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U-37883A potently inhibits dopamine-modulated K^+ channels on rat striatal neurons

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Abstract

An 85 pS K^+ channel of rat caudate–putamen neurons, which is activated by dopamine D_2 receptors and inhibited by sulfonylurea drugs, was studied using cell-attached patch-clamp electrophysiology. This channel was inhibited by externally-applied U-37883A (4-morpholinecarboximidine-*N*-1-adamantyl-*N′*-cyclohexyl hydrochloride), a blocker of vascular ATP-sensitive K^+ channels, with a half-maximal effect at a concentration of approximately 0.1 μM. Channel inhibition occurred in a time-dependent fashion when U-37883A was applied to the membrane from a back-filled patch pipette. Inhibition was associated with a decrease in fractional open time, but was voltage-insensitive and did not alter channel conductance, suggesting an effect on channel gating at a site largely insensitive to the electrical field of the channel. U-37883A was about 50 times more potent at inhibiting this channel than was the sulfonylurea drug glibenclamide. This relative potency, opposite to that found in pancreatic tissue, indicates that U-37883A is a useful tool to distinguish amongst different subtypes of sulfonylurea-sensitive K^+ channels.

Keywords: ATP-sensitive K^+ channel; Caudate–putamen; Dopamine D_2 receptor; Patch-clamp; Sulfonylurea; U-37883A

1. Introduction

Sulfonylurea drugs, which are widely used for the treatment of Type II diabetes mellitus, inhibit ATP-sensitive K^+ channels by binding to an associated membrane protein (Ashcroft and Ashcroft, 1992; Aguilar-Bryan et al., 1995). K^+ channel subunits may combine with these sulfonylurea binding proteins (or sulfonylurea 'receptors') in a one-to-one stoichiometry (Shyng and Nichols, 1997). Association of the sulfonylurea binding protein with the K^+ channel appears to be necessary for channel function (Inagaki et al., 1995). Evidence from a variety of tissue systems and cloning studies suggests that there may be more than one subtype of sulfonylurea receptor (Boden et al., 1989; Zini et al., 1991; Rajan et al., 1993; Benz and Kohlhardt, 1994; Freedman and Lin, 1996; Inagaki et al., 1996; Isomoto et al., 1996; Nelson et al., 1996; Dunn-Meynell et al., 1997; Tricario et al., 1997). The ability to distinguish pharmacologically between different subtypes of the sulfonylurea binding protein may facilitate the development of more selective K^+ channel drugs.

The non-sulfonylurea K^+ channel blocker U-37883A (4-morpholinecarboximidine-*N*-1-adamantyl-*N′*-cyclohexyl hydrochloride) is selective for ATP-sensitive K^+ channels in vascular smooth muscle, where it is more potent than the sulfonylurea drug glibenclamide (glyburide) (Ludens et al., 1995; DeWitt et al., 1996). This is in contrast to ATP-sensitive K^+ channels in the pancreas, where glibenclamide is more potent (Guillemare et al., 1994; Ludens et al., 1995). These tissue-related differences in relative potency suggest that sulfonylurea binding proteins are different in vasculature than in the pancreas (Ludens et al., 1995). There is indirect evidence that glibenclamide and U-37883A bind to different sites on the sulfonylurea binding protein (Ohrnberger et al., 1993), and so it is plausible that different sulfonylurea receptors might differ in their relative affinities for these two compounds.

Here, we have studied the effects of U-37883A at an 85 pS K^+ channel of rat caudate–putamen (corpus striatum) neurons. This channel is activated by dopamine D_2 receptors (Greif et al., 1995). Pharmacological characterization

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of agents that block this channel's pore have revealed some distinctive features of its conductance properties. It is highly sensitive to blockade by quinine, with inhibition occurring at low nanomolar concentrations (Freedman and Weight, 1989). The channel also displays anomalous conductance behaviors when partially permeated by cesium ions (Lin et al., 1996). In its gating, this channel shares some features of ATP-sensitive K^+ channels, in that it is activated by rotenone and so appears to be metabolically sensitive, and is inhibited by sulfonylureas (Lin et al., 1993). However, it displays an atypical rank-order potency for sulfonylurea blockade, with tolbutamide more potent than glibenclamide, and is relatively insensitive to diazoxide activation, and so might represent a novel ATP-sensitive K^+ channel subtype (Lin et al., 1993; Freedman and Lin, 1996). We now present data, utilizing U-37883A, which further suggest the involvement of an atypical subtype of sulfonylurea binding protein.

