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Glyburide-Sensitive K^+ Channels in Cultured Rat Hippocampal Neurons: Activation by Cromakalim and Energy-Depleting Conditions

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SUMMARY

Previous studies in our laboratory have shown that cromakalim activates a tetraethylammonium-sensitive K^+ current in cultured embryonic rat hippocampal neurons. This phenomenon was further characterized using whole-cell voltage-clamp and single-channel recording techniques. Glyburide (1–25 μM), an antagonist of ATP-sensitive K^+ channels, produced a concentration-dependent depression of the cromakalim-activated current. In contrast, charybdotoxin (100 nM), an antagonist of some Ca^{2+}-dependent and other K^+ channels, not only failed to block the effect of cromakalim but actually produced a moderate enhancement of the cromakalim-activated K^+ current. Neither glyburide nor charybdotoxin affected resting or voltage-activated K^+ currents in the absence of cromakalim. Exposure of the cells to energy-depleting conditions (0.24 μg/ml oligomycin and 10 mM 2-deoxy-D-glucose) also activated an outward current. Single-channel recordings in the cell-attached configuration showed that cromakalim (100 μM) stimulated the opening of flickery single channels having a unitary conductance of ~26 pS and a prolonged burst duration (mean open time, ~131 msec); similar channel openings were observed in patches from cells exposed to energy-depleting conditions. In patches containing a single K^+ channel, the open probability in the presence of cromakalim was ~0.6 and in the presence of energy-depleting conditions was ~0.8; in the absence of either of these treatments, channel openings were not observed. Glyburide produced a reversible inhibition of the channels activated by cromakalim and energy-depleting conditions. These data provide additional support for the existence of ATP-sensitive K^+ channels in central neurons and indicate that the K^+ channels whose opening is stimulated by cromakalim are likely to be of the ATP-sensitive type.

Potassium channels that are inhibited by intracellular ATP were originally described in cardiac muscle by Noma (1) but have now been identified in a wide variety of peripheral tissues (2). Under normal physiological conditions, these ATP-sensitive K^+ channels are in the closed state because the intracellular ATP levels are higher than the submillimolar levels required to inhibit the channels, but their activity is dramatically increased when intracellular ATP levels are reduced by hypoxia or treatment with uncouplers of oxidative phosphorylation. In some cell types, such as pancreatic β-cells and cardiac myocytes, ATP-sensitive K^+ channels are specifically inhibited by sulfonylurea antidiabetic drugs such as glyburide (3). Further progress in understanding the pharmacological regulation of ATP-sensitive K^+ channels has come with the recognition that the channels in smooth muscle (4, 5), heart (6–8), and pancreatic islets (9) appear to be targets for potassium channel opener drugs, a novel class of chemically unrelated vasodilating agents that have the common property of enhancing membrane K^+ permeability (10).

Recently, it has become apparent that K^+ channel openers such as cromakalim (BRL 34915) can have centrally mediated behavioral actions (11), including anticonvulsant activity in a variety of animal seizure models (12–14), and that they have the ability to block epileptiform discharges in *in vitro* preparations (15, 16). Moreover, we have observed that cromakalim activates a sustained K^+ current in cultured embryonic rat hippocampal neurons that can be inhibited by high concentrations of the K^+ channel blocker tetraethylammonium (17).

A related line of investigation has provided evidence for the existence of ATP-sensitive K^+ channels in the central nervous system. Thus, using radioligand binding techniques it has been possible to specifically label and partially purify a high affinity sulfonylurea acceptor site in brain membranes (18–20). These binding sites have a heterogeneous distribution within the brain and are present at particularly high densities in certain brain regions, including some parts of the hippocampus (21, 22). Moreover, it has been observed that, whereas glyburide has little effect on the electrophysiological properties of hippocampal neurons under ordinary conditions, the drug can modify cellular responses to anoxia (21, 23, 24). Finally, studies of

ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; CTX, charybdotoxin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid.

$^{86}Rb^+$ efflux from brain slices (25, 26) and whole-cell (27) and single-channel recordings (28–30) have provided additional evidence for the existence of ATP-sensitive K^+ channels in brain neurons.

In the present study, we further investigated the cromakalim-activated current in hippocampal neurons, in an attempt to evaluate the hypothesis that the K^+ channels opened by cromakalim are of the ATP-sensitive type. In support of this idea, we observed that a current similar to the one stimulated by cromakalim could be activated by conditions that reduce the energy store of the cell. Moreover, we found that both cromakalim and energy-depleting conditions activate identical glyburide-sensitive unitary currents in cell-attached single-channel recordings, providing additional support for the hypothesis.

Materials and Methods

Cell culture. Neurons from the hippocampi of 17–19-day-old Sprague-Dawley rat embryos were grown in primary culture, as previously described by Segal (31). We used a culture medium consisting of modified minimal essential medium (Advanced Biotechnologies) supplemented with 10% horse serum, 10% fetal calf serum, and 1% glutamine (all from GIBCO). The cells were plated onto 35-mm polyornithine-coated polystyrene dishes (Falcon), which were incubated at 37° in a humidified atmosphere containing 10% CO_2. Fresh medium was added every 7 days. Cultures were used 7–15 days after plating. Electrophysiological recordings were performed at room temperature (22–24°) on the stage of an inverted phase contrast microscope. We generally selected the smaller (~12-μm diameter) cells with smooth surfaces, because these appeared to allow higher resistance patch-electrode seals.

