0026-895X/93/050907-04$03.00/0

MOLECULAR PHARMACOLOGY, 44:907-910

ACCELERATED COMMUNICATION

Multiple Sulfonylurea-Sensitive Potassium Channels: A Novel Subtype Modulated by Dopamine

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Received July 7, 1993; Accepted August 11, 1993

SUMMARY

In single channel patch-clamp recordings from freshly dissociated rat corpus striatum (caudate-putamen) neurons, the sulfonylurea drugs tolbutamide and glibenclamide caused a concentration-dependent blockade of a K^+ channel that is activated by D_2 dopamine receptor agonists. Tolbutamide was about 10–100 times more potent than glibenclamide, a rank-order potency opposite to that seen at previously described adenosine triphosphate-sensitive K^+ channels. The channel also was poorly activated by diazoxide, which is a known opener of adenosine triphosphate-sensitive K^+ channels. However, like adenosine triphosphate-sensitive channels, it opened in the absence of dopaminergic agonist when the cells were treated with the metabolic inhibitor rotenone, indicating that channel openings occur under energy-depleting conditions. This suggests the existence of a novel, pharmacologically distinct class of sulfonylurea-sensitive K^+ channels, regulated metabolically and also mediating dopaminergic neurotransmission.

Hypoglycemic sulfonylurea drugs are the major therapeutic agents for the treatment of type II diabetes mellitus (1). These drugs are known to enhance insulin release from the pancreas by inhibiting K^+ channels that open when cytoplasmic levels of ATP decrease (2). These K_{ATP} channels are blocked more potently by "second generation" sulfonylureas such as glibenclamide (glyburide) than by "first generation" drugs such as tolbutamide, with potencies differing by over 100-fold (1, 2). At higher concentrations, sulfonylureas also block voltage-gated (3) and Ca^{2+}-activated (4) K^+ channels. K_{ATP} channels have been implicated in a variety of other functions in endocrine, muscle, and nervous tissues (2). In brain, it has been reported that sulfonylureas block the K^+ conductance activated by presynaptic D_2 dopamine receptors in the substantia nigra (5), although other investigators were unable to reproduce this result (6). This raises the question of whether D_2 receptors might modulate K_{ATP} channels during synaptic transmission in the brain.

Therefore, we studied whether sulfonylureas could block an 85-pS K^+ channel that is activated by postsynaptic dopamine receptors in rat corpus striatum (caudate-putamen) neurons (7). Various dopamine receptor subtypes have now been cloned, some of which are classified as belonging to a "D_2-like" group (8). The 85-pS K^+ channel is activated by dopaminergic drugs with a D_2-like profile (7). In recordings from over 300 cells (9), openings of this channel showed an absolute dependence on the presence of dopamine or of quinpirole, a D_2 agonist (10), in the cell-attached patch pipette. Blockade studies showed that this channel is inhibited by low nanomolar concentrations of quinine (11, 12). In the present report, we extend these blockade studies to sulfonylureas, using cell-attached patch-clamp recordings. Patch-clamp electrophysiology can resolve single channel currents, and so could be a useful tool to compare this channel with other known K^+ channels.

Materials and Methods

Cell preparation. Caudate-putamen neurons were freshly dissociated from 30- to 45-day-old male Sprague-Dawley rats by enzymatic and mechanical means (7). Coronal sections (200 μm) of caudate-putamen were dissociated as described (7), with the following modifications. The medium for trypsin treatment was (in mM) sucrose, 235; KCl, 2; $CaCl_2$, 1; $MgCl_2$, 1; $MnCl_2$, 0.02; D-glucose, 25; and piperazine-*N*,*N*′-bis-(2-ethanesulfonic acid)(PIPES)-Na, 20, pH 7.0, equilibrated with 100% O_2. The trypsin concentration was decreased to 26,000 benzoyl-arginine ethyl ester units, and the incubation time to 1 hr. Cell dissociation was by trituration. The cells were plated on poly-L-ornithine-coated petri dishes and used for patch-clamping the same day.