2. Materials and methods

2.1. Cell preparation and patch-clamp recording

Neurons were freshly dissociated from the caudate–putamen of 30- to 45-day-old rats by methods previously described (Greif et al., 1995). All animal procedures were approved by an institutional review committee. Patch-clamp recordings were made in the cell-attached configuration, from multipolar cells with diameters ≥ 10 μm, by methods previously described in greater detail (Lin et al., 1993; Greif et al., 1995). The cells were superfused with (in mM): NaCl, 149; KCl, 3.5; $CaCl_2$, 2.5; $MgCl_2$, 1; D-glucose, 10; HEPES-Na, 10, pH 7.4; adjusted with sucrose to 330–340 mosM/kg and equilibrated with 100% O_2. Patch pipettes contained (in mM): KCl, 140; $CaCl_2$, 2.5; $MgCl_2$, 1; quinpirole (a dopamine D_2 receptor agonist), 0.01; HEPES-K, 10, pH 7.4, plus glibenclamide or

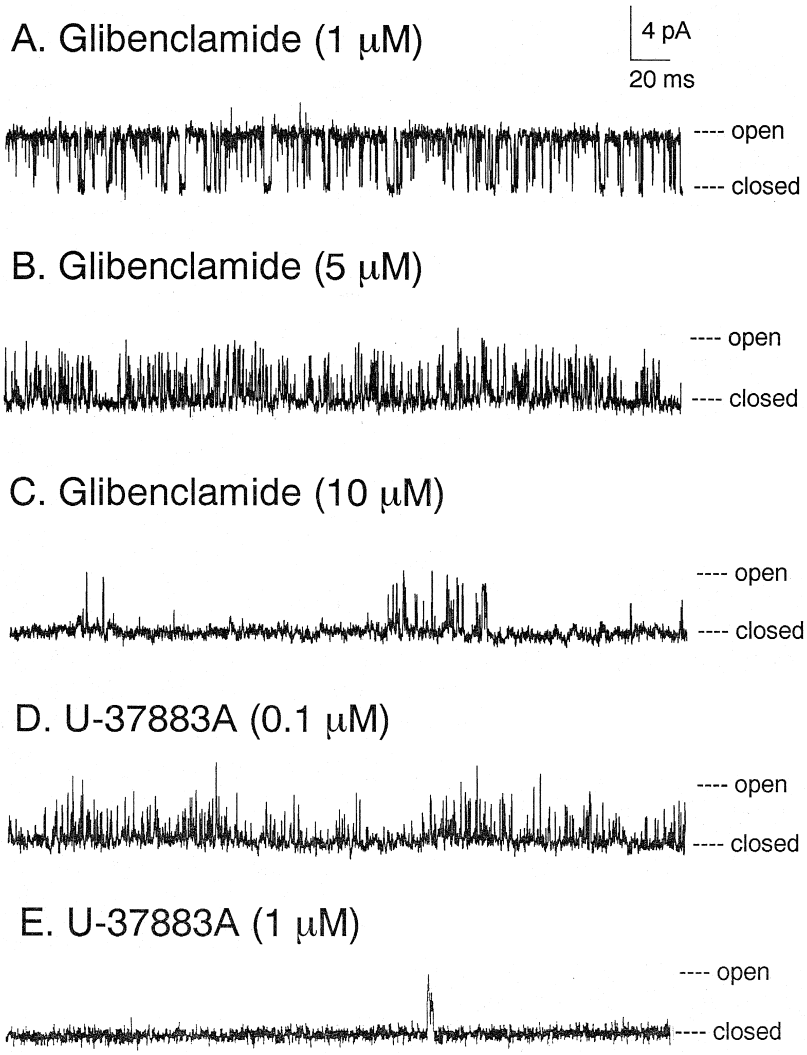

Fig. 1. Cell-attached patch-clamp recordings from five striatal neurons, showing the effects of 1 μM glibenclamide (A), 5 μM glibenclamide (B), 10 μM glibenclamide (C), 0.1 μM U-37883A (D), and 1 μM U-37883A (E) on the 85 pS K^+ channel, recorded in the presence of 10 μM quinpirole. Drugs were applied from within the patch pipette, at a uniform concentration. All records are at resting membrane potential.

U-37883A as indicated. K^+ currents were measured as inward currents, and one patch per cell was tested. In some experiments, we applied U-37883A by a back-fill method (Auerbach, 1991; Lin et al., 1996). The patch pipette was tip-filled with 0.5–1 μl of the above solution without U-37883A, and back-filled with the same solution also containing U-37883A, and blockade was monitored during recording as the drug diffused to the membrane patch. The 85 pS channel was distinguished from other channels present on these cells by criteria described previously (Lin et al., 1993; Greif et al., 1995). Channel reversal potentials occurred at 50–60 mV depolarized from resting membrane potential, corresponding to approximately 0 mV patch potential in the presence of symmetrical K^+ (Greif et al., 1995). Data were filtered at 2 kHz lowpass and acquired to a computer at 100 μs/point. Consequently, the limit of time resolution in our measurements was about 500 μs.

