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Classic D1 Dopamine Receptor Antagonist *R*-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH23390) Directly Inhibits G Protein-Coupled Inwardly Rectifying Potassium Channels

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ABSTRACT

R-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) is a widely used, highly selective antagonist of D1 dopamine receptors. While investigating the crosstalk between D1 and D3 dopamine receptor signaling pathways, we discovered that in addition to being a D1 receptor antagonist, SCH23390 and related compounds inhibit G protein-coupled inwardly rectifying potassium (GIRK) channels. We present evidence that SCH23390 blocks endogenous GIRK currents induced by either somatostatin or D3 dopamine receptors in AtT-20 cells (IC_{50}, 268 nM). The inhibition is receptor-independent because constitutive GIRK currents in Chinese hamster ovary cells expressing only GIRK channels are also blocked by SCH23390. The inhibition of GIRK channels is somewhat selective because members of the closely related Kir2.0 family of inwardly rectifying potassium channels, as well as various endogenous cationic currents present in AtT-20 cells, are not affected. In addition, in current clamp recordings, SCH23390 can depolarize the membrane potential and induce AtT-20 cells to fire action potentials, indicating potential physiological significance of the GIRK channel inhibition. To identify the chemical features that contribute to GIRK channel block, we tested several structurally related compounds [SKF38393, *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (nor-methyl-SCH23390), and *R*-(+)-2,3,4,5-tetrahydro-8-iodo-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrochloride (iodo-SCH23390)], and our results indicate that the halide atom is critical for blocking GIRK channels. Taken together, our results suggest that SCH23390 and related compounds might provide the basis for designing novel GIRK channel-selective blockers. Perhaps more importantly, some studies that have exclusively used SCH23390 to probe D1 receptor function or as a diagnostic of D1 receptor involvement may need to be reevaluated in light of these results.

We have previously shown that the D3 dopamine receptor couples to and activates G protein-coupled inwardly rectifying potassium (GIRK) channels (Werner et al., 1996; Kuzhikandathil et al., 1998) and inhibits P/Q-type calcium channels (Kuzhikandathil and Oxford, 1999). Given the coexpression of D1 and D3 dopamine receptors in central nervous system neurons, particularly in a subset of nucleus accumbens neurons (Le Moine and Bloch, 1996), we were interested in exploring the functional consequence of D1 receptor activation on D3 receptor–GIRK channel coupling. To address this issue, we used two ligands that are highly selective for D1 receptors and have long served as diagnostic probes of D1 receptor function. One of these ligands, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390), is a highly selective antagonist of D1 dopamine receptors with at least a 1000-fold higher affinity for D1 receptors than for D3 receptors (Iorio et al., 1983; Neve and Neve, 1997). SCH23390 is a member of the phenyltetrahydrobenzazepines, a structural class that also includes the partial D1 agonist *R*-(+)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride (SKF38393). SCH23390 has been widely used both in vivo and in vitro to characterize D1 receptor function and

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ABBREVIATIONS: GIRK, G protein-coupled inwardly rectifying potassium channel; SCH23390, *R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; SKF38393, *R*-(+)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride; nor-methyl-SCH23390, *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride; iodo-SCH23390, *R*-(+)-2,3,4,5-tetrahydro-8-iodo-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hydrochloride; U50488H, *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide; VTA, ventral tegmental area; QP, quinpirole; CHO, Chinese hamster ovary cells; 30 K-ES, 30 mM potassium.

to distinguish "D1-like" (D1 and D5) receptors from "D2-like" (D2, D3, and D4) receptors. Although SCH23390 inhibits D1 receptor-mediated activation of adenylyl cyclase with an IC_{50} of 0.01 μM (Iorio et al., 1983), most published studies use SCH23390 at a concentration of 0.1 to 10 μM to antagonize D1 receptor function (Yamamoto et al., 1994; Aosaki et al., 1998; Cai et al., 1999). We report that at these concentrations, SCH23390 also directly inhibits currents through GIRK channels.

The GIRK channels are a subfamily of inwardly rectifying potassium channels that consists of five major isoforms (GIRK1 through GIRK5, or Kir3.1 through Kir3.5). Functional GIRK channels are tetramers composed of either four identical (homomeric) or nonidentical (heteromeric) subunits. All known GIRKs form heteromultimers in vivo (Kofuji et al., 1995; Liao et al., 1996), although recent evidence suggests that GIRK2 and GIRK4 form functional homomultimers (Corey and Clapham, 1998; Kuzhikandathil et al., 1998; Inanobe et al., 1999). GIRK channels are activated by the $\beta\gamma$ subunits of G proteins that are released from the heterotrimeric G$\alpha\beta\gamma$ complex when G protein-coupled receptors are bound by ligands (Logothetis et al., 1987; Huang et al., 1995). Since the cloning of the GIRK channel family, numerous reports have indicated their coupling to a variety of G protein-coupled receptors (Yamada et al., 1998).

Despite their extensive functional and biochemical characterization, no selective GIRK channel blockers have yet been reported. Although many toxins block voltage-dependent K^+ channels, tertiapin, a peptide from bee venom, is the only known distinctive blocker of inwardly rectifying potassium channels (Jin and Lu, 1998; Kitamura et al., 2000). Unfortunately this peptide does not discriminate between GIRK channels and renal outer medullary (ROM) K1 (Kir1.1) channels (Jin and Lu, 1998). The lack of selective GIRK channel blockers has limited the characterization of their role in neurotransmission. We have recently used an alternative dominant-negative strategy to establish a role for these channels in maintaining the resting membrane potential as well as modulating neurosecretory activity (Kuzhikandathil and Oxford, 2000).