Patch-clamp electrophysiology. The cells were superfused continuously with (in mM) NaCl, 149; KCl, 3.5; $CaCl_2$, 2.5; $MgCl_2$, 1; D-glucose, 10; and HEPES-Na, 10, pH 7.4, adjusted with sucrose to 330–340 mOsm/kg, and equilibrated with 100% O_2 (plus rotenone where indicated). The cells were viewed under phase-contrast optics. Patch pipettes contained (in mM) KCl, 140; $CaCl_2$, 2.5; $MgCl_2$, 1; and HEPES-K, 10, pH 7.4 (plus quinpirole, tolbutamide, glibenclamide, or diazoxide as indicated). Patch-clamp recordings were made in the cell-attached configuration. Criteria for satisfactory recordings were seal resistance

ABBREVIATIONS: ATP, adenosine triphosphate; K_{ATP}, ATP-sensitive K^+ channel; HEPES, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid).

greater than 5 GΩ, channel current reversal potentials greater than 50 mV depolarized from resting membrane potential (when currents were present), and stable phase-bright cell appearance for the duration of the recording, which normally was at least 10 min. Currents were measured with an Axopatch-1D patch-clamp system and analyzed with pCLAMP software (Axon Instruments). After filtering at 2 kHz low pass, currents were acquired at 100 μsec/point to a 386-based desktop computer, and were viewed on-line with an analog oscilloscope to verify accurate digital acquisition. During each experiment, we varied the pipette potential in order to determine channel conductance, to assess whether channel activation was voltage sensitive, and to identify the reversal potential and the zero-current level. Membrane potential is expressed relative to resting potential; whole cell recordings from these cells indicated resting potentials around −55 mV (7). (Since channel current reversal potentials thus occurred near 0 mV membrane potential with symmetrical K^+ concentrations, this implies that K^+ was the primary charge carrier in the channels that we recorded.) The number of channels in the patch was estimated from simultaneous openings detected throughout the observation period of approximately 10 min. Digital records of at least 25,600 points were used to determine fractional open and closed times. We verified that these records were long enough to encompass bursting periods and give representative results by performing selected longer acquisitions and by repeating and comparing acquisitions during the experiment.

Drugs. Quinpirole was from Research Biochemicals. Tolbutamide, glibenclamide, diazoxide, and rotenone were from Sigma. Sulfonylureas were freshly diluted before use from 1 mM stock solutions in either dilute KOH or 50% ethanol; dilutions of these solvents had no effect by themselves on channel currents, and equivalent results were obtained with either solvent.

Results and Discussion

We performed cell-attached patch-clamp recordings from multipolar neurons of at least 10 μm in diameter. Under our present cell preparation conditions, approximately 26% of patches from cells of this morphology display the 85-pS channel when dopamine or D_2 agonists are present in the patch pipette (9). However, when dopamine agonists are omitted from the patch pipette, the open probability of the 85-pS channel is close to zero (9). Recordings in the absence of drugs reveal two other classes of resting K^+ channels on these cells: small inwardly rectifying K^+ channels of 8–30 pS, which could be distinguished from the 85-pS channel by their conductances, and voltage-sensitive channels of 100–200 pS (which we presume to be large-conductance Ca^{2+}-activated K^+ channels), which could be distinguished by their conductances and voltage sensitivity (13).

Fig. 1 shows the effects of tolbutamide and glibenclamide on the 85-pS channel, measured in the presence of 10 μM quinpirole. Compared with control currents in the presence of quinpirole alone, both sulfonylureas were associated with concentration-dependent shifts to shorter channel open times and longer channel closed times when present in the patch pipette. These effects were sometimes progressive over the first few minutes after gigaseal formation, but reached maximum within 2–6 min (not shown). Tolbutamide (50–100 nM) blocked channel currents at lower concentrations than did glibenclamide (1–10 μM).

The concentration dependence of this blockade is shown for a greater number of cells in Fig. 2. Although application of sulfonylureas through the patch pipette precluded control recordings from the same patches, the conductances of the channels that were partially blocked ranged between 80–89 pS, and thus were similar to the channels measured with quinpirole only, which had a conductance range of 82–87 pS. As shown, tolbutamide was about 10–100 times more potent than glibenclamide. (A possible source of error could be underestimation of the number of channels in the patch due to channel blockade; however, this would tend to understate rather than overstate the extent of blockade.) The rank-order potency we observed is opposite to that seen at previously described K_{ATP} channels (2). Thus, although the 85-pS channel shared with K_{ATP} channels a sensitivity to these two drugs, it differed in their relative potencies at blockade. This raises the question of whether this is a different subtype of sulfonylurea-sensitive K^+ channel.