2.2. Data analysis

Inward currents are shown as upward deflections. Membrane potential has been expressed relative to the resting membrane potential of the cell (as the pipette potential multiplied by -1). The fractional conducting time of the channel (analogous to fractional open probability, but without distinction between channel closure and channel blockade) was defined as $1 - NP_c$, where N was the number of active channels in the patch, as determined from simultaneous openings during a 10 min observation on-line and verified from the number of peaks in an all-points amplitude histogram, and P_c was the fraction of time that no channel current was passed (channel closed or inhibited). P_c was determined from the relative areas under the peaks of all-points amplitude histograms, obtained from records of at least 25 600 points (this calculation of conducting time tends to collapse to values near zero when blockade is extensive; however, N will be underestimated under these conditions because simultaneous openings are rare even when there are multiple channels. This error will tend to understate the true extent of channel blockade, but is unlikely to affect the relative potency of two blockers). For preliminary analyses of channel dwell-times, we utilized patches with only one active channel, and interactively verified all transitions across a 50% threshold. We used 0.4 ms as a minimum event duration, and 5 ms as a minimum interburst interval, which were previously found to be appropriate values for characterizing dwell-times in the absence of inhibitors (unpublished results). Data are expressed as mean $\pm$ S.D. Statistical comparisons were made by a χ^2 test.

2.3. Drugs

Quinpirole was obtained from Research Biochemicals (Natick, MA, USA), glibenclamide was from Sigma (St. Louis, MO), and U-37883A was from Upjohn Laboratories (Kalamazoo, MI, USA).

3. Results

3.1. Channel inhibition by U-37883A

When applied to the external surface of the membrane at a uniform concentration within the cell-attached patch

