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Two different types of channels are targets for potassium channel openers in *Xenopus* oocytes

Eric Honoré and Michel Lazdunski

Institut de Pharmacologie Moléculaire et Cellulaire du CNRS, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France

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K^+ channel openers elicit K^+ currents in follicle-enclosed *Xenopus* oocytes. The most potent activators are the pinacidil derivatives P1075 and P1060. The rank order of potency to activate K^+ currents in follicle-enclosed oocytes was: P1075 ($K_{0.5}$:5 μM) > P1060 ($K_{0.5}$: 12 μM) > BRL38227 (lemakalim) ($K_{0.5}$:77 μM) > RP61419 ($K_{0.5}$:100 μM) > (−)pinacidil ($K_{0.5}$:300 μM). Minoxidil sulfate, nicorandil, RP49356 and diazoxide were ineffective. Activation by the K^+ channel openers could be abolished by the antidiabetic sulfonylurea glibenclamide. It was not affected by the blocker of the Ca^{2+}-activated K^+ channels charybdotoxin. The various K^+ channel openers failed to activate glibenclamide-sensitive K^+ channels in defolliculated oocytes, but BRL derivatives ($K_{0.5}$ for BRL38226 is 150 μM) and RP61419 inhibited a background current. The channel responsible for this background current is K^+ permeable but not fully selective for K^+. It is resistant to glibenclamide. It is inhibited by Ba^{2+}, 4-aminopyridine, Co^{2+}, Ni^{2+} and La^{3+}.

Follicular *Xenopus* oocyte; Potassium channel opener; Glibenclamide; ATP-sensitive potassium channel

1. INTRODUCTION

The discovery that the vasorelaxant effects of BRL34915, most commonly called cromakalim (CK), are associated with an hyperpolarization of vascular smooth muscle due to K^+ channel opening, has recently attracted considerable interest [1–3]. There are now many different chemical classes of K^+ channel openers (KCOs). The best known compounds in each class are cromakalim, pinacidil, nicorandil, minoxidil sulfate, diazoxid and RP49356 [4].

KCOs have now been shown to have an action not only on the vascular smooth muscle cell but also on β-pancreatic cells [5] or other types of smooth muscle cells [6,7], on cardiac cells [8], on neurons [9], and on skeletal muscle cells [10]. Their target seems to be an important class of K^+ channels, the ATP-sensitive K^+ (K_{ATP}) channels [11].

Abbreviations: CK, cromakalim; KCOs, K^+ channel openers; K_{ATP} channels, ATP-sensitive K^+ channels; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; EGTA, ethyleneglycol-bis-(β-aminoethylether)*N,N,N',N'*,-tetraacetic acid; *I–V* relationship, current–voltage relationship; TEA^+, tetraethylammonium; 4AP, 4-aminopyridine; MCD peptide, mast cell degranulating peptide; RP49356, *N*-methyl,2-[-3-pyridil]-tetrahydrothiopyran-2-carbothiamide-1-oxide; BRL34915, 6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-[2-oxo-1-pyrrolidyl]-2*H*-benzo[b]-pyran-3-ol; P1060, *N-tert*-butyl-*N''*-cyano-*N'''*-3-pyridinylguanidine; P1075, *N-tert*-pentyl-*N''*-cyano-*N'''*-3-pyridinylguanidine.

Correspondence address: E. Honoré, Institut de Pharmacologie Moléculaire et Cellulaire du CNRS, 660 routes des Lucioles, Sophia Antipolis, 06560 Valbonne, France. Fax: (33) (93) 957704.

The purpose of this paper is to describe the pharmacological properties of action of KCOs in the *Xenopus* oocyte system which turns out to be one of the best systems for studying this class of drugs.

