IV action (blockade of the voltage-dependent Ca^{2+} channel). Several Ca^{2+} antagonists are reported to suppress the glibenclamide-sensitive K^+ currents in oocytes (Sakuta *et al.*, 1992a). The Ca^{2+} antagonism might change intracellular Ca^{2+} concentration, inhibit Ca^{2+} -dependent processes and subsequently affect glibenclamide-sensitive K^+ currents. The Ca^{2+} channel blockade by this means, however, is unlikely to be contributing to the suppressive effect of antiarrhythmic drugs on oocyte glibenclamide-sensitive K^+ current for the following reasons: (1) the weak Ca^{2+} antagonists, bepridil and prenylamine, block oocyte glibenclamide-sensitive K^+ currents much more potently than strong Ca^{2+} antagonists, diltiazem and verapamil (Sakuta *et al.*, 1992a); (2) typical Ca^{2+} antagonists, verapamil and diltiazem, require much higher concentrations to block the K^+ current than to block Ca^{2+} channels (Sakuta *et al.*, 1992a); (3) both the active Ca^{2+} antagonist, ($-$)-verapamil, and the inactive isomer, ($+$)-verapamil, equipotently block the K^+ current (our unpublished observation); and (4) cibenzoline blocked KRN2391-induced K^+ currents in oocytes 19 times more potently than ($\pm$)-verapamil (cf. Figure 4 and Sakuta *et al.*, 1992a), whilst the potency of cibenzoline in blocking Ca^{2+} channels is only 1/50 of that of ($\pm$)-verapamil (Matsuoka *et al.*, 1991).

Clinical significance of the blockade of glibenclamide-sensitive K^+ channels by antiarrhythmic drugs

Myocardial ATP/glibenclamide-sensitive K^+ channels are opened by an ischaemic condition and are thought to play a major role in the ischaemia-induced shortening of action potential duration and consequent generation of ischaemia-related arrhythmia (Noma, 1983). Therefore, any drug which can block this type of K^+ channel theoretically has the potential to suppress ischaemia-induced arrhythmia (Lynch *et al.*, 1992). In support of this idea, ATP-sensitive K^+ channel blockers glibenclamide and 5-hydroxydecanoate have been reported to suppress experimental ventricular arrhythmia (Kantor *et al.*, 1990; Notsu *et al.*, 1992). Furthermore, some class I, class II and class IV antiarrhythmic drugs in Vaughan Williams' classification block glibenclamide-sensitive K^+ channels (Sakuta *et al.*, 1992a). The present study shows that the class III antiarrhythmic drug, clofilium, and some novel class I antiarrhythmic drugs also block the K^+ channels. Thus, it appears that the inhibition of ATP/glibenclamide-sensitive K^+ channels may underlie a part of antiarrhythmic effects of some antiarrhythmic drugs.

Disopyramide, quinidine and ($\pm$)-cibenzoline are related to rare hypoglycaemic attacks during treatment of arrhythmia (Semel *et al.*, 1983; Phillips *et al.*, 1986; Jeandel *et al.*, 1988). Disopyramide and quinidine suppress glibenclamide-sensitive K⁺ currents in *Xenopus* oocytes (Sakuta *et al.*, 1992a). ($\pm$)-Cibenzoline also potentially blocked the K⁺ currents (cf. Figures 4 and 6). Thus, it is conceivable that these drugs might inactivate ATP/glibenclamide-sensitive K⁺ channels in pancreatic β -cells, and facilitate the secretion of insulin. In support of this, disopyramide, quinine (an optical isomer of quinidine) and imidazoline derivatives have been reported to stimulate the secretion of insulin from islet cells (Henquin *et al.*, 1975; Nakabayashi *et al.*, 1989; Schulz & Hasselblatt, 1989).