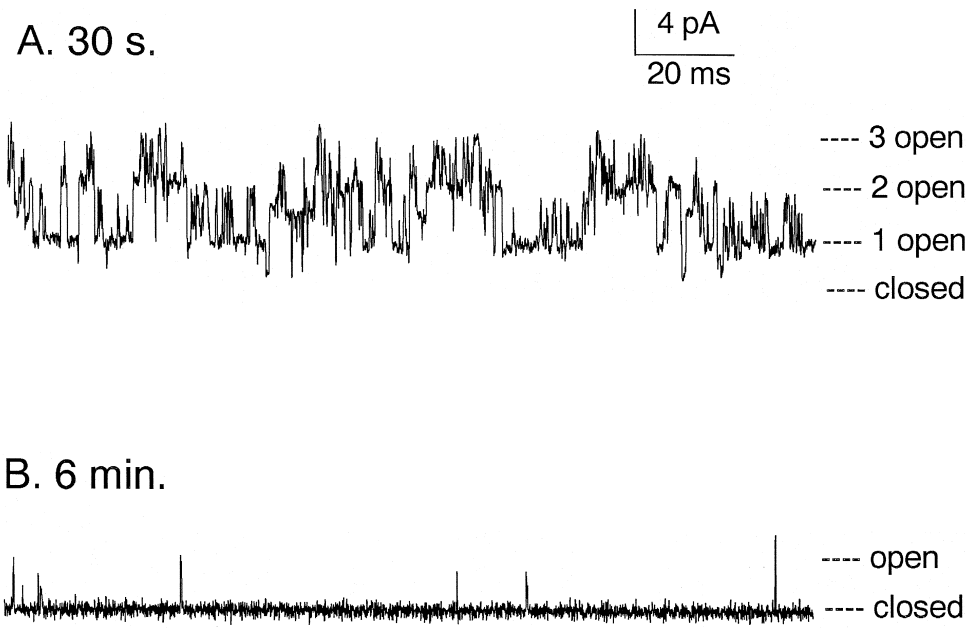

Fig. 2. Cell-attached patch-clamp recording of 85 pS channels, using an electrode whose tip was nominally inhibitor-free at the start of the recording, but which was back-filled with 0.2 μM U-37883A. Records from the same patch are shown 30 s (A) and 6 min (B) after formation of a gigaseal. Both records are at resting membrane potential.

pipette, U-37883A inhibited the dopamine-modulated K^+ channel in a manner similar to the effects previously described for sulfonylureas such as glibenclamide. As previously reported (Lin et al., 1993), glibenclamide inhibited the 85 pS channel in a dose-dependent manner at concentrations between 1–10 μM (Fig. 1A–C). Partially-blocked single channel openings were often briefer than the time resolution set by the filter frequency of our recordings, as manifested by foreshortening and corner-rounding of the openings. Channel current was only slightly blocked relative to control at 1 μM glibenclamide (Lin et al., 1993; Greif et al., 1995). U-37883A at 0.1 μM had effects similar to 5–10 μM glibenclamide (Fig. 1D). At 1 μM, U-37883A blocked the channel almost completely (Fig. 1E). In recordings from 38 cells in the presence of 1 μM U-37883A, a sample size large enough that we would have expected to see 85 pS channel openings in about 25% of patches recorded in the absence of blocker (Greif et al., 1995; $P < 0.001$), only one cell showed a few brief openings (Fig. 1E).

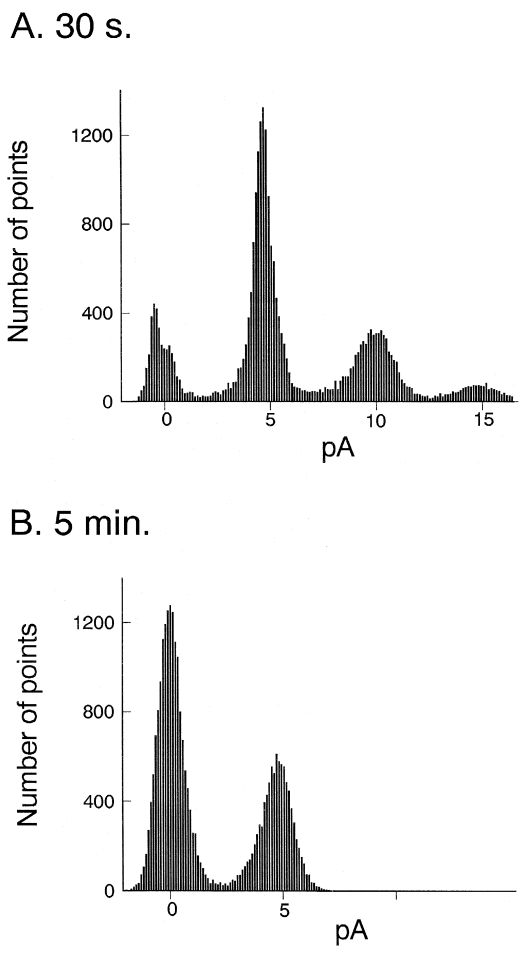

Fig. 3. All-points amplitude histograms of recordings from a patch where the electrode was back-filled with 0.5 μM U-37883A. Data were collected at resting membrane potential 30 s (A) and 5 min (B) after formation of a gigaseal.