2. MATERIALS AND METHODS

Xenopus laevis were purchased from C.R.B.M. (Montpellier, France). Pieces of the ovary were surgically removed and individual oocytes were dissected away in a modified Barth's solution: 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (HEPES), pH 7.4 with NaOH. To discard follicular cells, stage V and VI oocytes were treated for 2 h with collagenase (1 mg · ml^{-1}, Boehringer Mannheim, Germany) in Barth's medium and then the follicular layer was manually removed with fine forceps. Oocytes were kept for 2 to 7 days in Barth's medium supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin.

In a 0.3 ml perfusion chamber, a single oocyte was impaled with two standard glass microelectrodes (0.5–2.0 MΩ resistance) filled with 3 M KCl and maintained under voltage clamp using a Dagan 8500 amplifier. Stimulation of the preparation, data acquisition and analyses were performed using the pClamp software (Axon Instruments, USA). Drugs were applied either externally by addition to the superfusate (Gilson peristaltic pump; flow rate: 3 ml · min^{-1}). EGTA was internally applied by pressure injection (Inject + Matic, Switzerland) using an additional micropipette (2–4 μm in diameter). The injected volume was 0.01–0.02% of the cell volume. Saline solution (ND 96) of the following composition was used in all procedures unless otherwise stated: 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM HEPES (pH 7.4 with NaOH). All chemicals used were from Sigma unless otherwise stated. The variability of the results was expressed as SEM with n indicating the number of cells contributing to the mean.

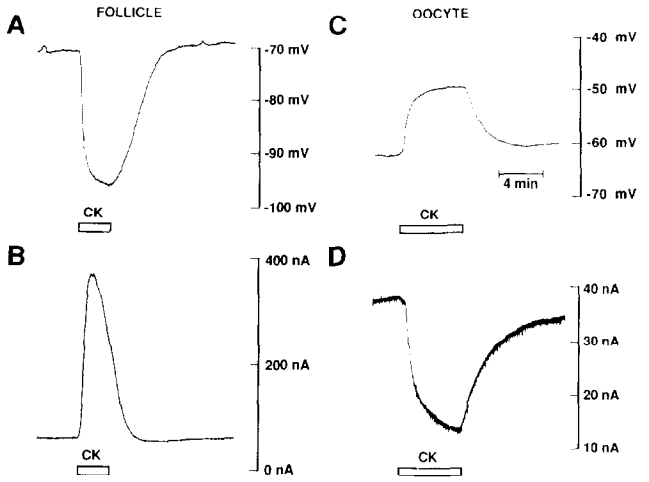

Fig. 1. (A) In a follicle-enclosed *Xenopus* oocyte 100 μM CK induced an hyperpolarization. (B) In the same follicle-enclosed oocyte voltage clamped as −20 mV, 100 μM CK elicited a large outward current. (C) In a defolliculated oocyte, 100 μM CK induced a depolarization. (D) In the same defolliculated oocyte voltage clamped at −20 mV, 100 μM CK induced an inward current.