In conclusion, the antiarrhythmic drugs, ($\pm$)-cibenzoline and clofilium block the glibenclamide-sensitive K⁺ channel in *Xenopus* oocytes at concentrations comparable to their therapeutic plasma concentrations. These drugs are as potent as bepridil which is the strongest blocker of glibenclamide-sensitive K⁺ channels among the antiarrhythmic drugs studied so far.

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References

- ADANIYA, H. & HIRAOA, M. (1990). Effects of a novel class III antiarrhythmic agent, E-4031, on reentrant tachycardias in rabbit right atrium. *J. Cardiovasc. Pharmacol.*, **15**, 976–982.
- ASHCROFT, F.M., HARRISON, D.E. & ASHCROFT, S.J.H. (1984). Glucose induces closure of single potassium channels in isolated rat pancreatic β -cells. *Nature*, **312**, 446–448.
- ASHFORD, M.L.J., STURGEON, N.C., TROUT, N.J., GARDNER, N.J. & HALES, C.N. (1988). Adenosine-5'-triphosphate-sensitive ion channels in neonatal rat cultured central neurones. *Pflügers Arch.*, **412**, 297–304.
- BOKVIST, K., RORSMAN, P. & SMITH, P.A. (1990). Block of ATP-regulated and Ca²⁺-activated K⁺ channels in mouse pancreatic β -cells by external tetraethylammonium and quinine. *J. Physiol.*, **423**, 327–343.
- COOK, D.L. & HALES, C.N. (1984). Intracellular ATP directly blocks K⁺ channels in pancreatic β -cells. *Nature*, **311**, 271–273.
- DUNNE, M.J. (1991). Block of ATP-regulated potassium channels by phentolamine and other α -adrenoceptor antagonists. *Br. J. Pharmacol.*, **103**, 1847–1850.
- EARL, C.Q., PROZIALECK, W.C. & WEISS, B. (1984). Interaction of alpha adrenergic antagonists with calmodulin. *Life Sci.*, **35**, 525–534.
- ESCANDE, D., THURINGER, D., LEGUERN, S. & CAVERO, I. (1988). The potassium channel opener cromakalim (BRL34915) activates ATP-dependent K⁺ channels in isolated cardiac myocytes. *Biochem. Biophys. Res. Commun.*, **154**, 620–625.
- HAWORTH, R.A., GOKNUR, A.B. & BERKOFF, H.A. (1989). Inhibition of ATP-sensitive potassium channels of adult rat heart cells by antiarrhythmic drugs. *Circ. Res.*, **65**, 1157–1160.
- HENQUIN, J.C., HOREMANS, B., NENQUIN, M., VERNIERS, J. & LAMBERT, A.E. (1975). Quinine-induced modifications of insulin release and glucose metabolism by isolated pancreatic islets. *FEBS Lett.*, **37**, 280–284.
- HIDAKA, H. & TANAKA, T. (1983). Naphthalenesulfonamides as calmodulin antagonists. *Methods Enzymol.*, **17**, 185–194.
- HIMMEL, H.M. & RAVEN, U. (1990). TMB-8 as a pharmacological tool in guinea pig myocardial tissues. I. Effects of TMB-8 on force of contraction and on action potential parameters in atrial and papillary muscles. *J. Pharmacol. Exp. Ther.*, **255**, 293–299.
- HONORÉ, E. & LAZDUNSKI, M. (1991). Hormone-regulated K⁺ channels in follicle-enclosed oocytes are activated by vasorelaxing K⁺ channel openers and blocked by antidiabetic sulfonylureas. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 5438–5442.
- ISHIGURO, M., MIURA, Y. & INUI, J. (1992). Electrophysiological analysis of the effects of Y-26763, an active metabolite of a new K channel opener Y-27152, in the guinea-pig ventricular muscle. *Jpn. J. Pharmacol.*, **58**, 185.
- JEANDEL, C., PREISS, M.A., PIERSON, H., PENIN, F., CUNY, G., BANNWARTH, B. & NETTER, P. (1988). Hypoglycemia induced by cibenzoline. *Lancet*, **i**, 1232–1233.
- KAMIYA, J., ISHII, M. & KATAKAMI, T. (1992). Antiarrhythmic effects of MS-551, a new class III antiarrhythmic agent, on canine models of ventricular arrhythmia. *Jpn. J. Pharmacol.*, **58**, 107–115.