In order to rule out the possibility that the altered recordings in the presence of U-37883A resulted either from misidentification of some other channel as the 85 pS channel, or from non-stationary behavior of the channel independent of the blocker, we applied U-37883A by means of a back-fill method (Auerbach, 1991; Lin et al., 1996). Fig. 2A shows a recording of a patch expressing three relatively unblocked 85 pS channels, obtained 30 s after obtaining a membrane seal with a patch pipette whose tip was nominally free of blocker, but which was back-filled with solution containing 0.2 μM U-37883A. After allowing 6 min for the drug to diffuse to the surface of the membrane, channel openings were markedly decreased (Fig. 2B). Fig. 3 shows all-points amplitude histograms of another patch containing three channels where 0.5 μM U-37883A was applied by back-filling. As inhibition increased over time, the channels shifted to spending a higher fraction of time in the nonconducting state, without a change in the current amplitude of channel openings. Patches from five different cells were tested with electrodes back-filled with 0.2–1 μM U-37883A, and in which unblocked 85 pS channels could be clearly resolved at the start of the recording, and in every case a 50%–100% decrease in openings occurred within 5 min. Openings consistently remained inhibited for 30 min or more; in contrast, recordings of this channel in the absence of inhibitor have previously been found to be stable for at least this long (Greif et al., 1995).

3.2. Characteristics of inhibition

Fig. 4 shows the current–voltage relationships of 85 pS channels under control conditions, and in the presence of 0.1 μM U-37883A at a uniform concentration within the patch pipette. As shown, the current amplitude was unchanged by the inhibitor at any voltage, and consequently this compound had no effect on the channel conductance. In addition, there was no effect on the extrapolated reversal potential of the channel.

As previously found for sulfonylurea effects at this channel (Lin et al., 1993), U-37883A block was instead associated with a decrease in channel fractional conducting time (Fig. 5). Also in a manner similar to sulfonylureas, this effect of U-37883A was largely voltage-insensitive (Fig. 5). In the absence of blockers, 85 pS channels activated by 10 μM quinpirole have a fractional open time of about 0.6 at voltages negative to the reversal potential, while single channel currents are not detectable at more depolarized voltages (Greif et al., 1995). At a uniform concentration of 0.1 μM within the patch pipette, U-37883A reduced fractional conducting time to approximately 50% of control at voltages ranging from 40 mV hyperpolarized to 20 mV depolarized from resting potential. At more depolarized voltages, the channel displayed decreased conduction due to inward rectification, which is

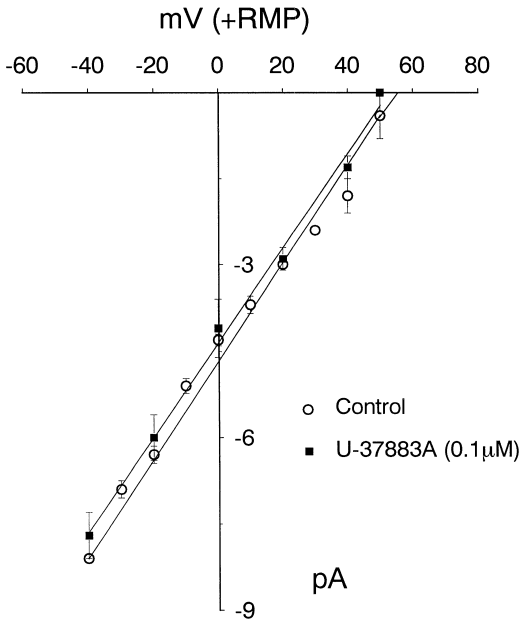

Fig. 4. Current–voltage relationships of 85 pS channels in the absence (control) and the presence of a uniform concentration of 0.1 μM U-37883A in the patch pipette. Membrane potential is expressed relative to resting membrane potential (RMP). Each point is the mean ± S.D. of 4–8 recordings. The lines were fit by linear regression.

also observed in the absence of blocker (Greif et al., 1995), and so is unlikely to have been due to U-37883A.