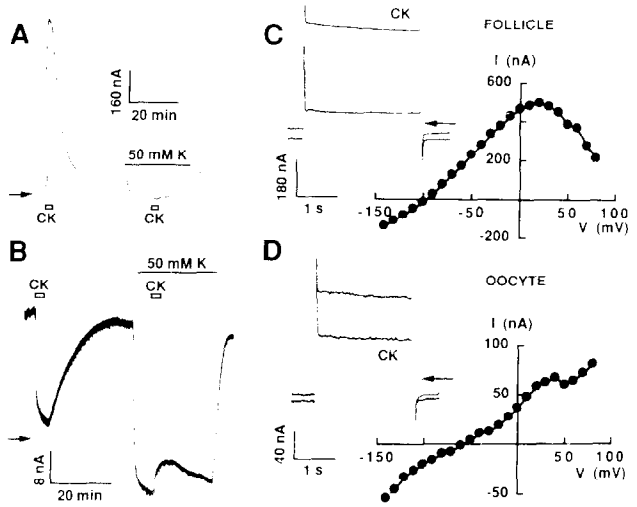

Fig. 2. (A) In the follicle-enclosed oocyte, CK induced an outward current in normal saline solution containing 2 mM K^+. In the presence of a K^+-rich solution (50 mM K^+ substituting Na^+), the CK-induced current reversed direction, becoming inward. (B) In a defolliculated oocyte, the CK-induced inward current became outward in the K^+-rich solution (50 mM K^+ substituting Na^+). In these experiments the holding potential was −20 mV and the zero current level is indicated by an arrow. (C) *I–V* relationship of the CK-induced current in a follicle-enclosed oocyte. The current before CK was digitally subtracted. Inset: current traces in the control and in the presence of CK. The holding potential was −80 mV and the cell was depolarized to −10 mV. (D) *I–V* relationship of the current altered by CK in a defolliculated oocyte. The *I–V* curve was determined during the reduction in membrane conductance, by stepping clamp potential to different levels, and currents were subtracted from those obtained in the absence of CK, thus, giving the voltage dependence of currents actually being blocked by CK. Inset: current traces in control and in the presence of CK. The holding potential was −80 mV and the cell was depolarized to 60 mV. In these experiments, CK concentration was 300 μM.

3. RESULTS

Fig. 1 compares the effects of KCOs on the electrophysiological properties of follicle-enclosed and defolliculated oocytes.

Superfusion of a follicle-enclosed *Xenopus* oocyte with 100 μM CK induced a large hyperpolarization (Fig. 1A). Under control conditions, in the absence of CK, the resting membrane potential was −57 ± 6 mV (n = 8). In the presence of 100 μM CK, the cell hyperpolarized to −88 ± 8 mV (n = 8). In the same cell under voltage-clamp conditions, at a holding potential of −20 mV, CK induced a large outward current (Fig. 1B). Conversely, CK induced a membrane depolarization in the defolliculated oocyte. In the typical experiment presented in Fig. 1C, the resting membrane potential of a control defolliculated oocyte was −61 ± 4 mV (n = 7). In the presence of 100 μM CK the cell depolarized to −49 ± 3 mV (n = 7). Voltage-clamp experiments at a holding potential of −20 mV show (Fig. 1D) that CK application to the defolliculated oocyte reveals an inward current. This inward current will actually be shown later to result from the inhibition of an outward background current.

Fig. 2A shows that if in the follicle-enclosed oocyte, an outward current was elicited by CK in the presence of 2 mM external K^+, the current direction was reversed when the cell was bathed in a 50 mM K^+ medium. The CK-induced inward current observed in 2 mM K^+ with the defolliculated oocyte became outward in the presence of 50 mM K^+ (Fig. 2B).

Current-voltage (*I–V*) relationships for the CK-modulated currents in follicle-enclosed and defolliculated oocytes are presented in Fig. 2C and 2D. The CK-induced outward current measured at −10 mV in the follicle-enclosed oocyte is shown in Fig. 2C (inset). The *I–V* curve in that case was linear between −140 and 20 mV and then rectified in the inward direction for positive potentials (Fig. 2C). The reversal potential was −97 ± 5 mV (n = 9). The inset in Fig. 2D shows that CK decreased the amplitude of the outward current measured after a voltage jump from −80 to 60 mV in a defolliculated oocyte. In that case CK clearly decreased a membrane conductance. The *I–V* curve of the current component which is blocked by CK is linear over all the range of potentials tested and the reversal potential was observed at −51 ± 5 mV (n = 12). The inhibitory effects of CK remained after removal of extracellular Ca^{2+} or loading with intracellular EGTA.