Whole-cell voltage-clamp recording. Immediately before each experiment, the tissue culture medium was aspirated and replaced with a buffered salt solution containing (in mM): NaCl, 140; KCl, 5; $CaCl_2$, 5; $MgCl_2$, 1; and HEPES, 10. The bath solution was adjusted to an osmolality of 315–320 mOsm/kg of H_2O with sucrose and to a pH of 7.40 with NaOH. Patch-clamp recording electrodes were prepared from filament-fused, borosilicate glass capillaries (1.5-mm o.d., TW150F-4; WPI Instruments). The electrodes were filled with a solution containing (in mM): potassium acetate, 150; $MgCl_2$, 1; EGTA, 1.1; and HEPES, 10. The electrode solution was adjusted to an osmolality of 305–310 mOsm/kg of H_2O with sucrose and to a pH 7.40 with KOH. Electrode tip resistances were 6.5–10 MΩ. Whole-cell currents were recorded with an Axopatch 1B patch-clamp amplifier, using the 500-MΩ headstage feedback resistor (Axon Instruments, Burlingame, CA). Voltages corresponding to the membrane currents were displayed on a high-speed ink pen recorder with a direct current input stage (Gould Electronics, Cleveland, OH). Reported membrane potentials are uncorrected for liquid-junction potentials.

Whole-cell recordings were begun in current-clamp mode, which allowed assessment of the resting potential. Only cells with resting potentials more negative than −40 mV and with input resistances under voltage clamp of >500 MΩ were utilized. In addition, cells were excluded if, at any time during the experiment, the resting current at −60 mV drifted more than 20 pA or if, before drug application, the evoked current fluctuated more than 10%. In a typical voltage-clamp experiment, the cells were subjected to 500-msec depolarizing voltage steps from a holding potential of −60 mV.

Single-channel recording. Unitary current recordings were carried out in the cell-attached configuration, using the 50-GΩ headstage of the Axopatch amplifier. Electrodes were coated with silicone elastomer (Sylgard 184) but were not fire polished. Electrode resistances were 4–8 MΩ. Recordings were made by touching the electrode to the cell membrane under visual control and applying gentle suction to the electrode to form a seal with resistance of >10 GΩ. The bath and patch electrode usually contained (in mM): NaCl, 140; KCl, 5; $CaCl_2$, 5; $MgCl_2$, 1; and HEPES, 10. Tetrodotoxin (2 μM) (Sigma Chemical Co., St. Louis, MO) was added to block Na^+ channel currents. The solution was adjusted to an osmolality of 315–320 mOsm/kg of H_2O with sucrose and to a pH of 7.40 with NaOH. In some experiments, the KCl concentration in the patch electrode solution was raised to 25 mM, in which case there was a corresponding reduction in NaCl. Membrane currents were filtered at 1 kHz (−3 dB, four-pole Bessel filter), digitally sampled at 10 kHz using the Axotape data acquisition system (Axon Instruments), and stored on magnetic media for later analysis. Data were collected in epochs of 10–20 sec. Several epochs were acquired during the control period before drug application. Single-channel current amplitude and open time (burst duration) determinations were made from hard-copy printouts of the single-channel current records. Only records containing the openings of a single channel (i.e., no superimposed channel openings) were used for analysis. In the determination of burst durations, closings of <2 msec in duration were ignored. The channel amplitudes reported are the arithmetic means of the amplitudes of all openings in the epoch. Open time probability (P_o) was estimated by dividing the total channel open time by the time of the recording epoch.

Drug application. Drug solutions were applied to the cell surface with blunt micropipettes (tip diameter, 1–2 μm), using a pneumatic pressure ejection system (Medical Systems, Greenvale, NY). The tip of the pressure pipette was situated 150–200 μm from the cell membrane (20 μm in single-channel recording experiments), and the drug solution was expelled by applying a back pressure of ~0.5 psi. Drug concentrations given are the nominal concentrations in the pressure pipette.

Stock solutions of cromakalim (50 mM), glyburide (3 mM), and oligomycin (0.24 mg/ml) were prepared in 70% (v/v) ethanol. Final dilutions to the appropriate concentrations were made in bathing solution. Previous experiments have indicated that the diluted ethanol vehicle is inactive (17). Cromakalim solutions were protected from light with aluminum foil. CTX and 2-deoxy-D-glucose were diluted to the final concentration in bathing solution.

Cromakalim [(±)-6-cyano-3,4-dihydro-2,2-dimethyl-(*trans*)-4-(2-oxo-1-pyrrolidyl)-2*H*-benzo[*b*]pyran-3-ol] was obtained from Beecham Pharmaceuticals (Harlow, Essex, UK). Glyburide was from Hoechst-Roussel Pharmaceuticals (Somerville, NJ), CTX was from Latoxan (Rosans, France), and oligomycin (mixture of oligomycins A, B, and C) and 2-deoxy-D-glucose were from Sigma.

