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Functional Receptors in *Xenopus* Oocytes for U-37883A, a Novel ATP-Sensitive K^+ Channel Blocker: Comparison with Rat Insulinoma Cells

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SUMMARY

Follicle-enclosed *Xenopus* oocytes were used to describe the ATP-sensitive K^+ (K_{ATP}) channel-blocking properties of U-37883A (4-morpholinecarboximidine-*N*-1-adamantyl-*N'*-cyclohexyl), in comparison with glibenclamide. In follicular oocytes, the K_{ATP} channel opener P1060 (30 μM), a pinacidil analog, activated a large outward K^+ current that was blocked by glibenclamide (IC_{50} = 0.33 μM) and U-37883A (IC_{50} = 0.26 μM). P1060 activation was inhibited by both U-37883A and glibenclamide in a noncompetitive manner. U-37883A also blocked the K_{ATP} channel activation by cAMP (300 μM) and adenosine (10 μM). Single-channel studies on isolated follicular cells showed that U-37883A (10 μM) reduced the open probability of the K_{ATP} channel by 76%, without significantly modifying the single-channel current amplitude. Receptor binding studies with [^{3}H]U-37883 in membranes from follicle-enclosed oocytes demonstrated a single class of low affinity binding sites, with a K_d of 450 nM and a B_{max} of 17 pmol/mg of protein. Studies with analogs of U-37883A showed that U-52090A inhibited K_{ATP} current and displaced [^{3}H]U-37883 from its binding site with similar potencies. In contrast, U-42069D neither inhibited K_{ATP} current nor competed with [^{3}H]U-37883 binding. In RINm5F cells (an insulinoma cell line), U-37883A, unlike glibenclamide, failed to inhibit K_{ATP} current. Furthermore, there was no significant specific binding of [^{3}H]U-37883 in RINm5F cell membranes, which displayed high levels of specific binding of [^{3}H]glibenclamide. These data demonstrate the presence of a receptor for U-37883A-type guanidines that controls the activity of the endogenous K_{ATP} channels in follicle-enclosed oocytes. The available data collectively suggest that U-37883A is a more selective blocker of the follicular K_{ATP} channel, which is very similar to that in smooth muscle, than of the pancreatic β cell K_{ATP} channel.

Follicle-enclosed *Xenopus* oocytes have been shown in recent years to contain endogenous K^+ channels that are modulated by various hormones and receptor agonists (1–3). Thus, this system has been used as a valuable model for investigating the mechanisms of regulation of K^+ channels by a large variety of hormones and transmitters (4, 5). More recently, our laboratory has provided identification and detailed electrophysiological characterization of a K^+ channel that is sensitive to blockade by glibenclamide (glyburide) and is activated by structurally diverse K^+ channel openers (6–8). Based on the electrophysiological and pharmacological characteristics (6, 8, 9), it appears that this K^+ channel belongs to the class of K_{ATP} channels. The presence of K_{ATP} channels in follicle-enclosed oocytes has also been implicated by other laboratories (10). The follicular K_{ATP} channel appears to be quite similar to the vascular smooth muscle K_{ATP} channel. Both are similarly activated by various K^+ channel openers, and this activation is blocked by relatively high concentrations of glibenclamide, compared with K_{ATP} channels in β cells and cardiac cells (6–9, 11–14). Both are activated by vasodilators such as adenosine and vasoactive intestinal peptide.[2]

Characterization of the *in vitro* and *in vivo* pharmacology of various K_{ATP} channel openers has required extensive use of appropriate blockers of the K_{ATP} channel (11, 12). One such potent K_{ATP} channel blocker is glibenclamide, a sulfonylurea that has been used in a variety of cell systems to characterize K_{ATP} channels (12–14). We have recently described the *in vitro* and *in vivo* pharmacology of U-37883A (see Fig. 1 for structure), a nonsulfonylurea vascular K_{ATP} channel blocker (15, 16). Thus, U-37883A (a guanidine) selectively blocks vasorelaxation, stimulation of ^{42}K efflux, and the hypotension produced by various K_{ATP} channel openers. One interesting feature of U-37883A is that, unlike glibenclamide, U-37883A is a selective vascular

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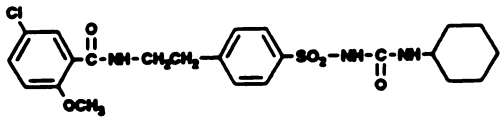
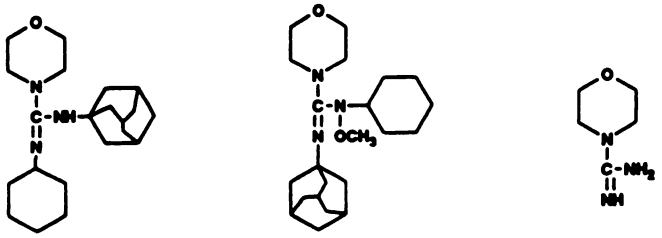
[2] E. Honore and M. Lazdunski, unpublished observations.