Here, we report a selective and direct inhibition of GIRK1/GIRK2 and GIRK2 channels by the D1 dopamine receptor antagonist SCH23390. Our results suggest that at concentrations routinely used to study D1 receptor function, SCH23390 effectively blocks GIRK channels, depolarizes membrane potential, and induces action potential activity in cells. By testing several different phenyltetrahydrobenzazepine compounds, we also demonstrate that the halide atom in these compounds is critical for this blockade.

Materials and Methods

Cell Culture. CHO cells were grown in Ham's F12 medium with 10% fetal calf serum and 100 U/ml of penicillin/streptomycin. AtT-20 mouse pituitary cells were grown in Ham's F10 medium with 5% fetal calf serum, 10% heat-inactivated horse serum, 2 mM glutamine, and 50 μg/ml gentamicin. AtT-20 cells stably expressing human dopamine receptors were maintained in 500 μg/ml geneticin. For transient transfections and subsequent electrophysiological characterization, cells were plated onto glass coverslips coated with 40 μg/ml poly(L-lysine).

Transfection of Receptors and Channels into AtT-20 and CHO Cells. AtT-20 cells stably expressing the human D3 or D2S receptors were generated by clonal selection after a transfection with expression plasmids (pcDNA3; Invitrogen, Carlsbad, CA) mediated by Pfx-2 reagent (Invitrogen) into which the coding sequences for each receptor was subcloned (Kuzhikandathil et al., 1998). CHO-K1 cells were transiently transfected using LipofectAMINE (Invitrogen) with pcDNA3.1 vectors encoding the various potassium channels (a gift from ICAgen, Research Triangle Park, NC) and the enhanced green fluorescent protein (BD Biosciences Clontech, Palo Alto, CA). Expression efficiency of 15 to 30%, assessed by the enhanced green fluorescent protein marker and K^+ currents, was achieved routinely.

Electrophysiology. Agonist-activated currents were measured in AtT-20 or CHO cells by the whole-cell patch clamp technique using an Axopatch 200 amplifier (Axon Instruments, Union City, CA). Patch pipettes were constructed from N51A (Drummond, Broomall, PA) and polished on a homemade microforge at 600× magnification. Cells were held at −60 mV, and currents were elicited by ramp voltage commands (−120 to +40 mV) and hyperpolarizing steps (−100 mV) or other indicated protocols. The current responses were normalized to the cell capacitance (picoamperes/picofarads) to account for variation in cell size. The standard external solution used was: 145 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose. The pipette solution contained 130 mM potassium-aspartate, 20 mM NaCl, 10 mM HEPES, 10 mM glucose,

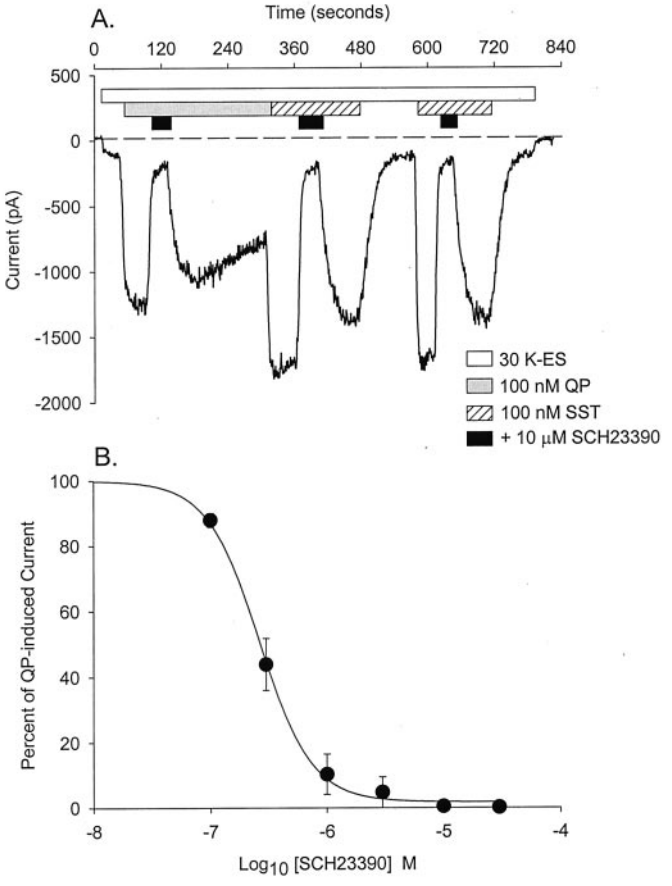

Fig. 1. A, SCH23390 (10 μM) blocks inward currents induced by 100 nM quinpirole (QP) or 100 nM somatostatin (SST) in an AtT-20 cell stably expressing the human D3 dopamine receptor. The representative trace shown was obtained in the whole-cell recording mode from a cell held at −60 mV. The duration of drug application is indicated by the coded bars above the trace. B, concentration-dependent inhibition of endogenous GIRK currents induced by 100 nM QP in AtT-20 cells expressing the human D3 receptor. The smooth curve represents the fit of the data set to the Hill equation, yielding an IC_{50} of 268 nM and a Hill coefficient of 1.92. Each data point represents the percentage of QP-induced current [e.g., the current in QP − the current in 30 mM K^+ (30 K-ES); mean ± S.E.M.] from six AtT-20 cells held at −60 mV.

0.1 mM GTP, 5 mM Mg-ATP, and 1 mM EGTA. To enhance inwardly rectifying K^+ currents, controls and drug exposures were carried out in solutions with elevated extracellular potassium (30 mM) by substitution for Na^+.

Data Acquisition and Analysis. Whole-cell macroscopic currents in response to ramp and step commands were sampled via a Digidata 1200b interface using Axotape and pClamp 7.0 software (Axon Instruments). Data files are then imported into SigmaPlot (SPSS, Chicago, IL) for display or analysis. The Student's *t* test was performed on relevant data using SigmaPlot. In the *t* test, the data were considered statistically different at $p < 0.05$.