- KANTOR, P.F., COETZEE, W.A., CARMELIET, E.E., DENNIS, S.C. & OPIE, L.H. (1990). Reduction of ischemic K⁺ loss and arrhythmia in rat hearts. Effects of glibenclamide, a sulfonylurea. *Circ. Res.*, **66**, 478–485.
- KOZLOWSKI, R.Z. & ASHFORD, M.L.J. (1991). Barbiturates inhibit ATP-K⁺ channels and voltage-activated currents in CRI-G1 insulin-secreting cells. *Br. J. Pharmacol.*, **103**, 2021–2029.
- KUMAZAWA, T., HARAKAWA, H., OBASE, H., OIJI, Y., NITO, M., KUBO, K., ISHII, A., TOMIOKA, S. & YAMADA, Y. (1990). Synthesis and antiarrhythmic activity of 5,11-dihydro[1]benzoxepino [3,4-b]pyridines. *J. Med. Chem.*, **33**, 3095–3100.
- KUSANO, K., MILEDI, R. & STINNAKRE, J. (1982). Cholinergic and catecholaminergic receptors in the *Xenopus* oocyte membrane. *J. Physiol.*, **328**, 143–170.
- LYNCH, J.J. Jr., SANGUINETTI, M.C., KIMURA, S. & BASSETT, A.L. (1992). Therapeutic potential of modulating potassium currents in the diseased myocardium. *FASEB J.*, **6**, 2952–2960.
- MANNHOLD, R., VOIGT, W., BAST, A. & TIMMERMAN, H. (1990). Interaction of class I compounds with calmodulin. *Arch. Pharmacol. (Weinheim)*, **323**, 487–491.
- MATSUOKA, S., NAWADA, T., HISATOME, I., MIYAMOTO, J., HASEGAWA, J., KOTAKE, H. & MASHIBA, H. (1991). Comparison of Ca²⁺ channel inhibitory effects of cibenzoline with verapamil on guinea-pig heart. *Gen. Pharmacol.*, **22**, 87–91.
- MÜLLER, M., DE WILLE, J.R. & LAZDUNSKI, M. (1991). Chlorpromazine and related phenothiazines inhibit the ATP-sensitive K⁺ channel. *Eur. J. Pharmacol.*, **198**, 101–104.
- NAKABAYASHI, N., ITO, T., IGAWA, T., HIRAIWA, Y., IMAMURA, T., SETA, T., KAWATO, M., USUKURA, N. & TAKEDA, R. (1989). Disopyramide induces insulin secretion and plasma glucose diminution: studies using the in situ canine pancreas. *Metabolism*, **38**, 179–183.
- NAKAJIMA, T., SHINOHARA, T., YAOA, O., FUKUNARI, A., SHINAGAWA, K., AOKI, K., KATOH, A., YAMANAKA, T., SETOGUCHI, M. & TAHARA, T. (1992). Y-27152, a long-acting K⁺ channel opener with less tachycardia: antihypertensive effects in hypertensive rats and dogs in conscious state. *J. Pharmacol. Exp. Ther.*, **261**, 730–736.
- NOACK, T., DEITMER, P., EDWARDS, G. & WESTON, A.H. (1992). Characterization of potassium currents modulated by BRL38227 in rat portal vein. *Br. J. Pharmacol.*, **106**, 717–726.
- NOMA, A. (1983). ATP-regulated K⁺ channels in cardiac muscle. *Nature*, **305**, 147–148.
- NOTSU, T., TANAKA, I., TAKANO, M. & NOMA, A. (1992). Blockade of the ATP-sensitive K⁺ channel by 5-hydroxydecanoate in guinea pig ventricular myocytes. *J. Pharmacol. Exp. Ther.*, **260**, 702–708.
- PHILLIPS, R.E., LOOAREESUWAN, S., WHITE, N.J., CHANTHAVANICH, P., KARBWANG, J., SUPANARANOND, W., TURNER, R.C. & WARRELL, D.A. (1986). Hypoglycaemia and antimalarial drugs: quinidine and release of insulin. *Br. Med. J.*, **292**, 1319–1321.
- PLANT, T.D. & HENQUIN, J.C. (1990). Phentolamine and yohimbine inhibit ATP-sensitive K⁺ channels in mouse pancreatic β -cells. *Br. J. Pharmacol.*, **101**, 115–120.
- REDER, R.F., DANILO, P. Jr. & ROSEN, M.R. (1980). Effects of pirlmenol HCl on electrophysiological properties of cardiac Purkinje fibers. *Eur. J. Pharmacol.*, **61**, 321–333.
- SAKUTA, H., OKAMOTO, K. & WATANABE, Y. (1992a). Blockade by antiarrhythmic drugs of glibenclamide-sensitive K⁺ channels in *Xenopus* oocytes. *Br. J. Pharmacol.*, **107**, 1061–1067.
- SAKUTA, H., SEKIGUCHI, M., OKAMOTO, K. & SAKAI, Y. (1992b). Inactivation of glibenclamide-sensitive K⁺ channels in *Xenopus* oocytes by various calmodulin antagonists. *Eur. J. Pharmacol.*, **226**, 199–207.