ABBREVIATIONS: K_{ATP} channel or current, ATP-sensitive K^+ channel or current; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid.



Glibenclamide (or Glyburide)



U-37883 U-52090 U-42069

Fig. 1. Chemical structures of compounds used in this study. Glibenclamide is a sulfonylurea, whereas the other three compounds are guanidines. U-37883A is 4-morpholinecarboximidine-*N*-1-adamantyl-*N'*-cyclohexyl hydrochloride.

K_{ATP} channel blocker, because it does not stimulate insulin secretion (15). However, no direct electrophysiological characterization of the inhibitory effects of U-37883A on the K_{ATP} channel in any cell type is available. Thus, the primary objective of this study was to provide a detailed electrophysiological characterization of the effects of U-37883A on follicular oocytes, using whole-cell current and single-channel analyses. Furthermore, we have carried out a comparative study of the inhibitory effects of U-37883A and glibenclamide on the follicular K_{ATP} channel and the pancreatic β cell K_{ATP} channel, to define the selectivity of U-37883A. Finally, we also provide the initial identification of a functional receptor by characterizing the binding of [^{3}H]U-37883 in follicular oocyte membranes.

Materials and Methods

Electrophysiological Experiments

***Xenopus* oocytes.** *Xenopus laevis* were purchased from Centre de Recherches de Biochimie Macromoleculaire (Montpellier, France). Pieces of the ovary were surgically removed and individual oocytes were dissected away in a saline solution (ND96) containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, at pH 7.4 (with NaOH). Stage V and VI oocytes were then kept for 2–6 days at 19° in saline solution (ND96) supplemented with 50 μg/ml gentamycin. The standard saline solution (ND96) was used for all procedures. All experiments were performed at room temperature (22–24°). The two-microelectrode voltage-clamp technique has been described previously (6).

Effect of U-37883A on P1060 responses and comparison with glibenclamide. The first series of experiments were designed to study the inhibitory effects of U-37883A or glibenclamide on the follicular oocyte K^+ currents activated by a known K_{ATP} channel opener, P1060, a pinacidil analog (8). As shown in Fig. 2A, in a follicular oocyte voltage-clamped at −20 mV, K^+ current could be reproducibly activated by a 2-min exposure to 30 μM P1060. After two such exposures to P1060, the oocyte was pretreated with U-37883A (0.01–100 μM) or glibenclamide (0.05–10 μM) for 5 min and then rechallanged with P1060 for 2 min in the continuing presence of the inhibitor. Each oocyte was exposed to only one concentration of the inhibitor. The percentage inhibition produced was calculated compared with the pretreatment control K^+ current. Dose-response curves were fitted with the equation $y = ax/(b + x)$, where y is the percentage inhibition of P1060-activated current, a is the maximum inhibition, x is the logarithm of the drug concentration, and b is the drug concentration for 50% inhibition. In another series of experiments, the effects of U-37883A (0.3, 3, and 30 μM) or glibenclamide (0.3 and 3 μM) pretreatment on the dose-response curve for P1060 (0.3–300 μM)-activated K^+ current were studied.

Effects of U-37883A and glibenclamide on K^+ currents activated by cAMP and adenosine. Based on previously reported data (6), inhibitory effects of U-37883A and glibenclamide (both at 10 μM) on K^+ currents activated by chlorophenylthio-cAMP (300 μM) and adenosine (10 μM) were studied. The drugs were superfused for 2 min with a peristaltic pump at a flow rate of 0.3 ml/min.

The data are presented as mean ± standard error, with n indicating the number of cells contributing to the mean. The Student t test was used for statistical analysis.

Single-channel experiments. We have recently described single-channel properties of P1060-activated and glibenclamide-inhibitable K^+ channels in follicular cells from *Xenopus* oocytes (8). To obtain isolated follicular cells, follicles were treated for 2 hr with 1 mg/ml collagenase (type IA; Sigma), and the follicular layer was manually dissected (8). The outside-out patch-clamp configuration was used to record single-channel activity from isolated follicular cells. The effect of U-37883A (10 μM) on K_{ATP} channels was recorded in the absence of internal Ca^{2+} and in the presence of a low internal ATP concentration (0.2 mM). The details of the electrophysiological procedures have been described previously (8).