Drugs. Quinpirole and somatostatin (Sigma/RBI, Natick, MA) were made as 10 mM stock solutions in distilled water and were used at a final concentration of 100 nM unless otherwise indicated. Stock solutions of SCH23390 (Sigma/RBI or TOCRIS Cookson, St. Louis, MO), *R*-(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (nor-methyl-SCH23390; Sigma/RBI), *R*-(+)-2,3,4,5-tetrahydro-8-iodo-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrochloride (iodo-SCH23390; Sigma/RBI), and SKF38393 (Sigma/RBI) were made at a concentration of 10 mM in distilled water. For electrophysiological experiments, drug solutions were delivered at indicated concentrations to cells via a multibarreled micropipette array (Microcaps, 3 μl; Drummond).

Results

SCH23390 Blocks Agonist-Activated GIRK Currents in AtT-20 Cells. In experiments investigating the crosstalk between D1 and D3 dopamine receptor signaling pathways, we discovered that SCH23390 blocked GIRK currents. We have shown previously that AtT-20 cells express only GIRK1 and GIRK2 isoforms and that both transfected D3 dopamine receptors and native somatostatin receptors activate endogenous GIRK currents in these cells (Kuzhikandathil et al., 1998; Kuzhikandathil and Oxford, 2000). Figure 1A shows that GIRK currents activated by either D3 dopamine receptors or by somatostatin receptors are completely inhibited by 10 μM SCH23390. The inhibition by SCH23390 is rapid and reversible. Furthermore, the representative recording in Fig. 1A demonstrates that the kinetics of inhibition and recovery are quite different, with the latter being much slower. GIRK currents induced by 100 nM quinpirole (a D2/D3 receptor agonist) in AtT-20 cells are inhibited by SCH23390 in a dose-dependent manner (Fig. 1B) with an EC_{50} of 268 nM. SCH23390 also inhibits somatostatin-induced GIRK currents with a similar efficacy and potency (representative example shown in Fig. 1A). SCH23390 from two different manufacturers (Sigma/RBI and TOCRIS Cookson) were used in all studies and yielded identical results, suggesting that the inhibition is not an artifact of supplier-specific contaminants.

SCH23390 Inhibition of GIRK Currents Is Receptor-Independent. The ability of SCH23390 to inhibit GIRK currents induced by two very different G protein-coupled receptors (D3 dopamine and somatostatin receptors) suggests that the inhibition may not reflect ligand interactions with either receptor but is receptor-independent. To assess this possibility, we examined the ability of SCH23390 to directly block currents in CHO cells only expressing human GIRK channels without coexpression of any G protein-coupled receptor. We and others demonstrated previously that robust constitutive inwardly rectifying currents can be observed in elevated extracellular potassium in cells overexpressing GIRK channels without receptors and/or receptor agonist application (e.g., Lesage et al., 1994; Chan et al., 1996; Kuzhikandathil et al., 1998). This feature permits a direct test of receptor-independent block of GIRK channels by SCH23390. Figure 2A shows representative current responses to voltage steps from −120 to +40 mV in a CHO cell coexpressing only the human GIRK1 and GIRK2 isoforms. Inward currents are enhanced upon changing from the normal external potassium concentration (5 mM, upper records) to 30 mM external potassium (middle records). Application of 10 μM SCH23390 to this cell dramatically suppressed the constitutive GIRK currents (lower records). Figure 2B is a current-voltage plot derived from the raw current traces in Fig. 2A indicating that the block is not appreciably voltage-dependent. Interestingly, Fig. 2B also shows that SCH23390 blocks the small but physiologically relevant outward GIRK currents. Untransfected CHO cells or CHO cells transfected with only D3 receptors do not express currents associated with GIRK channels and are correspondingly unaffected by SCH23390 (data not shown). Taken together, these results strongly suggest that SCH23390 directly inhibits GIRK channels, and this inhibition is receptor-independent.

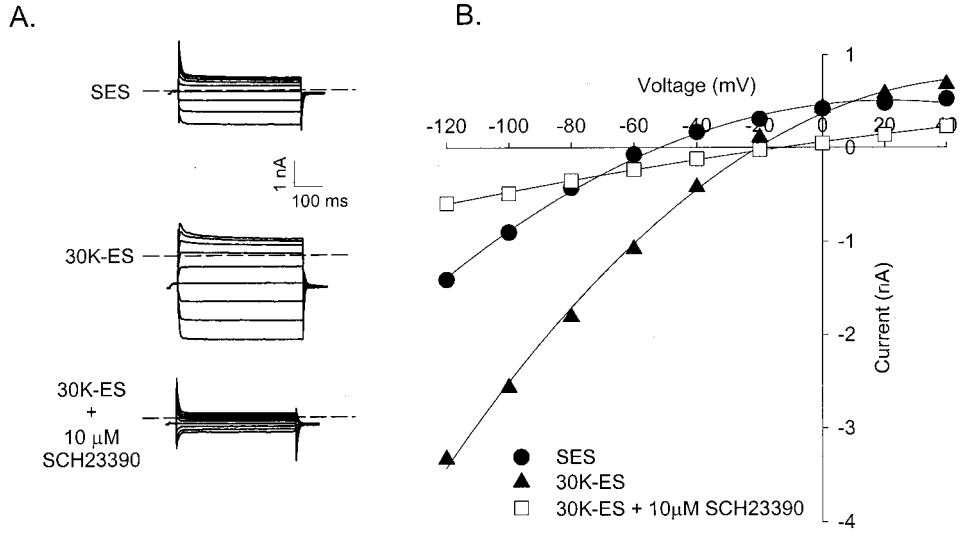

Fig. 2. A, representative current traces from a CHO cell transiently transfected with human GIRK1- and GIRK2-expressing plasmids. The traces represent current responses to voltage steps from −120 to +40 mV and were obtained in the whole-cell recording mode from a holding potential of −60 mV. The three sets of current responses were from the same cell exposed to standard external solution, external solution with 30 K-ES, and 30 K-ES solution with 10 μM SCH23390. The broken lines indicate the zero current level. B, a current-voltage plot generated from the current responses shown in A demonstrates the block of constitutive GIRK currents by 10 μM SCH23390 in a CHO cell expressing human GIRK1 and GIRK2. The smooth curves represent fits of a second-order linear equation to the data.