Results

Cromakalim-activated whole-cell current. In cultured hippocampal neurons, depolarizing voltage steps from a holding potential of −60 mV to potentials more positive than −20 mV typically elicited a fast transient inward current, followed by a small rapidly inactivating (transient) outward current component. After the decay of the transient outward current, a slowly activating, minimally inactivating (sustained), outward current was apparent. With repeated activation of the currents under the conditions used in these experiments, the transient current component usually diminished in amplitude, whereas the sustained current did not show such slow inactivation (i.e., the sustained current fluctuated by <6%; four cells). As previously reported (17), superfusion with cromakalim (100 μM) caused a ~60% enhancement in the amplitude of the sustained outward current (measured at the end of 500-msec depolarizing voltage steps) (Fig. 1A). The outward current activated by cromakalim showed a modest voltage dependency, as illustrated in Fig. 2, which plots the mean conductance, G, of the current activated by cromakalim in four cells at various potentials. G was calculated according to the formula $(I_{cromakalim} - I_{control})/(V - V_r)$, where $I_{control}$ and $I_{cromakalim}$ are the relative current amplitudes (at the end of 500-msec steps from −60 mV) before and during

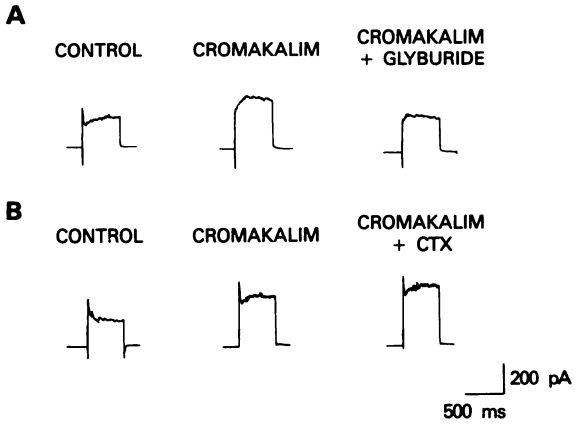

Fig. 1. Effects of glyburide and CTX on cromakalim-activated outward currents in cultured rat hippocampal neurons. Current records were obtained with 500-msec voltage steps from −60 to 0 mV. A, Superfusion with cromakalim (100 μM) causes an enhancement of the outward current, which is blocked by glyburide (100 μM) applied with a second superfusion pipette. Cromakalim was applied continuously after the acquisition of the control record; the record shown was obtained 10 min after the onset of the drug superfusion. The cromakalim plus glyburide (*right*) trace was obtained 10 min after the start of the glyburide superfusion and indicates the maximal blockade produced by the drug. The decrement in the early (transient) outward current (I_A) is unlikely to be a specific effect of cromakalim, because such "run-down" was also frequently observed in control neurons. B, In another neuron, CTX (100 nM) causes a modest augmentation of the cromakalim-enhanced outward current response.

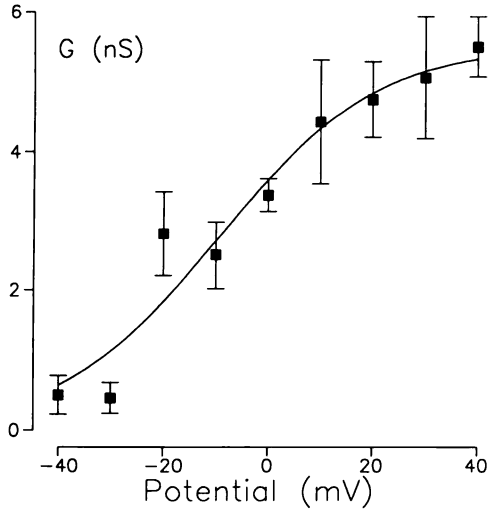

Fig. 2. Voltage dependence of the cromakalim-activated outward current. Outward currents were elicited with 500-msec voltage steps from −60 mV to the step potentials indicated on the *abscissa*, before and during the application of 500 μM cromakalim. The conductance, *G*, of the cromakalim-activated current was calculated according to the formula given in the text. For these calculations, the cromakalim-activated current was taken to be the difference in the net outward current at the end of the cromakalim and control steps. The *solid curve* was fit according to the equation $G = G_{max}\{1 + \exp[(V_{1/2} - V)/A]\}^{-1}$, where G_{max} = 5.5 nS, $V_{1/2}$ = −9.3 mV, and A = 15 mV.

the cromakalim superfusion, respectively, V is the membrane potential, and V_r is the experimentally determined K^+ reversal potential (−60 mV; see Ref. 32; evidence that the cromakalim-activated outward current is carried by K^+-selective channels is given below).