Fig. 3A compares the relative amplitudes of the outward currents elicited with 300 μM of various KCOs. Both (+) and (−)pinacidil were active although with a relatively weak potency. (−)Pinacidil was about twice more effective than the (+)enantiomer. The pinacidil derivatives P1075 and P1060 were about 20 times more potent than pinacidil. BRL38227 (also called

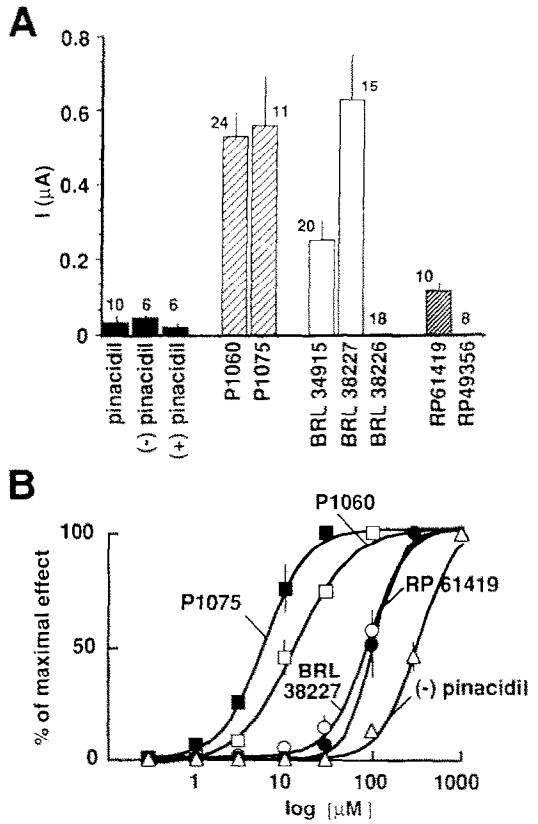

Fig. 3. (A) Amplitude of the currents elicited by 300 μM KCOs in follicles. Numbers of oocytes are indicated and the number of donors was at least 3. In all these experiments the holding potential was −20 mV. (B) KCOs dose–effect curves in follicle-enclosed oocytes. Every curve is the mean of at least 6 experiments on three different frogs.

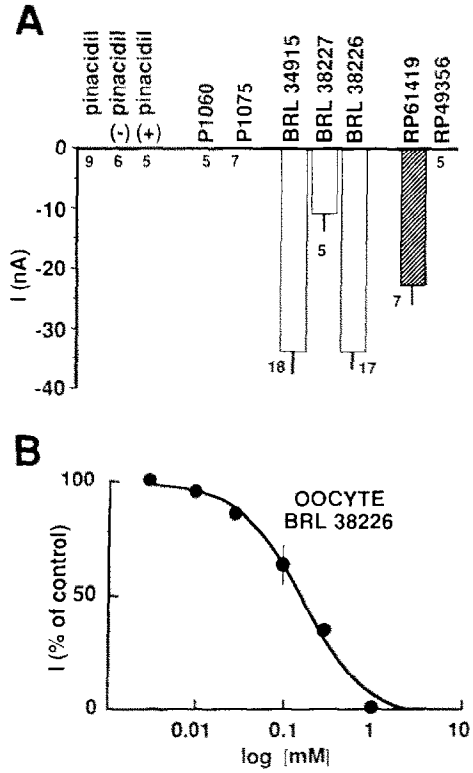

Fig. 4. (A) Amplitude of the current inhibited by 300 μM KCOs in defolliculated oocytes. Numbers of oocytes are indicated and the number of donors was 4. In all these experiments the holding potential was −20 mV. (B) Dose–effect curve of the inhibition of the background current by BRL38226 on a defolliculated oocyte. Every mean data point is the mean of at least 6 experiments on 4 donors.

lemakalim) induced a current of about the same amplitude as P1060 and P1075. The (+)enantiomer BRL38226 was totally inactive. The racemate BRL34915 (CK) was 2.5 times less potent than the (−)enantiomer BRL38227. Among the RP derivatives tested, only RP61419 could activate an outward current in follicle-enclosed oocytes with a potency of about half of that found for CK. Minoxidil sulfate, nicorandil and diazoxide at a concentration of 300 μM were also totally ineffective ($n=5$). The comparative dose–response curves for the activation of outward currents in follicular cells by different KCOs are presented in Fig. 3A. The rank order of potency was P1075 > P1060 > BRL38227 > RP61419 > (−)pinacidil.