We examined the dose-dependence of SCH23390 inhibition of constitutive GIRK currents in CHO cells expressing either human GIRK1/GIRK2 heteromultimers or human GIRK2 homomultimers. Figure 3 shows that SCH23390 seems to inhibit GIRK1/GIRK2 heteromultimeric channels with a significantly higher potency (EC_{50}, 7.8 μM) than it inhibits GIRK2 homomultimeric channels (EC_{50}, 83 μM). Both apparent affinities are lower than observed for the inhibition of native GIRK channels in mouse AtT-20 cells (Fig. 1B). This difference could reflect species-specific sequence differences between mouse and human GIRK channels. Alternately, given the differential ability of SCH23390 to block GIRK heteromultimers versus homomultimers, it is possible that for an unknown reason, transient coexpression of human GIRK1 and GIRK2 channels in CHO cells results in a higher proportion of GIRK2 homomultimers compared with the native GIRK channels in AtT-20 cells. We have shown previously that AtT-20 cells express endogenous GIRK1 and GIRK2 isoforms (Kuzhikandathil et al., 1998). Finally, in addition to inhibiting GIRK1/GIRK2 channels, we have observed that SCH23390 also inhibits GIRK1/GIRK4 channels expressed in CHO cells (data not shown).

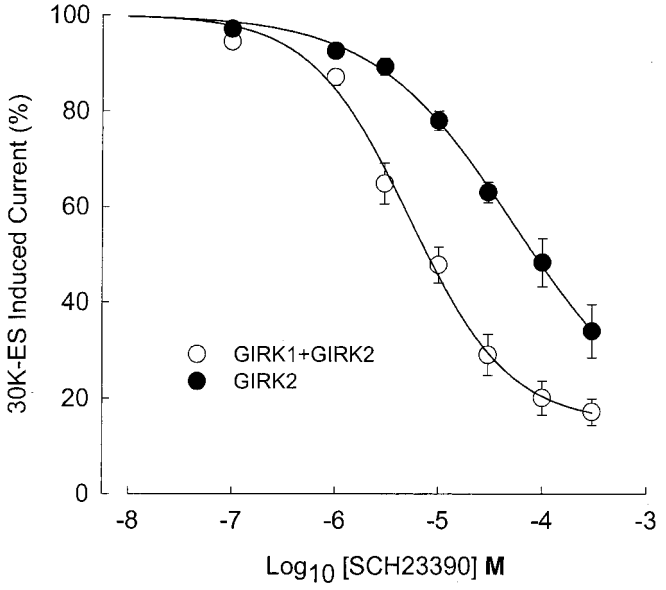

Fig. 3. Concentration-dependent inhibition of constitutive inward currents in CHO cells expressing either human GIRK1/GIRK2 channels (○, n = 7 cells) or human GIRK2 channels alone (●, n = 9 cells). Each data point represents the percentage (mean ± S.E.M.) of inward currents observed in 30 K-ES solution at −60 mV. Individual data sets were fitted to the Hill equation and yielded IC_{50} values of 7.78 μM (human GIRK1/GIRK2) and 83 μM (human GIRK2 alone).

Kir2.0 Family Potassium Channels Are not Significantly Inhibited by SCH23390. We were next interested in exploring whether the inhibition of GIRK (Kir3.x) channels by SCH23390 was unique to this class of inward rectifiers. To approach this question, we tested the ability of SCH23390 to block members of a related family of inwardly rectifying potassium channels (Kir2.0). CHO cells were transfected individually with plasmids expressing human Kir2.1, Kir2.2, Kir2.3, or Kir2.4. Figure 4 shows representative recordings of currents in response to voltage ramps from such transfected cells. At the highest concentration of SCH23390 tested (100 μM), the inward currents at −100 mV in CHO cells expressing Kir2.1, Kir2.2, Kir2.3, or Kir2.4 were inhibited by only