RINm5F cells. RINm5F cells from an insulin-producing cell line were grown as described previously (13). Current-clamp experiments were carried out using the whole-cell suction pipette technique (13). Membrane potential recordings were made in a RINm5F cell dialyzed with an internal ATP-free solution. Single-channel currents were recorded from outside-out membrane patches; their membrane potential was clamped at 0 mV with a voltage-clamp amplifier (Biologic, Grenoble, France). The intracellular solution contained 150 mM KCl, 1 mM $MgCl_2$, 2 mM EGTA, and 10 mM HEPES/KOH, pH 7.2. The extracellular solution contained 140 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM HEPES/NaOH, pH 7.3. Pipettes were coated with Slygard resin to reduce current noise or parasitic capacity.

Membrane Binding Experiments in Oocytes and RINm5F Cells

The binding studies were carried out using [^{3}H]U-37883 (specific activity, 50 Ci/mmol; a gift from The Upjohn Company, Kalamazoo, MI). The membranes from RINm5F cells were prepared as described before (13). Briefly, cells were homogenized in 40 mM HEPES/NaOH buffer with five strokes of a Potter-Elvehjem homogenizer, and the suspension was sonicated for 10 sec and centrifuged at 70,000 × g for 25 min. The microsomal pellet was suspended in 20 mM HEPES/NaOH buffer, pH 7.5. Follicle-enclosed oocytes from three frogs were used for the preparation of crude membranes for binding studies. Oocytes were suspended in 300 ml of 50 mM HEPES (containing 0.3 M sucrose and 1 mM EDTA) at 4° on ice. Oocytes were homogenized using a motorized Teflon homogenizer (five strokes, 15 sec each, at high speed). This was followed by sequential centrifugations at 1000 × g (10 min) and 10,000 × g (20 min); each time the pellet was discarded and the supernatant was used for the subsequent step. The final centrifugation was at 60,000 × g for 25 min. The microsomal pellet was resuspended in 20 mM HEPES buffer and used for the binding studies.

The binding experiments were carried out at room temperature (22–24°). Oocyte membranes were incubated in 1 ml of 50 mM HEPES, pH 7.4, at a protein concentration of 500 μg/ml. [^{3}H]U-37883 was used at a concentration of 1–2 nM. Incubations lasted for 60 min and were stopped by rapid filtration through Whatman GF/C filters under reduced pressure. Filters were washed with ice-cold 100 mM Tris·HCl buffer, pH 7.5. Nonspecific binding was measured using 10 μM U-37883A. Several control experiments were carried out without mem-

branes to determine the propensity of the radiolabel to adhere to the filters. The binding experiments in RINm5F cell membranes were similarly carried out using 500 μg/ml protein. For comparison, some binding experiments using [^{3}H]glibenclamide (50 Ci/mmol; NEN) were also carried out as described previously (13).

U-37883A Solutions

The unlabeled drug was U-37883A, which is the hydrochloride salt of U-37883. This HCl salt is freely water soluble, and thus U-37883A was used in all electrophysiology and competition experiments. The radiolabeled [^{3}H]U-37883 stock solution was in methanol.

Results

Effects of U-37883A and Glibenclamide on P1060 Responses in Follicle-Enclosed Oocytes

Follicular oocyte currents. As presented in Fig. 2A, the study of follicular oocyte currents showed that successive applications of P1060 induced repetitive large outward K^+ currents in follicle-enclosed oocytes. This tracing also shows that pretreatment with 0.3 μM U-37883A inhibited about 50% of the P1060 response. Similar effects were seen after glibenclamide pretreatment (tracing not shown). Fig. 2, B and C, shows dose-response curves for the inhibitory effects of U-37883A and glibenclamide, respectively. The majority of the K_{ATP} channel inhibitory response for both blockers occurred within the concentration range of 0.1–10 μM. The IC_{50} values (concentration required to produce 50% of maximum inhibition) for U-37883A and glibenclamide were 0.26 μM and 0.33 μM, respectively.