- SCHULTZ, A. & HASSELBLATT, A. (1989). An insulin-releasing property of imidazoline derivatives is not limited to compounds that block α -adrenoceptors. *Naunyn-Schmied Arch. Pharmacol.*, **340**, 321–327.
- SEMEL, J.D., WORTHAM, E. & KARL, D.M. (1983). Fasting hypoglycemia associated with disopyramide. *Am. Heart J.*, **106**, 1160–1161.
- SILVER, P.J., CONNELL, M.J., DILLON, K.M., CUMISKEY, W.R., VOLBERG, W.A. & EZRIN, A.M. (1989). Inhibition of calmodulin and protein kinase C by amiodarone and other class III antiarrhythmic agents. *Cardiovasc. Drugs Ther.*, **106**, 675–682.
- SPRUCE, A.E., STANDEN, N.B. & STANFIELD, P.R. (1985). Voltage-dependent ATP-sensitive potassium channel of skeletal muscle membrane. *Nature*, **316**, 736–738.
- STURGESS, N.C., ASHFORD, M.L.J., COOK, D.L. & HALES, C.N. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet*, **ii**, 474–475.
- SZEWCZYK, A., DE WILLE, J.R. & LAZDUNSKI, M. (1992). TMB-8 (8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate) inhibits the ATP-sensitive K^+ channel. *Eur. J. Pharmacol.*, **226**, 175–177.
- WEISS, B. & LEVIN, R.M. (1978). Mechanism for selectively inhibiting the activation of cyclic nucleotide phosphodiesterase and adenylate cyclase by antipsychotic agents. *Adv. Cycl. Nucleot. Res.*, **9**, 285–303.
- YORIKANE, R., MIZUNO, H., ITOH, Y., KOIKE, H., MIYAKE, S., SHIGA, H., KUMAKURA, S., FUKAMI, M., SHIMOJI, Y., HASHIMOTO, T. & TOMITA, K. (1991). Antiarrhythmic and cardiovascular profiles of the fused indole compound (3aR, 12R, 12aR, 12aS)-12-amino-2,3,3a,4,11,12,12a,12b-octahydro-10-hydroxyisoquinolo[2,1,8-lma]carbazol-5(1H)-one hydrochloride 1,5 hydrate. *Arzneim. Forsch.*, **41**, 1130–1136.

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