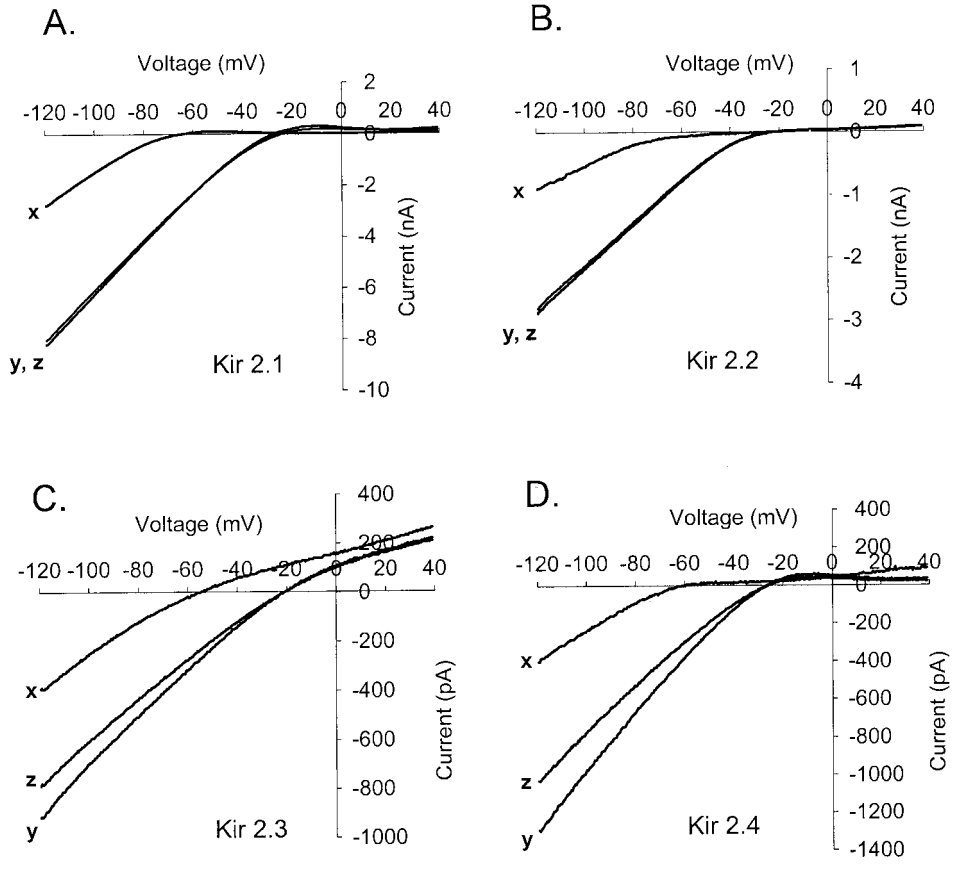

Fig. 4. Representative inward K^+ current responses from individual CHO cells transiently expressing the human isoforms of Kir2.1 (A), Kir2.2 (B), Kir2.3 (C), or Kir2.4 (D) during a voltage ramp (−120 to +40 mV). Whole-cell recording was performed 24 to 48 h after transfection. Cells were held at −60 mV, and responses were elicited in standard external solution (x), external solution with 30 mM K^+ (y), and external solution with 30 mM K^+ and 100 μM SCH23390 (z).

2.6, 3.2, 34.0, and 15.8%, respectively. Although both Kir2.3 and Kir2.4 were inhibited by this high concentration of SCH23390 (100 μM), the inhibition was much less than that of GIRK channels (see Fig. 3) and exhibited slower kinetics. Furthermore, 10 μM SCH23390 had no effect on Kir2.1 and Kir2.2 channels and blocked Kir2.3 and Kir2.4 channels by only 1.8 and 2.8%, respectively (n = 2).

We also tested the ability of SCH23390 to inhibit currents generated by voltage ramp commands (from −120 mV to +40 mV) through other native channels in AtT-20 cells (Fig. 5). We and others have shown previously that AtT-20 cells exhibit voltage-gated sodium, calcium, and outwardly rectifying potassium currents (Loechner et al., 1996; Kuzhikandathil et al., 1998; Kuzhikandathil and Oxford, 1999). Figure 5A shows representative current responses to a voltage ramp command in an AtT-20 cell expressing the D3 dopamine receptor. Although inwardly rectifying GIRK currents induced by 100 nM quinpirole are completely inhibited by 3 μM SCH23390, other currents elicited between −40 and +40 mV are unaffected. These latter currents represent sodium, calcium, and delayed-rectifier potassium channels that are resistant to SCH23390 at this concentration. A comparison of

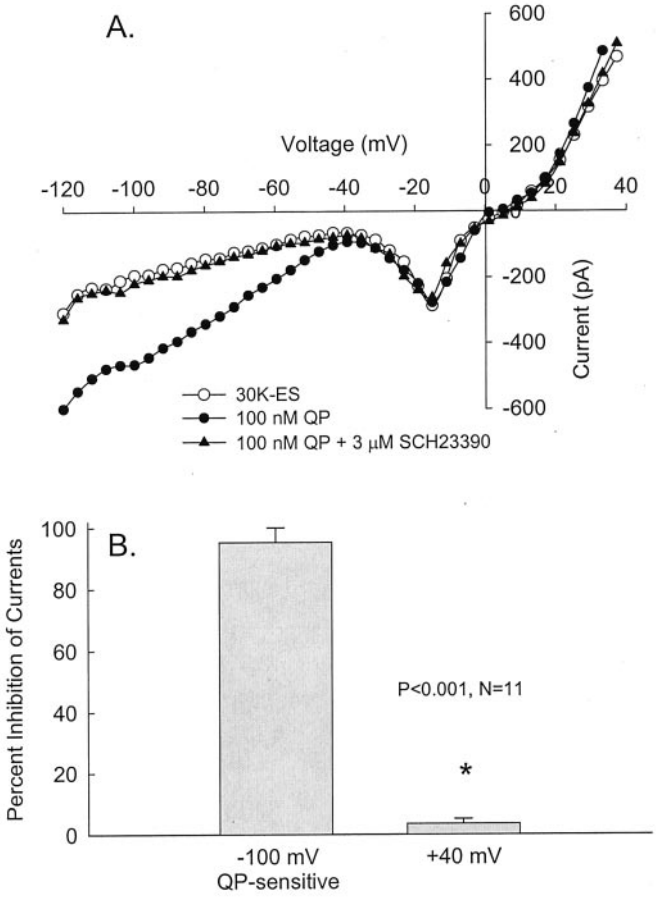

Fig. 5. A, whole cell currents obtained during a voltage ramp (−120 to +40 mV) from a single AtT-20 cell expressing the human D3 receptor. The cell was treated with an external solution containing (○), 100 nM QP (●), and 100 nM QP plus 3 μM SCH23390 (▲). Quinpirole and SCH23390 were applied in the 30 K-ES solution. Note that the currents elicited between −40 mV and +40 mV were not affected by SCH23390. B, bar graph comparing the percentage of inhibition (mean ± S.E.M.) of QP-sensitive currents at −100mV and voltage-gated currents at +40 mV by 3 μM SCH23390.

the relative sensitivity of quinpirole activated GIRK currents at −100 mV and voltage-gated potassium currents at +40 mV (Fig. 5B) to 3 μM SCH23390 documents this resistance. These results support the suggestion that SCH23390 inhibition of GIRK channels is specific among the many channels examined.