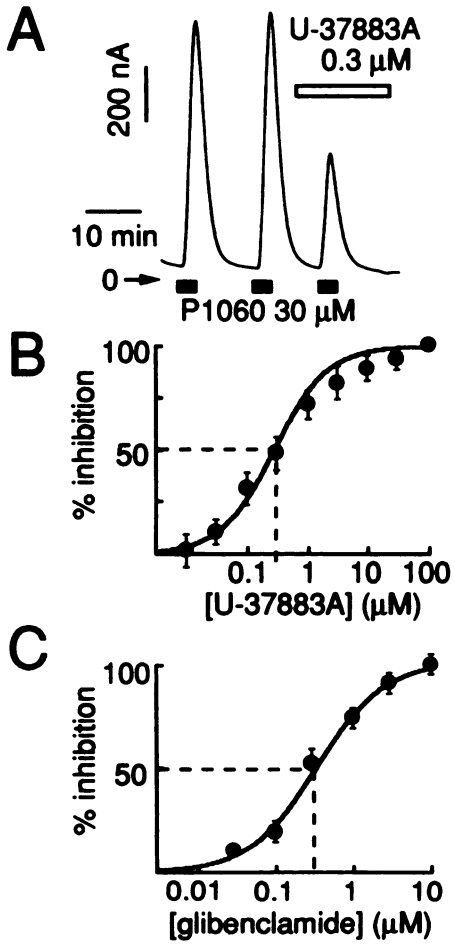


Fig. 2. A, Representative tracing showing P1060 (30 μM)-induced outward current in a follicle-enclosed oocyte. The whole-cell currents were recorded at −20 mV in the two-electrode voltage-clamp mode. The exposure to P1060 was for 2 min each time, and the total exposure to U-37883A (0.3 μM) was for 15 min, including a 5-min pretreatment period. B, Log dose-response curve for U-37883A-induced inhibition of P1060 (30 μM)-activated K^+ current. The data were generated using the protocol described for A. C, Log dose-response curve for glibenclamide-induced inhibition of P1060 (30 μM)-activated K^+ current. For both dose-response curves, each *data point* is the mean of observations from at least 10 oocytes from three different frogs.

Fig. 3 shows the shift in the P1060 dose-response curve for K^+ current activation after pretreatment with various concentrations of U-37883A or glibenclamide. The control P1060 EC_{50} value (concentration required to produce 50% of the maximum activation) was 10 μM. Increasing concentrations of either U-37883A (0.3, 3, and 30 μM) or glibenclamide (0.3 and 3 μM) caused a progressive inhibiton of the P1060 dose-response curve, such that higher concentrations of P1060 were required to produce similar levels of K^+ current activation. Furthermore, both U-37883A and glyburide also caused a significant decrease in the maximum response to P1060. Thus, both blockers appear to produce noncompetitive inhibition of the P1060 activation of K_{ATP} current in follicle-enclosed oocytes.

We have previously demonstrated that cAMP and various hormones and transmitters activate glibenclamide-sensitive K^+ channels in follicular cells (6, 7). Fig. 4 presents data on the comparative inhibitory effects of U-37883A and glibenclamide on adenosine- and cAMP-induced K^+ current activation. The response to 30 μM P1060 was used as the reference for 100% activation. As shown in Fig. 4, this response was reduced to 0% by 10 μM glibenclamide and to about 20% by 10 μM U-37883A. The control cAMP response was about 80% of the P1060 response. The 300 μM cAMP response was reduced by both blockers, to similar degrees. The control 10 μM adenosine response was about 20% of the P1060 response. This adenosine response was susceptible to inhibition by U-37883A, and to a lesser extent by glibenclamide.

Single-channel studies. Fig. 5 presents the single-channel data obtained from isolated follicular cells using the outside-

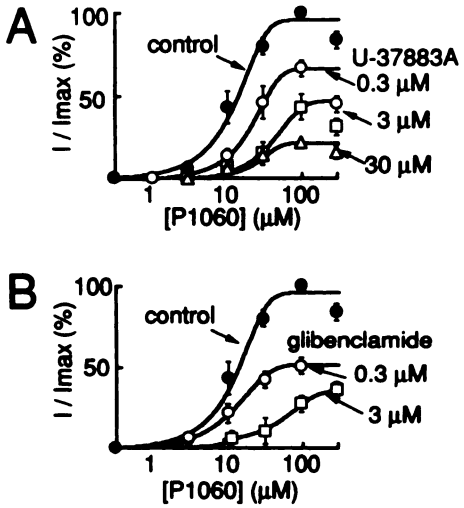


Fig. 3. Dose-response curves for P1060-activated K^+ currents in the presence of varying concentrations of U-37883A (A) or glibenclamide (B). U-37883A or glibenclamide was added 5 min before the 2-min P1060 application. I_{max} is the mean amplitude current activated by 100 μM P1060 in control solution. Each *data point* is the mean of observations from at least eight oocytes from three different frogs.

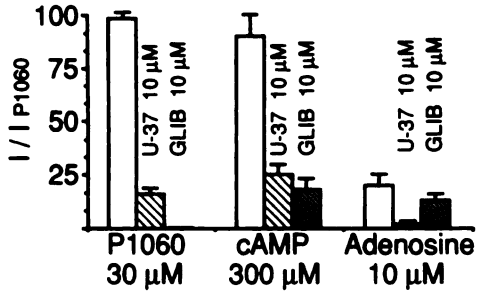


Fig. 4. Comparison of the inhibitory effects of U-37883A (*U-37*) (10 μM) and glibenclamide (*GLIB*) (10 μM) on K^+ currents activated by P1060 (30 μM), chlorophenylthio-cAMP (*cAMP*) (300 μM), or adenosine (10 μM). All responses were normalized to the response obtained with 30 μM P1060. Each *bar* is the mean of at least 12 oocytes from five different frogs.