In order to further characterize this difference, we examined whether the 85-pS channel could be activated in the absence of dopamine agonists, in the presence of other compounds that are known to open K_{ATP} channels. Diazoxide (50 μM) is a known activator of K_{ATP} channels (2), so we performed record-

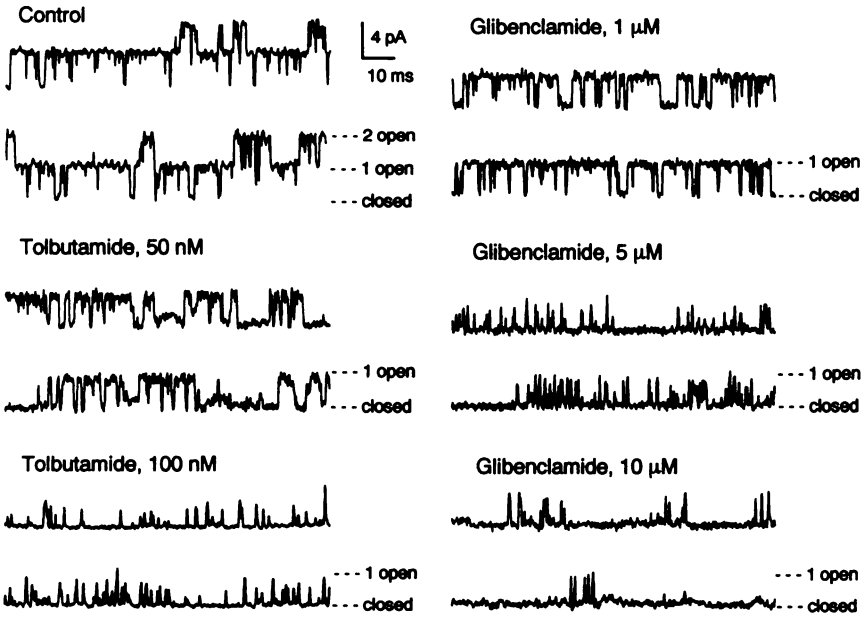

Fig. 1. Effects of tolbutamide (50 and 100 nM) and glibenclamide (1, 5, and 10 μM) on 85-pS single channel currents. Each record is from a different cell. In all cases, cell-attached patch pipettes contained 10 μM quinpirole, and other drugs were added in the pipette as indicated. All records are at resting membrane potential. Upward deflections correspond to inward currents. The scale bars apply to all records.

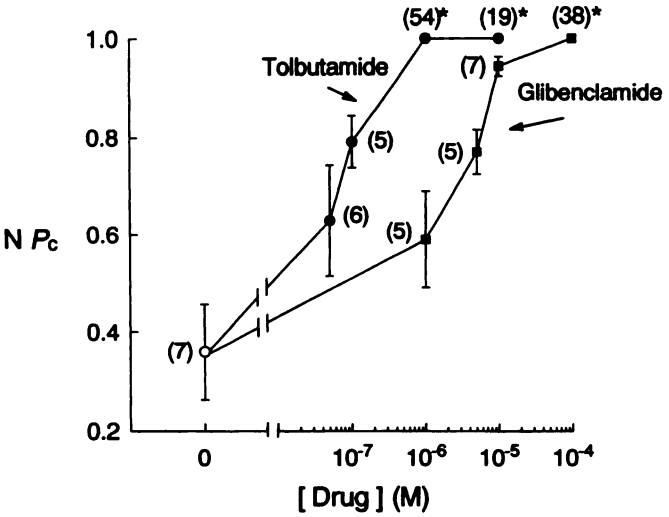

Fig. 2. Concentration-response relationship for channel blockade by sulfonylureas. All data are in the presence of 10 μM quinpirole, and at resting membrane potential (open circle, quinpirole only; closed circles, tolbutamide; squares, glibenclamide). The x-axis is log scale. The concentrations tested were 50 nM, 100 nM, 1 μM, and 10 μM for tolbutamide, and 1 μM, 5 μM, 10 μM, and 100 μM for glibenclamide. Blockade is expressed as NP_c, where N is the number of active channels in the patch, as determined from simultaneous openings during a 10-min observation, and P_c (closed probability) is the fraction of time all channels in the patch were closed or blocked (i.e., not conducting). Data are expressed as mean ± standard deviation. Numbers in parentheses indicate the number of patches expressing 85-pS channel activity, from which the mean values were determined; a total of 25–35 cells were tested at each concentration in order to obtain this number of patches expressing channels. When followed by an asterisk, numbers in parentheses indicate the total number of cells tested, and no 85-pS channel openings were observed.