The Chlorine Atom in SCH23390 Is Critically Involved in GIRK Channel Block. Several compounds that are closely related structurally to SCH23390 are available (Fig. 6A). Of these, SKF38393 is particularly interesting. Whereas SCH23390 is a D1 dopamine receptor antagonist, SKF38393 is a partial agonist of D1 receptors that is widely used to activate the receptor in vivo and in vitro. The chemical structure of these two compounds exhibits two key differences. First, SKF38393 lacks a methyl group and substitutes the chlorine atom in SCH23390 with a hydroxyl group (Fig. 6A). Furthermore, an intermediate compound, nor-methyl-SCH23390, lacks only the methyl group that is present in SCH23390 (Fig. 6A).

We tested whether these structural analogs could block human GIRK1/GIRK2 channels expressed in CHO cells. Figure 6B shows, for a representative experiment, that constitutive GIRK currents were completely inhibited by both SCH23390 and nor-methyl-SCH23390 (100 μM) but were only marginally reduced by 100 μM SKF38393. For all cells examined, both SCH23390 and nor-methyl-SCH23390 blocked constitutive GIRK currents by approximately 80%, whereas SKF38393 only blocked approximately 10% of the currents (Fig. 6C). Furthermore, the small block by SKF38393 exhibited much slower kinetics compared with either SCH23390 or nor-methyl-SCH23390 (Fig. 6B).

We also tested these compounds using native GIRK currents induced by D3 receptor activation in mouse AtT-20 cells. In agreement with the results on constitutive currents, agonist-induced GIRK currents in AtT-20 cells were significantly inhibited by SCH23390 and nor-methyl-SCH23390 compared with either SKF38393 or iodo-SCH23390 (Fig. 6D). Although iodo-SCH23390 was less effective than SCH23390 or nor-methyl-SCH23390, it was significantly more effective than SKF38393 at blocking quinpirole-induced current. These results suggest that the halide atom at position 7 in SCH23390 is critically involved in mediating the GIRK channel blockade.

SCH23390 Depolarizes and Excites AtT-20 Cells. To evaluate the potential physiological significance of SCH23390 block, we then tested the effect of these compounds on membrane potential in AtT-20 cells. It has been demonstrated previously that GIRK channels can play an important role in maintaining resting membrane potential (Ehrengruber et al., 1997; Kuzhikandathil and Oxford, 2000). Furthermore, the activation of endogenous GIRK channels by heterologously expressed D3 dopamine receptors in AtT-20 cells hyperpolarizes membrane potential and inhibits neurosecretory activity (Kuzhikandathil and Oxford, 2000). We hypothesized that if SCH23390 inhibits GIRK channels, then it should prevent or even reverse the D3 receptor-induced hyperpolarization in AtT-20 cells. To test this hypothesis, we recorded membrane potential responses to the D2/D3 selective agonist quinpirole (100 nM) under current clamp in AtT-20 cells stably expressing the human D3 receptor. As expected, the application of quinpirole hyperpolarized the membrane potential and inhibited spontaneous action poten-

tials in these cells. Both 10 μM SCH23390 and 10 μM nor-methyl-SCH23390 reversed the response to quinpirole, depolarizing the membrane potential and inducing the resumption of spontaneous action potentials (Fig. 7, A and B). In contrast, 10 μM SKF38393, which does not significantly alter GIRK currents, did not alter the quinpirole-induced hyperpolarization (Fig. 7B). These results are in agreement with experiments shown in Fig. 6 and support the premise that a SCH23390-mediated block of GIRK channels directly affects the electrical activity of cells. More importantly, it also indicates that SCH23390 inhibits the small but physiologically relevant outward current through GIRK channels (Fig. 2B).

Discussion

In this article, we present evidence that the classic D1 dopamine receptor antagonist SCH23390, widely used as a definitive probe of D1 receptor involvement in brain function, also effectively and completely inhibits GIRK channels. SCH23390 inhibits both native GIRK channels in AtT-20 cells and human GIRK channels heterologously expressed in CHO cells in a dose-dependent manner. The inhibition has rapid kinetics (Figs. 1A and 6B) and an IC_{50} of approximately 0.3 μM in AtT-20 cells (Fig. 1B). Two features of the inhibition suggest that the dramatic reduction of current reflects a direct interaction of SCH23390 with GIRK channels. First, the inhibition is quite rapid, which is consistent with a direct block of the channel. Second, the inhibition is observed for constitutively active GIRK channels in the absence of agonists or coexpressed G protein-coupled receptors. The nature of constitutive GIRK currents in cells overexpressing Kir3.0 subunits is not entirely clear because of the complexity of the signaling components linking G protein-coupled receptors to effectors. The expression systems examined here were unlikely to reflect spontaneous isomerizations of receptors because constitutive currents are insensitive to sulpiride, a specific D2 receptor antagonist, in cells expressing both D2 receptors and Kir3.2 channels (unpublished observations). Constitutive currents are also unlikely to reflect spontaneous dissociations of G protein heterotrimers containing Gαi/o subunits because pretreatment with pertussis toxin blocks agonist-induced GIRK currents but not constitutive currents (Kuzhikandathil et al., 1998; Leaney and Tinker, 2000; Leaney et al., 2000; Zhou et al., 2001; Chan et al., 1996). Although our data are consistent with channel block, we cannot definitively rule out the possibility that SCH23390 allosterically interferes with gating induced by either free G$\beta\gamma$ dimers and/or phosphatidylinositol-4,5-bisphosphate (Mark and Herlitze, 2000). With regard to the latter mechanism, Zhou et al. (2001) recently suggested that blockade of GIRK channels expressed in *Xenopus laevis* oocytes by the membrane-permeable local anesthetic bupivacaine may involve the destabilization of phosphatidylinositol-4,5-bisphosphate binding to the channel.