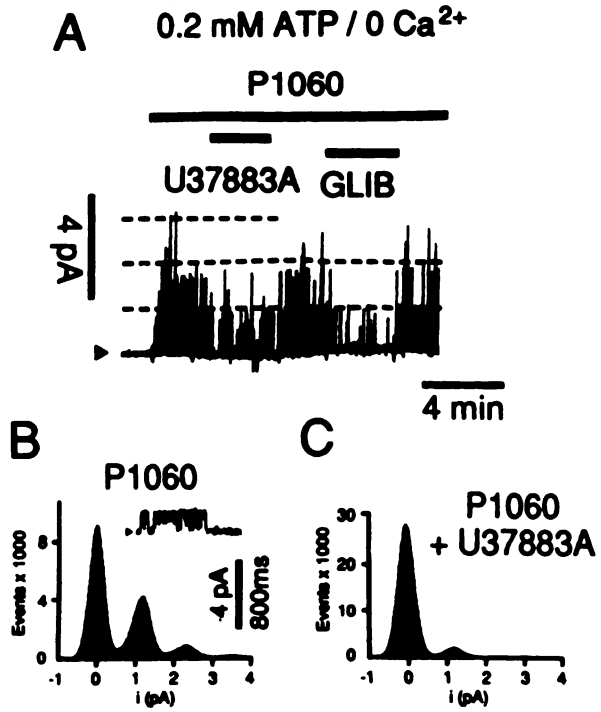


Fig. 5. Effects of U-37883A and glibenclamide on single K^+ channels in isolated follicular cells. A, Outside-out patch recording of a K_{ATP} channel with Ca^{2+}-free internal medium containing a low ATP concentration of 0.2 mM. Activation by P1060 (30 μM) and its inhibition by U-37883A (10 μM) or glibenclamide (*GLIB*) (10 μM) are shown. The external medium (ND96) contained 2 mM K^+ and the internal medium contained 130 mM KCl. The membrane potential was 0 mV. *Arrowhead*, closed state. B and C, Amplitude histograms of P1060-activated K_{ATP} channel at 0 mV in control solution (B) and in the presence of 10 μM U-37883A (C). Note that in the presence of U-37883A the channel remains mostly in the closed state.

out patch-clamp configuration. Fig. 5A shows a K_{ATP} channel recording obtained in the presence of a low internal ATP concentration of 0.2 mM and in the absence of free internal Ca^{2+}. This tracing shows the activation of the K_{ATP} channel by 30 μM P1060 and its blockade by U-37883A (10 μM) or glibenclamide (10 μM). Fig. 5, B and C, presents amplitude histograms of P1060-activated K_{ATP} channels in the absence and in the presence of 10 μM U-37883A. The single-channel current amplitude was 1.25 ± 0.03 pA (n = 7) at 0 mV in a physiological K^+ gradient. The open channel probability calculated from the amplitude histogram was 1.27 ± 0.63 (n = 7). The slope conductance determined from the I-V curve was 19 pS (8). U-37883A (10 μM) decreased the open channel probability by 76 ± 9% (n = 7), without significantly modifying the single-channel current amplitude (1.19 ± 0.03 pA, n = 7).

[^{3}H]U-37883 Binding

The characteristics of [^{3}H]U-37883 binding to a microsomal membrane preparation from follicle-enclosed oocytes are presented in Fig. 6. Fig. 6A shows the displacement of specific binding of [^{3}H]U-37883 by increasing concentrations of the unlabeled compound. Nonspecific binding was defined by using 10 μM U-37883A. Specific binding was found to be 62.5 ± 0.7% (three experiments) of the total binding. Because [^{3}H]U-37883 displayed low affinity binding, it was not experimentally possible to carry out saturation analysis. Thus, the data from the competition curve in Fig. 6A were transformed to generate a Scatchard plot (17). The specific activity of radiolabeled ligand at each point in the competition curve was calculated using the dilution factor from the known concentrations of radiolabeled drug and nonradiolabeled drug. The Scatchard plot presented in Fig. 6B shows a single class of binding sites, with a K_d of 454.1 ± 50.9 nM (three experiments) and a B_{max} of 17.3 ± 1.5 pmol/mg of protein (three experiments).