Effects of glyburide and CTX. Under control conditions, the sulfonylurea glyburide in concentrations as high as 50 μM failed to alter the resting current at −60 mV and did not affect the evoked current at any potential (five cells). However, as illustrated in Fig. 1A, glyburide substantially reduced the total sustained outward current recorded in the presence of cromakalim (similar results were obtained in a total of 20 cells). The inhibitory effect of glyburide began after a lag period of 1–2 min, and maximum block was not obtained until approximately 8–10 min after the onset of the drug superfusion (Fig. 3A). Note that, in the absence of glyburide, the amplitude of the cromakalim-activated current remains constant during this time (Fig. 3A). As shown in Fig. 3B, which summarizes data from 15 cells, glyburide (1–25 μM) produced a concentration-dependent inhibition of the cromakalim-activated current. With the highest

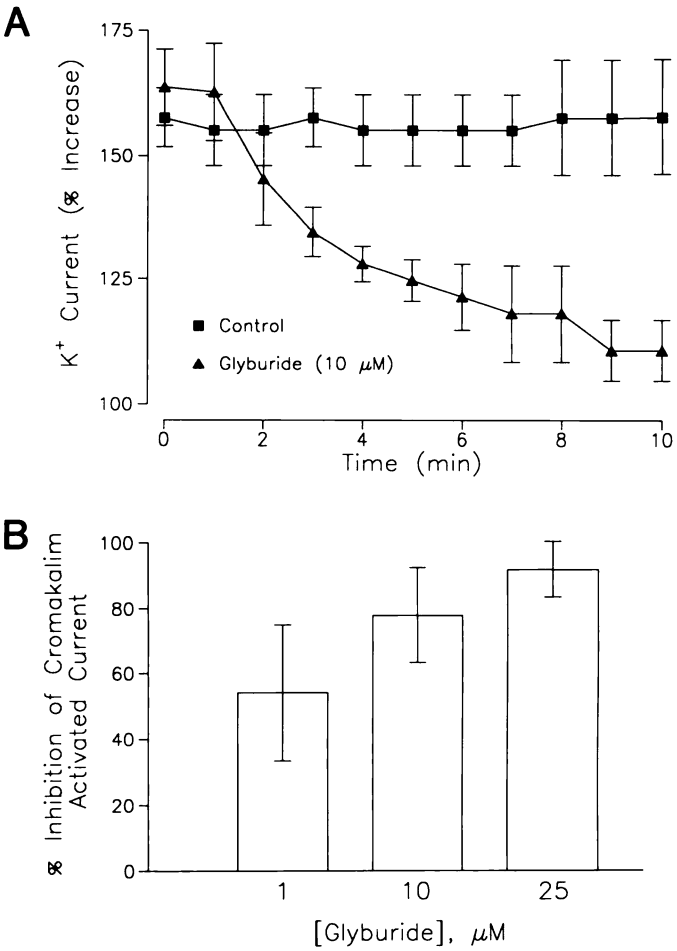

Fig. 3. A, Time course of glyburide blockade of cromakalim-activated outward current. Outward currents were elicited with 500-msec voltage steps from −60 to 0 mV at 1-min intervals. Cells were exposed to cromakalim (100 μM) until the increase in the outward current response (measured at the end of the 500-msec step) stabilized at a maximum of ~160% of the control response (10–13 min). The glyburide (10 μM) superfusion was begun at time 0 via a second pipette, while the cromakalim application was continued. The mean percentage increase (four cells) in the outward current (with respect to the precromakalim current level) is shown at each time point after the onset of the glyburide superfusion (▲). The inhibitory effect of glyburide is contrasted with the stability of the cromakalim-activated outward current in three control cells (■). (In the latter experiments, the cromakalim superfusion was terminated at time 0.) B, Concentration dependence of glyburide blockade of cromakalim-activated outward current. *Bars*, percentage of inhibition (mean ± standard error) of the maximum cromakalim-stimulated outward current obtained 10 min after the onset of a simultaneous glyburide superfusion.

concentration of glyburide, there was nearly complete suppression of the current. We were unable to detect any recovery from the glyburide inhibition of the cromakalim-activated current during a washout period of 3–6 min (four cells).

CTX (100 nM), an antagonist of some Ca^{2+}-dependent and other K^+ channels (33–36), failed to reduce the outward current facilitated by cromakalim. Instead, in three of four cells tested, CTX caused a 20–50% increase in the outward current during cromakalim administration (Fig. 1B). In four cells, CTX (100 nM) applied for up to 6 min did not alter the outward current in the absence of cromakalim and, in two cells, had no effect on the outward current facilitated by oligomycin/2-deoxy-D-glucose (see below).

Effects of oligomycin and 2-deoxy-D-glucose. Oligomycin, an inhibitor of oxidative phosphorylation, either alone or in combination with 2-deoxy-D-glucose, an inhibitor of glycolysis, caused a facilitation of the outward current similar to that produced by cromakalim. The effect of oligomycin alone (0.48 μg/ml) began with a lag period of 1–4 min after the onset of the superfusion, and the maximal response occurred after a delay of 10–15 min (56–88% increase in current at 0 mV, measured at the end of a 500-msec step from −60 mV; three cells). A combination of a lower concentration of oligomycin (0.24 μg/ml) and 2-deoxy-D-glucose (10 mM) produced an increase in the outward current of similar magnitude and time course (27–50%; three cells) (Fig. 4).