Studies in progress indicate that, in the absence of inhibitors, the major effect of dopaminergic agonists on the gating of the 85 pS channel is a shift into burst openings (multiple openings separated by brief closures) from a long interburst closed state (unpublished results). We performed

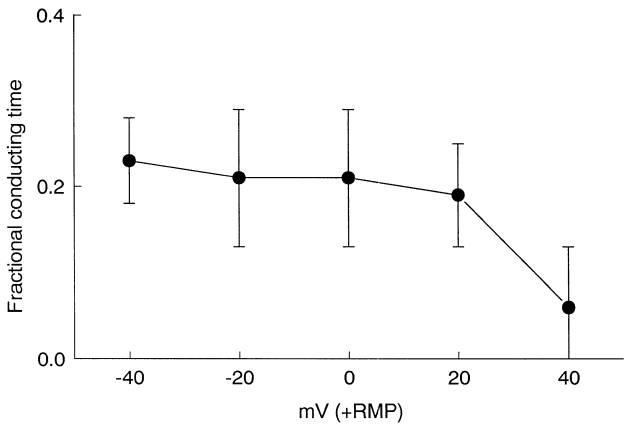

Fig. 5. The voltage-dependence of the effect of U-37883A on the fractional conducting time of the 85 pS K^+ channel. Recordings were performed with 0.1 μM U-37883A at a uniform concentration in the patch pipette. Membrane potential is expressed relative to resting membrane potential (RMP). The decrease in fractional conducting time at 40 mV depolarized reflects the decrease in channel current due to inward rectification as membrane potential approaches the K^+ reversal potential. Each point is the mean ± S.D. of 5–8 recordings.

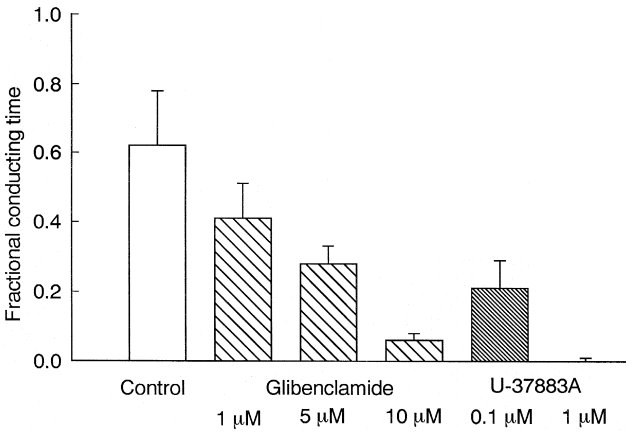

Fig. 6. The concentration-dependence of channel inhibition by glibenclamide (1, 5 and 10 μM) and by U-37883A (0.1 and 1 μM), present at uniform concentrations, compared to control recordings in the absence of blocking agent. All measurements were made at resting membrane potential, in the presence of 10 μM quinpirole. Each point is the mean ± S.D. of 5–8 recordings.

a preliminary gating analysis of brief recordings from a patch that appeared to have only one 85 pS channel, where the patch pipette was back-filled with 0.5 μM U-37883A. As inhibition progressed, there appeared to be an increase in the duration of the interburst nonconducting state, and possibly changes in other dwell-times as well (data not shown). In summary, U-37883A had no effect on channel conductance, but appeared to inhibit the channel by altering its gating.