A survey of studies that have used SCH23390 to probe D1 receptor function reveals that the compound is often used at concentrations ranging between 0.1 and 10 μM (Yamamoto et al., 1994; Aosaki et al., 1998; Cai et al., 1999). Our results

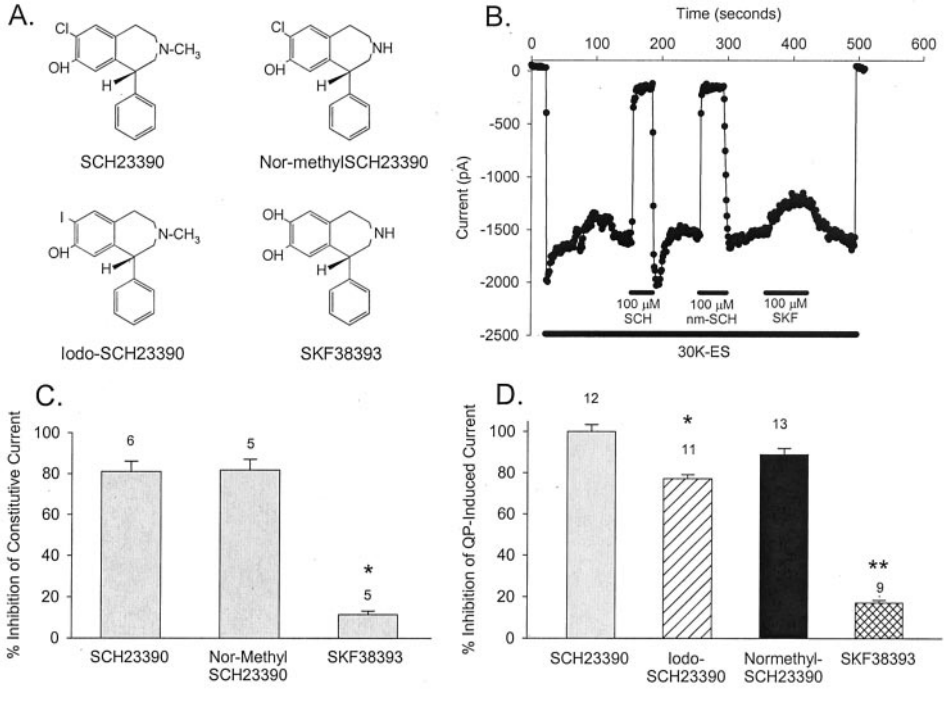

Fig. 6. A, chemical structure of the different phenyltetrahydrobenzazepines compounds used in this study. B, representative whole-cell current recording from a CHO cell transiently expressing human GIRK1 and GIRK2. The cell was held at −60 mV and was treated with 100 μM solutions of SCH23390 (SCH), nor-methyl-SCH23390 (nm-SCH), and SKF38393 (SKF). The black bars at the bottom of the trace indicate the duration of drug application. All drugs were applied in an external solution containing 30 K-ES. C, bar graph showing that SCH23390 and nor-methyl-SCH23390 block human GIRK1 and GIRK2 channel expressed in CHO cells with equal efficacy, whereas SKF38393 is much less effective (*, $P < 0.0001$, Student's t test). Each compound was used at a concentration of 100 μM. D, bar graph comparing the ability of various phenyltetrahydrobenzazepines compounds to inhibit QP-induced current in AtT-20 cells expressing the D3 receptor. Each compound was tested at a concentration of 3 μM. Although both iodo-SCH23390 (*, $P < 0.01$) and SKF38393 (**, $P < 0.0001$) were significantly less effective, iodo-SCH23390 was significantly better than SKF38393 at blocking the QP-induced current. The numbers above each bar in C and D represent the number of cells tested.

suggest that in this concentration range, SCH23390 also effectively blocks GIRK channels. This presents a potential problem, particularly in studies that exclusively use SCH23390 to differentiate between D1 and "D2-like" (D2, D3, and D4) receptors. Some of the physiological functions ascribed to D2-like receptors are because of their coupling with GIRK channels, including modulation of neuronal excitability and secretory activity. If in addition to being a D1 receptor antagonist, SCH23390 also blocks GIRK channels, its usefulness in distinguishing D1 receptor function from D2-like receptor function can become problematic in instances in which D1 and D2-like receptors are coexpressed. For example, the data in Fig. 7 demonstrate that SCH23390 inhibits the functional response to D3 dopamine receptor activation, depolarizing the cell and inducing action potentials. But for the evidence presented in this article, such a result in a native neuron would have normally and erroneously been interpreted to suggest that D1 receptors normally inhibit excitation. Thus, in light of the results presented here, it may be useful to reevaluate previous studies that have used SCH23390 exclusively to determine D1 receptor function.

Although we have no direct evidence indicating the involvement of GIRK channel block in any particular previous study of this type, examples of studies in which such a complication might exist are instructive. Ranaldi and Wise (2001) used SCH23390 to investigate the role of D1 receptors in the ventral tegmental area (VTA) in cocaine self-administration in rats. Injections of SCH23390 into the VTA at approximate final concentrations between 6 and 24 μM (assuming a 1000-fold dilution) increased cocaine self-administration, suggesting that dendritically released dopamine normally moderates reward behavior through D1 receptor activation. Considering that VTA neurons contain D2-like receptors coupled to GIRK channels (Momiyama et al., 1993; Liu et al., 1994), it is plausible that the SCH23390 actions might reflect the inhibition of D2 receptor-activated GIRK currents and subsequent excitation of VTA neurons.