Single-channel currents activated by cromakalim and oligomycin/2-deoxy-D-glucose. Single-channel recordings in the cell-attached configuration demonstrated that the continuous application of cromakalim (100 μM) induced the opening of flickery channels with a long burst duration (n = 30). Such channels were observed in roughly 65% of the patches. Within the voltage range of −60 to −100 (pipette potential), the mean burst duration was 131 ± 19 msec (mean ± standard error; n = 7), and there was a trend toward longer burst durations at more depolarized potentials. Channel openings with these characteristics were not observed in the absence of cromakalim (Fig. 5). Superfusion with oligomycin (0.24 μg/ml) and 2-deoxy-D-glucose (10 mM) also stimulated channel currents that were similar to those induced by cromakalim (Fig. 6). Single-channel conductances of the channels activated by the two treatments were determined from the slopes of straight line fits to plots of the mean single-channel amplitude versus pipette potential. The conductances determined in six patches from cells exposed to cromakalim ranged from 16 to 34 pS, with a mean ± standard error of 26 ± 3 pS (n = 4). The single-channel conductances of the channels activated by oligomycin/2-deoxy-D-glucose ranged from 26 to 34 pS, with a mean ± standard error of 29 ± 1 pS. The difference between the means was not statistically significant (t test).

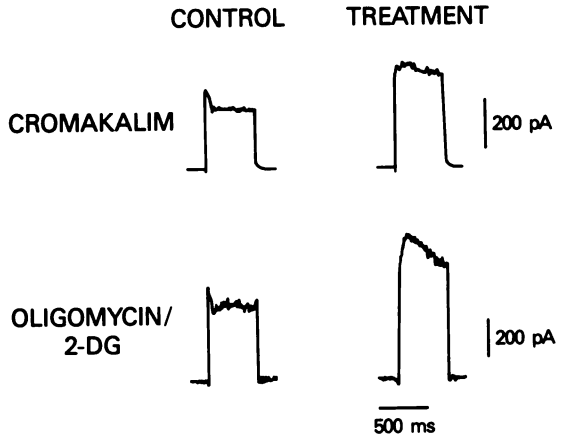

Fig. 4. Cromakalim and oligomycin/2-deoxy-D-glucose (*2-DG*) cause a similar increase in the outward current. Traces show current records obtained with 500-msec voltage steps from −60 mV to 0 mV. *Top*, recordings before and during superfusion with cromakalim (100 μM). *Bottom*, recordings in another cell, showing the effect of oligomycin (0.24 μg/ml)/2-deoxy-D-glucose (10 mM).

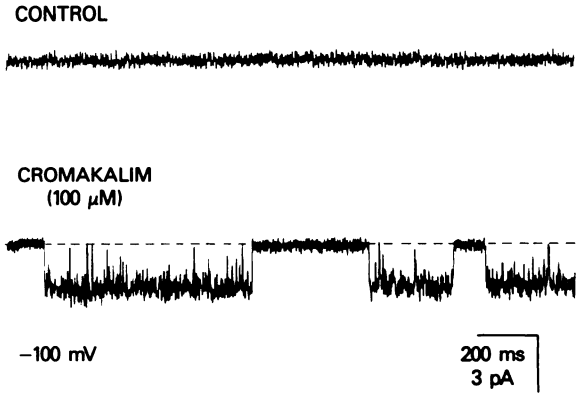

Fig. 5. Single-channel recordings in the cell-attached configuration before (control) and during superfusion of the cell with cromakalim (100 μM). In this Figure and in Fig. 6, the *dashed lines* indicate the zero current level. Outward currents are downward. The pipette potential was −100 mV.

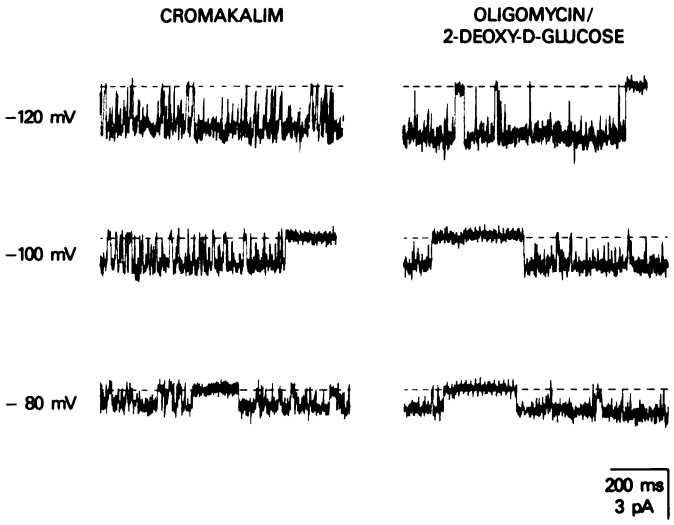

Fig. 6. Comparison of the single-channel currents activated by cromakalim and oligomycin/2-deoxy-D-glucose in two cell-attached recordings. The records shown were obtained 2–4 min after the onset of superfusion with either cromakalim (100 μM) or oligomycin (0.24 μg/ml)/2-deoxy-D-glucose (10 mM). The pipette potentials are indicated at the *left*.