Fig. 4A compares the efficacy of different KCOs used at the concentration of 300 μM to inhibit the background ionic conductance in the defolliculated oocyte. Pinacidil and derivatives were ineffective. CK, BRL38226 and RP61419 produced similar effects. BRL38227 was three times less effective than BRL38226. The other KCOs tested including RP49356, minoxidil sulfate, nicorandil and diazoxide did not have any inhibitory effect ($n=7$). Fig. 4B shows the inhibition of the background ionic current in defolliculated oocytes by the (+)enantiomer BRL38226. BRL38226 inhibited 50% of the current at a concentration of 150 μM.

Fig. 5 compares the effects of glibenclamide on both types of KCOs sensitive currents. The current stimulated by KCOs in the follicle-enclosed oocyte was inhibited in a dose-dependent manner by the sulfonylurea glibenclamide (Fig. 5A). The background current inhibited by KCOs was not altered by 10 μM glibenclamide (Fig. 5B). The less specific K^+ channel blockers tetraethylammonium (TEA^+), 4-aminopyridine (4AP), Cs^+ and Ba^{2+} are known to inhibit the outward current elicited by CK in different preparations [12,14]. The background current which is blocked by BRL38226 and CK was not inhibited by 30 mM TEA^+ and by 20 mM Cs^+ (data not shown). It was also depressed by 3 mM 4AP and abolished by 500 μM Ba^{2+} (Fig. 5B). Similar types of inhibitions were also found with 1 mM of Co^{2+}, Ni^{2+} and La^{3+} (data not shown).

The different polypeptides and toxins blocking voltage- and Ca^{2+}-dependent K^+ channels such as apamin, dendrotoxin I, MCD peptide, charybdotoxin and β-bungarotoxin (100 nM) were inactive on currents which are sensitive to KOCs activation or inhibition.

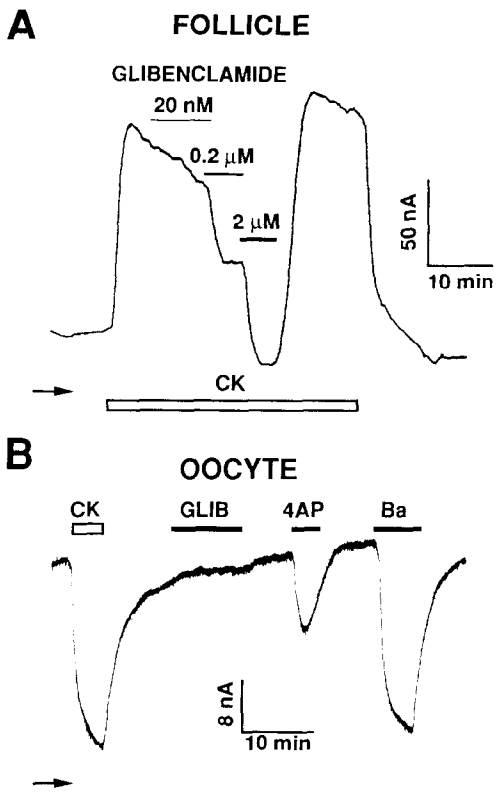

Fig. 5. (A) The 100 μM CK-induced outward current recorded at a potential of −20 mV was blocked by increasing concentrations of glibenclamide (as indicated on current trace) in a follicle-enclosed oocyte. (B) In a defolliculated oocyte, voltage-clamped at −20 mV, the background current was blocked by 300 μM CK, 3 mM 4AP and 500 μM Ba^{2+}. 10 μM glibenclamide did not affect this current. In these experiments the zero current levels are indicated by arrows.

4. DISCUSSION

This paper shows that both the follicle-enclosed *Xenopus* oocyte and defolliculated oocytes have ionic channels that are sensitive to KCOs.