Pharmacological Relevance of [^{3}H]U-378883 Binding in Oocytes

To establish the relevance of the above described [^{3}H]-U-37883 binding, experiments with two close analogs of U-37883A, i.e., U-52090A and U-42069D (the structures of which are presented in Fig. 1), were carried out. These two compounds were studied in the follicular oocyte K_{ATP} current assay as well as in the [^{3}H]U-37883 binding assay. Fig. 7A shows the dose-response curves for inhibition of P1060-induced

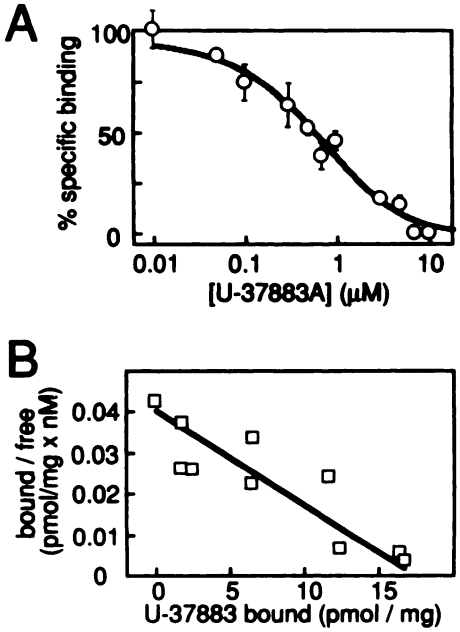


Fig. 6. Specific binding of [^{3}H]U-37883 in membranes from follicle-enclosed oocytes. A, Concentration-dependent displacement of [^{3}H] U-37883 by the unlabeled drug. Specific binding was defined by the use of 10 μM unlabeled drug and was 62.5% of the total binding. Each *data point* is a mean of three separate determinations. B, Scatchard analysis of the data from one of the competition curves. The best fit shows a single class of binding sites, with a K_d of 454.1 ± 50.9 nM (three experiments) and a B_{max} of 17.3 ± 1.5 pmol/mg of protein (three experiments).

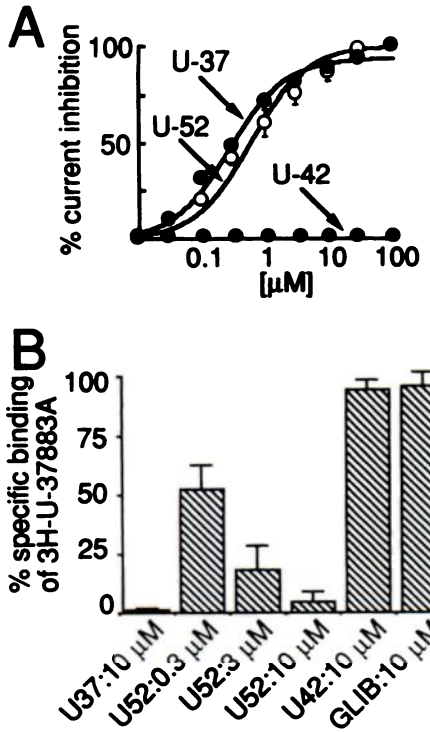


Fig. 7. A, Comparison of the dose-response curves of U-37883A (*U-37*), U-52090A (*U-52*), and U-42069D (*U-42*) for inhibition of P1060 (30 μM)-activated whole-cell K^+ currents in follicle-enclosed oocytes. The curve for U-37883A is taken from Fig. 2. B, Comparison of the inhibitory effects of U-52090A, U-42069D, and glibenclamide (*GLIB*) on the specific binding of [^{3}H]U-37883 in membranes from follicle-enclosed oocytes. Each *bar* is a mean of three determinations. U-52090A significantly ($p < 0.05$) reduced specific binding at all concentrations shown. U-42069D (10 μM) and glibenclamide (10 μM) did not significantly alter the specific binding of [^{3}H]U-37883.

TABLE 1

Comparison of electrophysiological and biochemical effects of U-37883A, U-52090A, and U-42069D in follicle-enclosed oocytes

Data presented are derived from Fig. 7.

Compound	K_i for K_{ATP} channel blockade	K_d for [^{3}H]U-37883 displacement
	μM	μM
U-37883A	0.26	0.45
U-52090A	0.30	0.30
U-42069D	No effect at 10 μM	No effect at 10 μM