K^+ selectivity of cromakalim-activated single-channel currents. The channels activated by cromakalim carried outward ionic current at all potentials depolarized from rest, suggesting that they are K^+ selective. To verify this, we carried out recordings in which the external K^+ concentration (in the patch electrode only) was raised to 25 mM. As illustrated in Fig. 7 (*top*), increasing the external K^+ in this manner resulted in a 36-mV, roughly parallel, shift in the single-channel current-voltage relationship. This value is close to the 41 mV predicted by the Nernst equation for a channel that is permeable only to

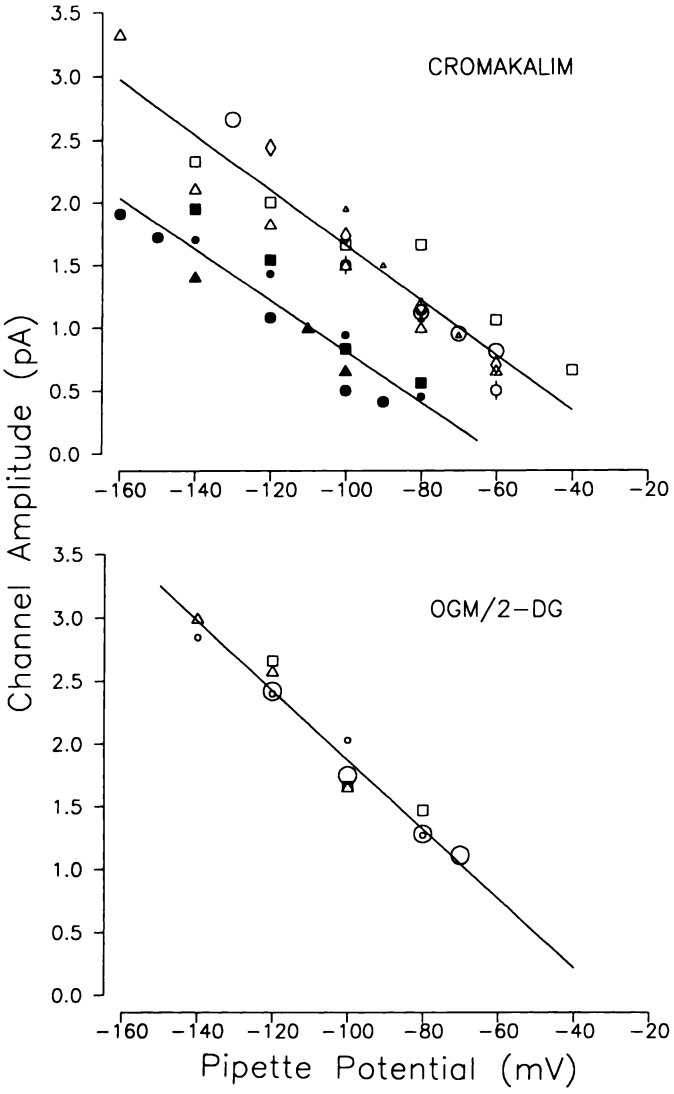

Fig. 7. Mean single-channel amplitude versus pipette potential in six patches (cell-attached recordings) from cells exposed to cromakalim (100 μM) and four patches from cells exposed to oligomycin/2-deoxy-D-glucose (*OGM/2-DG*) (10 mM). Each *symbol* indicates a different patch. *Top, open symbols*, experiments with 5 mM K^+ in the patch electrode; *closed symbols*, 25 mM K^+ in the patch electrode (in both cases, the bath K^+ concentration was 5 mM). The best-fit straight lines to the data are shown.

K^+. Assuming that the only ions in solution to which the channel is permeable are the monovalent cations K^+ and Na^+, we can use the Goldman-Hodgkin-Katz voltage equation to determine the permeability ratio P_K/P_{Na} from the observed reversal potential shift. This calculation indicates that the K^+ permeability of the channel is approximately 100-fold greater than its Na^+ permeability. Note that the extrapolated reversal potential values in Fig. 7 are near the cell resting potential (0 mV patch electrode potential). This may be explained by considering the fact that, in the presence of treatments that open K^+ channels, the membrane potential is likely to be "clamped" to the K^+ equilibrium potential. (According to constant-field theory, the single-channel current-voltage relationship shows outward rectification, and this presumably accounts for the fact that the linearly extrapolated reversal potential intersects the voltage axis at a somewhat more negative pipette potential value than expected.)