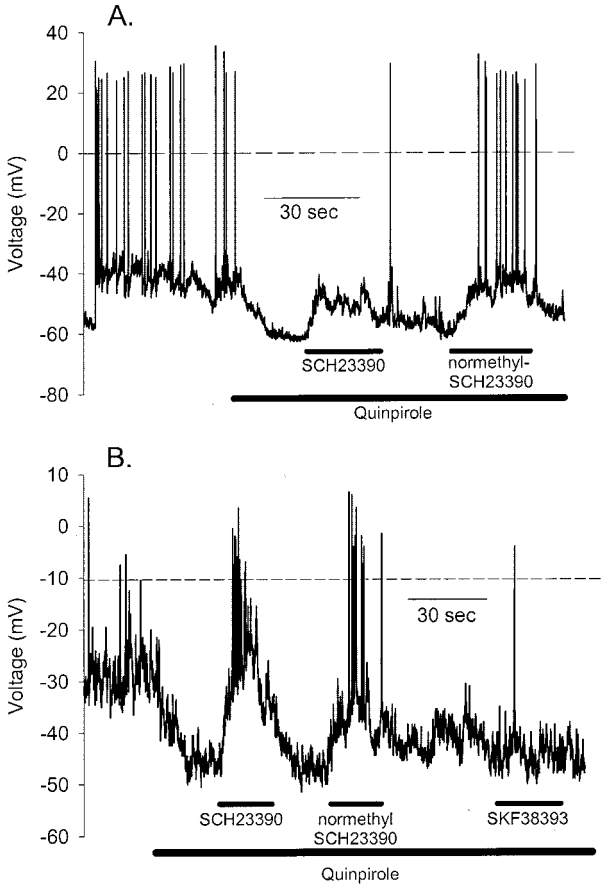

Fig. 7. Whole-cell current clamp recording from two representative AtT-20 cells expressing the D3 receptor demonstrating that SCH23390 and nor-methyl-SCH23390 (A and B), but not SKF38393 (B), block the hyperpolarization induced by 100 nM quinpirole. Each compound was used at a concentration of 10 μM. The black bar below the trace indicates the duration of drug application. Note that although quinpirole inhibits the spontaneous action potentials, both SCH23390 and nor-methyl-SCH23390 depolarize the membrane potential and induce the cells to fire action potentials.

Our identification of GIRK channels as an atypical target of SCH23390 is similar to findings from two recent reports of other receptor ligands inhibiting GIRK channel currents. Ulens et al. (1999) examined the activation of GIRK1/GIRK2 heteromultimeric channels by several opioid receptor ligands in *X. laevis* oocytes also expressing κ-opioid receptors. In addition to the expected activation by nanomolar concentrations of the κ selective agonist U50488H, they observed a reduction in GIRK currents at micromolar concentrations. This reduction was observed in oocytes expressing only Kir3.1 and Kir3.2 without κ-opioid receptors. Furthermore, as we observed for SCH23390, Kir2.1 (IRK1) channels were insensitive to the opioid agonist. Blockade of GIRK channels by U50488H, propoxyphene, and methadone exhibited EC_{50} values of 514, 53, and 56 μM, respectively. Another report indicates that several antipsychotic drugs (haloperidol, thioridazine, pimozide, and clozapine) also block GIRK channels expressed in *X. laevis* oocytes (Kobayashi et al., 2000). These authors reported that haloperidol, thioridazine, pimozide, and clozapine blocked GIRK channels with IC_{50} values of 75.5, 57.6, 2.96, and 178.9 μM, respectively. The potency of SCH23390 in blocking GIRK1/GIRK2 channels that we observed (IC_{50}, 0.3 μM) is greater than that of any of these other compounds. Taken together, these previous studies and our current observations reiterate the need to reevaluate previous studies that have used these "selective" ligands to determine the function of receptors in vivo at concentrations moderately exceeding the EC_{50} values for association with their respective G protein-coupled receptors.

The SCH23390-mediated block of GIRK channels exhibits several interesting properties. Although SCH23390 selectively blocks GIRK channels, its ability to block heteromultimeric GIRK1/GIRK2 channels is significantly different from its ability to block homomultimeric GIRK2 channels (Fig. 3). In addition to the species-specific differences in GIRK channel sequence, this differential potency might partly explain the significantly different IC_{50} values for native mouse GIRK channels in AtT-20 cells (Fig. 1B) and transiently expressed human GIRK channels in CHO cells (Fig. 3). It is possible that native GIRK channels in AtT-20 cells might be composed predominantly of GIRK1/GIRK2 heteromultimers, whereas CHO cells transiently transfected with both GIRK1 and GIRK2 plasmids might express a higher percentage of GIRK2 homomultimers than GIRK1/GIRK2 heteromultimers. Experiments heterologously expressing tandem GIRK1/GIRK2 constructs would be required to explore this speculation. A practical consequence of this observation is the intriguing possibility that SCH23390

or its derivatives might be useful pharmacological tools for probing the subunit composition of GIRK channels in vivo.

The results shown in Fig. 2B combined with the ability of SCH23390 to depolarize membrane potential (Fig. 7) indicate that the compound blocks not only the inward potassium current, but also the smaller, physiologically relevant outward current through GIRK channels. This property is particularly important if compounds structurally related to SCH23390 are to be used in pharmacotherapeutic strategies to block GIRK channels in vivo.

Our results also suggest that the phenyltetrahydrobenzazepines are potential candidates for designing novel blockers of GIRK channels. Toward this end, we have demonstrated that the halide atom present in phenyltetrahydrobenzazepine compounds is essential for mediating GIRK channel block. Although iodo-SCH23390 also blocks GIRK channels, it does so less effectively (Fig. 6D), indicating a preference for chlorine in this position. This suggests that the size of the atom at position 7 in phenyltetrahydrobenzazepines might be an important determinant of GIRK channel block. To examine this hypothesis, it will be interesting to test the effect of removing the chlorine atom at position 7 or substituting it with a fluorine or bromine atom. In addition, it is important to evaluate the contribution of the phenyl group at position 1 in the phenyltetrahydrobenzazepine compounds.

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