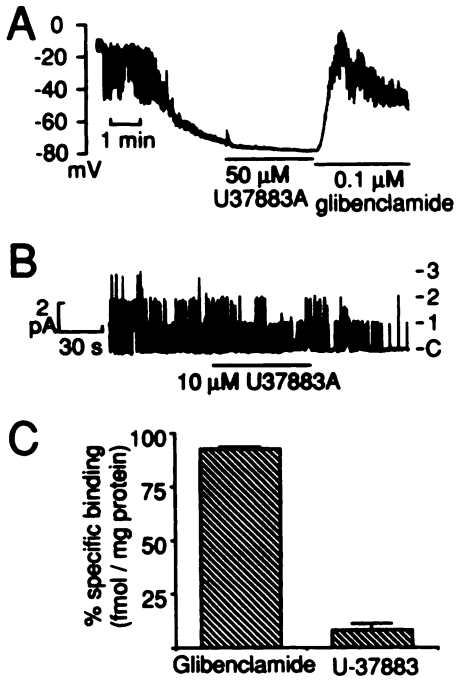


Fig. 8. Comparison of the effects of U-37883A and glibenclamide in RINm5F cells. A, Membrane potential recording in a RINm5F cell dialyzed with an ATP-free internal solution. U-37883A (50 μM) addition had no effect on the membrane potential, whereas glibenclamide (0.1 μM) addition caused a dramatic depolarization. B, Outside-out patch recording with an ATP-free internal medium. The K_{ATP} channel openings were not affected by the addition of 10 μM U-37883A. C, Specific binding of [^{3}H] glibenclamide and [^{3}H]U-37883A in membranes from RINm5F cells. Details are given in Materials and Methods. Glibenclamide displayed large amounts of specific binding, whereas U-37883A displayed no significant specific binding in RINm5F cell membranes.

follicular oocyte K_{ATP} current. The U-37883A curve is the same as that presented in Fig. 2B. U-52090A was found to be an active inhibitor, with roughly the same dose-response curve as U-37883A. In contrast, U-42069D was found to be inactive, with up to 100 μM having no significant inhibitory effect. Fig. 7B presents the [^{3}H]U-37883 binding data. Unlabeled U-37883A at 10 μM was used to define the specific binding of [^{3}H]U-37883. U-52090A produced a dose-dependent displacement of [^{3}H]U-37883 binding, with roughly the same potency as in the electrophysiology experiments, i.e., 0.3 μM producing 50% inhibition and 10 μM producing maximal 100% inhibition. In contrast, U-42069D at 10 μM was without any significant effect on the specific binding of [^{3}H]U-37883. Finally, we found that glibenclamide at 10 μM also did not displace [^{3}H]U-37883 from its binding site. A summary of the pharmacological significance of the [^{3}H]U-37883 binding in oocytes is presented in Table 1.

Effect of U-37883 on RINm5F Cells

Fig. 8A shows that a rat insulinoma cell dialyzed with an ATP-free solution in the whole-cell patch-clamp configuration hyperpolarized to about −75 mV. Addition of 50 μM U-37883A did not alter the resting membrane potential. In sharp contrast, addition of 0.1 μM glibenclamide very effectively reversed this hyperpolarization. Fig. 8B shows the lack of an effect of U-37883A (10 μM) on single K_{ATP} channels recorded in an outside-out patch from RINm5F cells. In these experiments, the intracellular medium did not contain ATP, and the single-channel activity showed the classical progressive run-down (13). We have previously shown that this channel activity is very sensitive to blockade by glibenclamide (13). Finally, Fig. 8C shows a comparison of the specific binding of [^{3}H]glibenclamide and [^{3}H]U-37883 in the RINm5F cell membranes. Using the same experimental conditions (i.e., 500 μg/ml protein, pH 7.4, at room temperature), a considerable difference in the binding of these two K_{ATP} channel blockers was found. Consistent with our previously published report (13), it was found that in RINm5F cell membranes 0.5 nM [^{3}H]glibenclamide (50 Ci/mmol) displayed large amounts of specific binding (approximately 90 fmol/mg of protein). In contrast, 1 nM [^{3}H] U-37883 (also 50 Ci/mmol) did not show much specific binding in RINm5F cell membranes. In summary, unlike glibenclamide, U-37883A neither blocked K_{ATP} channels in RINm5F cells nor displayed significant specific binding in membranes from RINm5F cells.

Discussion

This study confirms as well as extends the characterization of U-37883A as a K_{ATP} channel blocker. The following new observations reported in this study are of significance: (i) this study provides the first direct electrophysiological evidence, using follicular oocyte current as well as single-channel current measurements, that U-37883A can block K_{ATP} channel activation, (ii) U-37883A, similarly to glibenclamide, can block K_{ATP} channels activated by K^+ channel openers, cAMP, and adenosine, (iii) U-37883A, like glibenclamide, works by decreasing the open probability of the K_{ATP} channel without reducing the channel conductance, (iv) there is a specific receptor site for U-37883A that appears to mediate the K_{ATP} channel-blocking activity of U-37883A, and finally (v) U-37883A, unlike glibenclamide, is selective, because it did not block the pancreatic β cell K_{ATP} channel. These observations are discussed below.