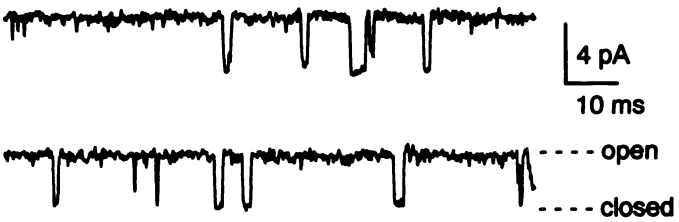

Fig. 3. Channel currents in the presence of rotenone. The experiment was performed as in Fig. 1, except that 5 μM rotenone was applied via the bath superfusing the cells, and no dopamine agonist was present in the patch pipette. The record is at resting membrane potential. Upward deflections correspond to inward currents.

ings with it in the patch pipette instead of quinpirole. We observed various channels of conductances smaller than 30 pS or larger than 100 pS with a greater frequency than under control conditions (not shown), although we did not further characterize these channels. However, we never elicited channel openings between 30–100 pS with diazoxide in recordings from 42 cells, a sample size large enough that we would have expected to see 85-pS openings when using dopamine or quinpirole (9). This suggests that diazoxide is a relatively poor activator of this channel.

Since some previously described sulfonylurea-sensitive K^+ channels are known to open under conditions of lowered intracellular ATP (2), we next asked whether the 85-pS channel could be activated metabolically in the absence of dopamine agonists. In order to avoid the run-down of currents that occurs in whole cell recordings from these cells (7), and which we seemed to encounter in preliminary attempts at cell-free patches, we performed recordings in the cell-attached configuration under conditions of metabolic depletion. This was accomplished by bath application of the NADH dehydrogenase inhibitor rotenone (5 μM) (14, 15). Openings of the 85-pS channel were seen in 5 of 24 cells tested (Fig. 3), a proportion similar to that obtained with dopaminergic agonists (9). These channel currents had conductances ranging from 85 to 87 pS, and thus were similar to those elicited with dopamine or quinpirole. They had fractional open times of 0.35–0.73, and fractional closed times of 0.27–0.65, reflecting similar or slightly smaller open probabilities than seen with dopamine agonists (cf. Fig. 2, control). Any difference in open probability might be consistent with different modes of channel activation. Although this result indicates that the 85-pS channel opens under conditions of metabolic depletion, it does not resolve whether it is gated by changes in the intracellular concentration of ATP or through some other mechanism.

We conclude that there are at least two classes of metabolically regulated K^+ channels that are inhibited by sulfonylureas. Type 1 would comprise the previously known K_{ATP} channels, whose actions include modulation of insulin release. These are activated by diazoxide and are inhibited more potently by glibenclamide than by tolbutamide. Type 2 would include the 85-pS channel, which appears to be coupled to dopamine receptors in brain. It is poorly activated by diazoxide, and is inhibited more potently by tolbutamide than by glibenclamide. It follows that caution must be used in attributing a sulfonylurea-sensitive effect to conventional K_{ATP} channels.

Previous studies by other groups have provided circumstantial evidence for such channel diversity in a variety of tissues. Cardiac K_{ATP} channels show heterogeneity in sensitivity to guanyl nucleotides (16). In brain, there appear to be two classes of [^{3}H]glibenclamide binding sites, with the low affinity site more abundant in the striatum (17). Also, sulfonylureas are often less potent at blocking K^+ currents in brain than in other tissues (2, 18). A 146-pS K_{ATP} channel in hypothalamic neurons was poorly activated by diazoxide and poorly inhibited by glibenclamide (19, 20).

Recognition that sulfonylurea-sensitive channels are heterogeneous may prove helpful in screening potential hypoglycemic drugs for treating diabetes. Presumably, since the K_{ATP} channel in the pancreas influences insulin release (1, 2), selectivity for this channel would be a desirable characteristic. However, since sulfonylurea binding to other channels might elicit side effects, it might be desirable that such drugs have lower affinity for these other channels.

The apparent sensitivity of metabolically regulated K^+ channels in brain to dopaminergic drugs may also have implications for neuronal function. Metabolically sensitive channels are thought to protect against excitoxicity-associated neuronal death (21), which is believed to occur in some diseases of the central nervous system. Huntington's disease, for example, is known to result from neuronal death in the striatum (22), and our results suggest that a metabolically sensitive channel is located postsynaptically to dopaminergic nerve terminals in this region of the brain. Also, differing hypotheses of the causes of schizophrenia have variously implicated either dopamine or neuronal degeneration. There is evidence that schizophrenic patients have elevated levels of D_2 dopamine receptors (23). However, it has been difficult to reconcile a dopaminergic model

of schizophrenia with the fact that some patients display degeneration of brain tissue apparently extending beyond dopaminergic projections (24, 25). Our results suggest that dopaminergic transmission in the striatum might be coupled to a mechanism that has been implicated in protection against neuronal degeneration.

Acknowledgments

This work was supported by National Institutes of Health FIRST award MH-48545, and by grants from the Tourette Syndrome Association and the Pharmaceutical Manufacturers Association Foundation.

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