Glyburide block of the single-channel currents. In order to identify the type of K^+ channel activated by cromakalim and oligomycin/2-deoxy-D-glucose in the cell-attached patch recordings, we studied the effects of the sulfonylurea glyburide, which in the first part of this study was found to inhibit the whole-cell currents activated by these treatments. It is believed that sulfonylureas gain access to their blocking site on (or associated with) ATP-sensitive K^+ channels by partitioning into the lipid plasma membrane (37). Consequently, it seemed appropriate to utilize the drug in cell-attached patch recordings, even though direct access of the drug to the external aqueous environment of the channel under study would be restricted by the patch electrode. Fig. 8 summarizes results for four patches from cells exposed to cromakalim and three patches from cells exposed to oligomycin/2-deoxy-D-glucose. As expected, both treatments markedly enhanced the open channel probability (P_o), although the mean P_o value for patches from cells exposed to oligomycin/2-deoxy-D-glucose (0.81) was somewhat greater than that of patches from cells exposed to cromakalim (0.60) (difference not statistically significant). In both cases, glyburide completely inhibited channel activity. The blocking effect of glyburide occurred slowly (requiring ~1 min), as is consistent with the requirement for the drug to diffuse into the membrane

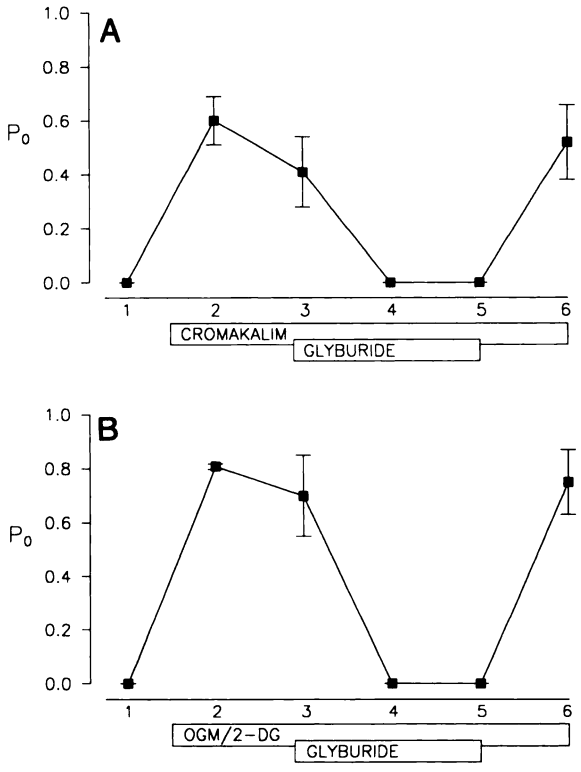

Fig. 8. Glyburide suppresses the opening of both cromakalim- and oligomycin/2-deoxy-D-glucose (*OGM/2-DG*)-activated single-channel currents. *Ordinate*, mean single-channel open probability (P_o), calculated as the total time the channel remained in the open state divided by the time of the recording epoch (each *point* represents an epoch of 20–40 sec). All data were from recordings in the cell-attached configuration, containing only a single active channel, and are expressed as the mean ± standard error of data from four cells in A and three cells in B. Epochs are numbered according to the following scheme: *1*, control; *2*, 60–240 sec after onset of cromakalim (100 μM) or oligomycin (0.24 μg/ml)/2-deoxy-D-glucose (10 mM); *3*, *4*, and *5*, 20, 60, and 120 sec, respectively, after onset of glyburide (10 μM); *6*, 20 sec after termination of glyburide. In all experiments, the pipette potential was −100 mV.

to reach its site of action. The inhibition was reversed upon discontinuation of the glyburide superfusion.

Discussion

In our previous study (17), we demonstrated that cromakalim can activate a K^+ current in cultured hippocampal neurons. We now provide evidence that the K^+ current is carried by ATP-sensitive K^+ channels. Our main results can be summarized as follows: (i) glyburide blocks the cromakalim-activated current, (ii) energy depletion activates an outward current similar to that generated by cromakalim, (iii) both cromakalim and energy-depleting conditions induce the opening of a 25–30-pS K^+ channel in cell-attached patch recordings, and (iv) the channels opened by either cromakalim or energy-depleting conditions are reversibly blocked by glyburide. Because glyburide is a selective antagonist of ATP-sensitive K^+ channels (2, 38, 39), our results suggest that the channels activated by cromakalim are of the ATP-sensitive type. Moreover, this conclusion is supported by the apparent identity of these channels and those opened by energy-depleting conditions. Obviously, however, definitive proof will require demonstration that the channel is in fact ATP sensitive, either by intracellular perfusion or in excised patch recordings.

In recent years, a great deal of indirect evidence for the existence of ATP-sensitive K^+ channels in brain has been provided (see introduction). However, there are only a few studies in which the activity of these channels has been recorded directly in brain neurons (28–30). In these cases, the channels were present in low density, and this may be attributed to the fact that the brain regions studied (neocortex and hypothalamus) fail to have substantial numbers of [^{3}H]glyburide binding sites (21). Moreover, in these previous studies, the ATP-sensitive channels had unusually high unitary conductances, compared with the conductances of ATP-sensitive channels in peripheral tissues, and did not show the inward rectification that is typical of these channels (2). On the other hand, the hippocampal formation exhibits a high density of [^{3}H] glyburide binding sites, and the presumed ATP-sensitive K^+ channels recorded in the present study have characteristics that are more or less similar to those obtained in single-channel recordings from cardiac muscle, skeletal muscle, and pancreatic β-cells. For example, the unitary conductance value of 25–30 pS obtained in the present study is similar to that reported for ATP-sensitive K^+ channels in cardiac muscle and pancreatic β-cells (under conditions of physiological $[K^+]_o = 5$ mM). Moreover, our preliminary determination of the permeability ratio P_K/P_{Na} (=100) approximates that observed for ATP-sensitive K^+ channels in skeletal muscle (for external high Na^+) (40). Based upon the whole-cell conductance measurements presented in Fig. 2, we estimate that cromakalim activates roughly 200 ATP-sensitive K^+ channels/cell.