The discovery that U-37883A might be a K^+ channel blocker originated from a serendipitous observation *in vivo* that U-37883A selectively blocked the hypotensive response to minoxidil, a K^+ channel opener vasodilator (15, 18). A subsequent study defined the K^+ channel-blocking properties of U-37883A against various, chemically diverse, K_{ATP} channel openers and showed that U-37883A is qualitatively quite similar to glibenclamide in vascular smooth muscle (15, 19). This earlier characterization was based on the functional data *in vitro* and *in vivo*, as well as the data obtained using $^{42}K^+$ efflux in smooth muscle. The present study provides the first direct electrophysiological evidence for the actions of U-37883A as a K_{ATP} channel blocker. As pointed out in the introduction, follicle-enclosed *Xenopus* oocytes have proven to be a valuable model system to investigate the mechanisms of K^+ channel regulation. This has been particularly true for the K_{ATP} channel. The endogenous follicular K_{ATP} channel in oocytes is qualitatively similar to the K_{ATP} channel described in vascular smooth muscle cells (20). Thus, the follicular K_{ATP} channel in oocytes is activated by structurally diverse, K^+ channel-opening vasodilators, is blocked by glibenclamide, and is not modulated by Ca^{2+} or voltage (6–10). The data with U-37883A indicate further similarity between the follicular and vascular smooth muscle K_{ATP} channels. The electrophysiological inhibition K_i of 0.26 μM for U-37883A in *Xenopus* oocytes reported here is in reasonable agreement with the pharmacological K_{ATP} channel-blocking IC_{50} of 0.78 μM in isolated rabbit mesenteric artery smooth muscle (15). Furthermore, in both systems the majority of the K_{ATP} channel-blocking effects of U-37883A are observed within the same concentration range of 0.1–10 μM. Thus, the potencies of U-37883A as a K_{ATP} channel blocker are fairly similar in these two assays. The present study provides additional evidence suggesting that U-37883A not only can block K_{ATP} channel activation by K^+ channel openers but also can block K_{ATP} channel activation via the cAMP second messenger system. The single-channel data provide additional direct evidence for the K_{ATP} channel-blocking actions of U-37883A.

Additional studies were aimed at investigating the effect of U-37883A on the pancreatic β cell K_{ATP} channel. It is well established that insulin secretion from pancreatic β cells is controlled by the K_{ATP} channel activity and that sulfonylureas such as glibenclamide cause enhancement of insulin secretion by blocking this K_{ATP} channel (13, 21). We reported previously that, in preliminary experiments, U-37883A was found to be without any significant effect on insulin secretion from pancreatic β cells (15).[3] Recently, it was shown that, unlike glibenclamide, U-37883A given intravenously to rats failed to alter plasma glucose levels (22). This study provides direct electrophysiological evidence for the lack of an effect of U-37883A on the β cell K_{ATP} channel. Thus, U-37883A, at a concentration that produces roughly maximal K_{ATP} channel inhibition in follicular oocytes or smooth muscle, was without effect on the RINm5F cell membrane potential or single-channel current. All of these data collectively provide a strong suggestion that, unlike glibenclamide, U-37883A is a selective blocker of the smooth muscle-type K_{ATP} channel. This is also consistent with the previous observations suggesting that the follicular K_{ATP} channel is similar to the vascular smooth muscle K_{ATP} channel, because both are activated by various K^+ channel openers and this activation is blocked by glibenclamide.

The final part of this study provides biochemical data to suggest the presence of a specific receptor site for U-37883A in the follicular membranes of *Xenopus* oocytes. The [^{3}H]U-37883 binding data show the presence of a single class of low affinity binding sites. Three types of data available from this study suggest that this binding site represents a functional and pharmacologically relevant receptor site for U-37883A. First, the K_d of 0.45 μM from [^{3}H]U-37883 displacement experiments is in excellent agreement with the electrophysiological K_i of 0.26 μM for inhibition of K_{ATP} channel activation. Second, we provide a structure-activity relationship using two analogs of U-37883A (Fig. 1; Table 1). U-52090A, an active analog, was found to be active in inhibition of K_{ATP} channel activation and active in [^{3}H]U-37883 displacement assays in similar concentration ranges and with roughly similar potencies. In contrast, U-42069D, an inactive analog, was found to be inactive in both assays. Consistent with this, we have observed that U-52090A is roughly equipotent to U-37883A in antagonizing *in vitro* vasorelaxation by a K_{ATP} opener, whereas U-42069D is quite inactive.[4] Finally, we did not find any specific binding of [^{3}H]U-37883 in RINm5F cells, a cell type in which U-37883A does not block K_{ATP} channels. Based on this collective evidence, we postulate that the observed specific binding represents the receptor for U-37883A-type guanidines, which controls the activity of the K_{ATP} channels in follicle-enclosed oocytes. No data are presently available regarding specific binding of sulfonylureas such as glibenclamide in this system. Our preliminary data presented here, showing that glibenclamide did not compete with [^{3}H]U-37883 binding, suggest that these two K_{ATP} blockers either interact with different proteins or interact at different sites on the same protein to produce their respective effects (also see Ref. 16). Further biochemical studies would be required to define the relationship between the receptors for these two chemical classes of K_{ATP} blockers.

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