CK, pinacidil and their derivatives activate a K^+ channel in the follicular oocyte. The opening of this channel produces an important hyperpolarization. The KCO-activated K^+ channels are blocked by the sulfonylurea glibenclamide. The current-voltage relationship of this channel indicates a rectification for membrane potentials higher than +20 mV. The pharmacological properties, i.e. the activation of KCOs and the inhibition by glibenclamide and the biophysical properties, i.e. the rectification at higher potentials, are similar to properties which have been previously established for the cardiac ATP-sensitive K^+ channel [8]. However the cardiac K^+ channel is much more sensitive to glibenclamide ($K_{0.5}$ = 0.3 nM; [13]) than the K^+ channel in follicle oocytes ($K_{0.5}$ = 200 nM). At a concentration of 300 μM, the most potent activators of the K^+ channel in follicular oocytes are the pinacidil derivatives P1060 and P1075 and the (−)enantiomer BRL38227 (lemakalim). However, other openers were also active and the rank order of potency is P1075 ($K_{0.5}$: 5 μM) > 1060 ($K_{0.5}$: 12 μM) > BRL38227 ($K_{0.5}$: 77 μM) > RP61419 ($K_{0.5}$: 100 μM) > (−)pinacidil ($K_{0.5}$: 300 μM). How does the efficacy of these different compounds on the follicular oocyte correspond to their known efficacy in other sensitive cell types? In smooth muscle both the affinities and the rank order of potency of KCOs are different from what is described here for the follicular oocyte. The pinacidil derivative P1060 and P1075 are more potent than pinacidil [14] but $K_{0.5}$ values for pinacidil ($K_{0.5}$: 0.3 μM) and cromakalim ($K_{0.5}$: 20 nM) are much lower than those described in this paper. Also nicorandil and minoxidil sulfate are active [2] in smooth muscle cells whereas they are inactive in follicular oocytes.

The potency of pinacidil and derivatives is also greater in cardiac cells than in follicular oocytes [15]. Although in this preparation again one finds that P1075 and P1060 are more potent than pinacidil.

In substantia nigra the order of potency of KCOs is lemakalim > nicorandil > cromakalim > pinacidil > P1075 [9]. Again it is clearly different from that found in follicular oocytes.

All these results taken together suggest that the *Xenopus* follicular oocytes contain a new subtype of KCOs-sensitive K^+ channel.

Defolliculation clearly indicates that the KCO/glibenclamide-sensitive K^+ channels are situated in follicles and are not present in the oocyte itself. The oocyte membrane, however, also contains a channel that is altered by some of the KCOs and particularly by CK (BRL34915) and by its two enantiomers BRL38227 and BRL38226. Interestingly while the (−)enantiomer BRL38227 is the most active opener of K^+ channels in follicles, the (+)enantiomer BRL38226 is the most active inhibitor of the oocyte channel. The RP61419 compound is, like compounds in the BRL series, an active opener of the follicular K^+ channels and an inhibitor of the oocyte channel. The channel inhibited in defolliculated oocytes is responsible for a background current since its inhibition reveals an inward current (Fig. 1D). Like many types of K^+ currents [16], the background current which is inhibited by some of the KCOs is also inhibited by Ba^{2+} and 4AP. However this current is not inhibited by glibenclamide. The channel responsible for this current however is probably not a completely selective K^+ channel since the reversal potential is −51 ± 5 mV (it should have been about −95 mV for a K^+ channel). The current is also blocked by Co^{2+} and Ni^{2+} as a background current which had been previously identified by Miledi et al. [17]. It seems that BRL38226 could be an adequate pharmacological tool to gain more information in the future on the properties of this particular background current.

The different molecules studied in this work will probably be useful to understand the respective physiological roles of the KCOs activated K^+ channels

in follicles and of the KCOs inhibited channel responsible for the background current in defolliculated oocytes.

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