3.3. Potency of inhibition relative to glibenclamide

As noted above, U-37883A appeared to inhibit the 85 pS channel at lower concentrations than did the sulfonylurea glibenclamide (Fig. 1). The concentration-dependence of this reduction in channel fractional conducting time by these two drugs is shown graphically in Fig. 6. The control fractional conducting time of about 0.6 was reduced by approximately 50% by 5 μM glibenclamide. U-37883A gave a similar effect at 0.1 μM. At 1 μM, U-37883A blocked the channel almost completely, whereas glibenclamide at the same concentration had a relatively small effect. Thus, U-37883A was approximately 50-fold more potent than glibenclamide in this system.

4. Discussion

The major finding of this study is that U-37883A is more potent than the sulfonylurea drug glibenclamide at inhibiting a K^+ channel in the brain, and therefore provides a tool to pharmacologically distinguish between multiple subtypes of sulfonylurea-sensitive K^+ channels. We performed these studies utilizing an 85 pS K^+ channel of rat caudate–putamen neurons, which is activated by

dopamine D_2 receptors (Greif et al., 1995). Previous experiments suggested that this channel might be associated with a novel subtype of sulfonylurea binding protein (Lin et al., 1993; Freedman and Lin, 1996), and our present results provide further support for this possibility. In order to identify this channel in cell-attached patch recordings under conditions of partial blockade, we have made measurements using a back-fill method to apply the U-37883A. In this way, we have been able to identify relatively unblocked channels and then observe the progressive inhibition of the same channels over time. We have also performed cell-attached recordings with U-37883A present at a uniform concentration within the patch pipette, in order to know more exactly the concentration of the drug present at the cell membrane, which is less precisely determined in the back-fill method. The finding that partially inhibited channels had the same 85 pS conductance as seen under control conditions (Fig. 4) further supports the conclusion that it was these channels that were affected, since these cells do not express any other channels of this conductance under these recording conditions (Greif et al., 1995).

The effects of U-37883A resemble the previously-reported inhibition of this channel by the sulfonylurea drugs glibenclamide and tolbutamide (Lin et al., 1993). Inhibition was largely voltage-insensitive, and had no effect on the single channel conductance or reversal potential. It is therefore improbable that blockade occurred by binding to a site within the electric field of the channel pore. In contrast, there appeared to be an alteration in the gating of the channel, with a reduction in channel fractional open time. This pattern is compatible with action at a site that modulates channel activity. In other systems, U-37883A is thought to interact with sulfonylurea binding proteins at a site distinct from the sulfonylurea binding site (Ohrnberger et al., 1993; Guillemare et al., 1994). Thus, it appears plausible that U-37883A may interact with a sulfonylurea binding protein associated with the dopamine-modulated ion channel.

However, U-37883A was approximately 50 times more potent than glibenclamide in this system. This result resembles the effects of U-37883A at vascular ATP-sensitive K^+ channels (Ludens et al., 1995; DeWitt et al., 1996), but differs from its effects at pancreatic ATP-sensitive K^+ channels (Guillemare et al., 1994; Ludens et al., 1995). It is, however, unlikely that the 85 pS channel is pharmacologically identical to most vascular ATP-sensitive K^+ channels, since the dopamine-modulated channel is more sensitive to tolbutamide than to glibenclamide (Lin et al., 1993). This result is further evidence that striatal dopamine receptors couple to a K^+ channel that has an atypical pharmacology relative to sulfonylurea-sensitive K^+ channels in other tissues.

Based upon these tissue differences in potency relative to sulfonylureas, U-37883A appears to be a useful tool for pharmacologic differentiation of ATP-sensitive K^+ channel subtypes. Drugs that inhibit such channels in the pancreas or activate such channels in vascular smooth muscle are known to be effective for treating hyperglycemia and hypertension, respectively (Ashcroft and Ashcroft, 1992; Freedman and Lin, 1996). There is recent evidence that effects on such channels in the brain may affect appetite (Spanswick et al., 1997). However, the development of more selective drugs will depend upon differences in sulfonylurea binding proteins between these different tissues, and upon the ability to distinguish them. Our results add to the evidence that such proteins exist in multiple forms, and suggest that U-37883A may be a useful tool in their characterization.

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