The question of whether the open state probability of the cromakalim-activated K^+ channel in hippocampal neurons is voltage dependent is an important one, for which a satisfactory answer is only partially available at this time. As shown in Fig. 2, the whole-cell conductance activated by cromakalim does show a gradual increase in the voltage range of −40 to +40 mV. On the other hand, within the voltage range studied, we did not detect a strong voltage dependence to the channel open state probability (data not shown). In cell-attached recordings, it was only possible to detect channel openings at strongly negative pipette potentials (i.e., at relatively depolarized transmembrane potentials), presumably because channel amplitudes at potentials closer to the K^+ equilibrium potential are too small to be discriminated from the baseline noise. However, as illustrated in Fig. 2, the whole-cell conductance saturates at 0 to +10 mV and shows little increase at more positive potentials, yet it is just at these positive potentials that our single-channel measurements were made. Consequently, our data are not inconsistent with the possibility that gating of the cromakalim-activated K^+ channel is voltage dependent, as is the case for ATP-sensitive K^+ channels in pancreatic islets (41) and skeletal muscle (40, 42). A definitive answer, however, awaits recordings in excised membrane patches.

Such recordings may also help to clarify whether cromakalim acts on the K^+ channel directly or on another cellular target to indirectly influence channel opening. Although our present data do not allow us to definitively exclude the possibility that cromakalim activates K^+ channels in hippocampal neurons via an indirect mechanism (or even that it trivially acts to reduce intracellular ATP levels), there are now several reports showing that cromakalim is active in excised patches (5, 43, 44), indicating that the drug target is the channel itself or a closely associated molecule.

The observation that the cromakalim-activated conductance is sensitive to glyburide forms the basis for our conclusion that the channels are of the ATP-sensitive type. Although perhaps not definitive, this inference is reasonable, because other K^+ channel types, including delayed rectifier K^+ channels, inward rectifier K^+ channels, and some Ca^{2+}-activated K^+ channels, are believed to be insensitive to sulfonylureas (Refs. 2, 38, and 39; however, see Ref. 45). As is the case in other cell types, glyburide was active when added to the bath solution in cell-attached recordings, indicating that the drug may reach its target site by dissolving in the lipid phase of the membrane (9, 37). The effective concentration of glyburide in blocking the cromakalim-activated K^+ current (EC_{50}, ~1 μM) (Fig. 3B) is comparable to the concentration of the drug that has previously been reported to antagonize the various effects of cromakalim in smooth muscle and cardiac tissue (6, 46, 47) and also to inhibit the cromakalim-induced increase in $^{86}Rb^+$ release from brain slices (26). As in these prior studies (see also Ref. 48), the effect of glyburide on the cromakalim-activated K^+ current in hippocampal neurons is achieved slowly, i.e., with a lag period of several minutes. It is interesting to contrast the slow time course of the glyburide effect with that of tetraethylammonium, which virtually instantaneously blocks the channel (17), presumably by an open channel mechanism in which access to the blocking site occurs via a hydrophilic pathway (49). The glyburide blockade of cromakalim-activated single-channel currents in cell-attached patch recordings was readily reversible, whereas it was difficult to demonstrate such reversibility in whole-cell recordings. These and other differences between the whole-cell and single-channel recordings may perhaps be explained by the fact that there is dilution of intracellular components during the whole-cell recording experiments, whereas this is not the case in the cell-attached single-channel recordings.

Although most recent investigations have concluded that cromakalim activates ATP-sensitive (Ca^{2+}-insensitive) K^+ channels, there are a few reports indicating that the K^+ channels opened by cromakalim are large conductance (BK-type)

Ca^{2+}-dependent K^+ channels (50–53). Consequently, we investigated whether CTX, an antagonist of some BK-type Ca^{2+}-dependent K^+ channels, could block the cromakalim-activated K^+ current in hippocampal neurons. Surprisingly, however, CTX not only failed to inhibit the cromakalim-activated K^+ current but actually produced a small enhancement of the current. Although the basis of this effect is unclear, it is interesting to note that Winquist *et al.* (46) have observed that CTX can produce a small augmentation of the relaxation response to cromakalim in vascular smooth muscle. In any case, the failure of CTX to antagonize the effect of cromakalim and the fact that the cromakalim-activated single-channel currents have a smaller unitary conductance suggest that BK-type Ca^{2+}-activated K^+ channels may not carry the cromakalim-activated outward current in hippocampal neurons.

In conclusion, our results support the existence of ATP-sensitive K^+ channels in rat hippocampal neurons, whose characteristics are similar to those previously studied in detail in peripheral tissues (pancreatic islets and muscle). The ability to pharmacologically regulate the activity of these channels with drugs such as cromakalim and glyburide should provide a basis for clarifying their functional role in central neurons.

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