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SULPHONYLUREAS REDUCE THE SLOWLY INACTIVATING D-TYPE OUTWARD CURRENT IN RAT HIPPOCAMPAL NEURONS

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SUMMARY

1. Using intracellular recording in hippocampal slices, we have examined, in CA3 pyramidal neurons, the effects of sulphonylureas (blockers of ATP-sensitive K^+ channels) on the slowly inactivating D-type K^+ current (I_D).

2. In the presence of TTX (1 μM) to block Na^+ currents, I_D had the following characteristics: activation by large depolarizing pulses from membrane potentials negative to -75 mV, slow inactivation kinetics, high sensitivity to 4-aminopyridine (4-AP, 3–40 μM), insensitivity to tetraethylammonium (TEA, 10 mM), Cs^+ (3 mM) and carbachol (50 μM).

3. Applications of glibenclamide (10 μM) did not modify the input conductance of the cell, but reduced the amplitude of I_D by $31{\cdot}2 \pm 5{\cdot}6\%$ ($n = 16$), without altering its voltage dependence and inactivation kinetics. The effects were usually reversible.

4. Glibenclamide also reduced I_D in the presence of TEA (10 mM), Cs^+ (3 mM) and carbachol (50 μM), to block several K^+ currents (I_K, I_C, I_Q, I_M), as well as kynurenate (1 mM) and bicuculline (10 μM) to block on-going synaptic currents mediated by activation of non-NMDA (*N*-methyl-D-aspartate) and GABA (γ-aminobutyrate)-A receptors, respectively.

5. Comparable depressions of I_D were produced by two other sulphonylureas: gliquidone (10 μM), $42{\cdot}6 \pm 7{\cdot}9\%$ ($n = 13$) and tolbutamide (500 μM), $39{\cdot}1 \pm 12{\cdot}8$ ($n = 8$).

6. It is concluded that, in the central nervous system, sulphonylureas can modulate K^+ currents which are not generated by ATP-sensitive K^+ channels.

INTRODUCTION

Sulphonylureas are hypoglycaemic agents that are widely used in the treatment of diabetes mellitus (Loubatières, 1977). Studies in pancreatic β-cells and various insulinoma cell lines have shown that sulphonylureas act principally by reducing K^+ permeability (Henquin & Meissner, 1982; Ferrer, Atwater, Omer, Goncalves, Croghan & Rojas, 1984), leading to a depolarization, the activation of voltage-dependent Ca^{2+} channels, a rise in intracellular Ca^{2+} and the release of insulin

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(Henquin & Meissner, 1984; Howell, 1984). Patch-clamp studies suggest that the target of sulphonylureas is a special class of K^+ channels (K_{ATP} channels) that are sensitive to intracellular ATP concentration $[K_{ATP}]_i$ (Ashcroft, 1988). First described in cardiac cells, these channels are blocked in normoxic–normoglycaemic conditions by high $[ATP]_i$ and activated when $[ATP]_i$ is decreased (Noma, 1983). In pancreatic β-cells, the rank order of inhibition of Rb^+ efflux and K_{ATP} channel activity by sulphonylureas shows a good correlation with sulphonylurea binding assays (i.e. glibenclamide > gliquidone $\gg$ tolbutamide), suggesting that the sulphonylurea binding site may be closely linked to K_{ATP} channels (Schmid-Antomarchi, De Weille, Fosset & Lazdunski, 1987*a*, *b*).

Sulphonylurea binding sites have also been described, in the central nervous system (CNS); glibenclamide binding sites are notably concentrated in the substantia nigra, cortex and CA3 region of the hippocampus (Mourre, Ben-Ari, Bernardi, Fosset & Lazdunski, 1989; Zini, Tremblay, Roisin & Ben-Ari, 1991*a*; Treherne & Ashford, 1991; Jiang, Xia & Haddad, 1992). As in peripheral tissues (Verspohl, Ammon & Mark, 1990; French, Linda, Mullins & Sarmiento, 1991; Gopalakrishnan, Johnson, Janis & Triggle, 1991; Zini, Ben-Ari & Ashford, 1991*b*), high and a low affinity binding sites have been found in several brain structures, with dissociation constants in the nanomolar and micromolar range, respectively (Gopalakrishnan *et al.* 1991; Zini *et al.* 1991*a*). Autoradiographic studies suggest that in the hippocampus the high affinity binding sites are located mainly presynaptically, on the mossy fibre terminals (Tremblay, Zini & Ben-Ari, 1991). Patch-clamp experiments have shown K_{ATP} channels in hippocampal, cortical and hypothalamic neurons (Trussel & Jackson, 1987; Ashford, Sturgess, Trout, Gardner & Hales, 1988; Ashford, Boden & Treherne, 1990; Politi & Rogawski, 1991). Intracellular studies in slices have also shown that sulfonylureas have important effects on neuronal excitability in normal and pathological conditions, including anoxia and epilepsy (Alzheimer & Bruggencate, 1988; Ben-Ari, 1989, 1990; Grigg & Anderson, 1989; Amoroso, Schmid-Antomarchi, Fosset & Lazdunski, 1990; Murphy & Greenfield, 1992; Jiang *et al.* 1992; Luhmann & Heinemann, 1992).

In all these previous studies, it has been assumed that in the CNS (as in pancreatic β-cells), sulfonylureas are selective markers of K_{ATP} channels and that the action of sulphonylureas is in fact mediated directly by K_{ATP} channels. This, however, has not been tested. CNS neurons possess a wide range of K^+ channels, which provide a fine regulation of neuronal discharge (Halliwell, 1990; Storm, 1990) and it is therefore important to examine the effects of sulfonylureas on these currents. We now report a depression by sulphonylureas of the slowly inactivating K^+ current, called D-current or I_D, which plays a significant role in the temporal integration of signals in pyramidal neurons (Storm, 1988). Since the properties of I_D (Storm, 1990) considerably differ from those of the ATP-sensitive potassium current ($I_{K(ATP)}$) (Rorsman & Trube, 1990; see Discussion), our observations suggest that sulphonylureas may negatively modulate different K^+ channels, and are thus not selective blockers of K_{ATP} channels. These results have been reported in a preliminary form (Crépel, Krnjević & Ben-Ari, 1992).

METHODS

Adult male Wistar rats were anaesthetized with ether and their brains rapidly removed. Hippocampal slices were cut with a McIlwain tissue chopper and kept in oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (ACSF) at room temperature. The ACSF had the following composition (mM): NaCl, 126; KCl, 3·5; $CaCl_2$, 2·0; $MgCl_2$, 1·3; NaH_2PO_4, 1·2; $NaHCO_3$, 25; and glucose, 11 (pH 7·3 at 34 °C). Individual slices were transferred to a recording chamber where they were fully submerged. The temperature was kept at 33±0·5 °C and the flow rate at 2–3 ml min^{-1}.

Data recording and analysis

CA3 pyramidal neurones were penetrated with 3 M KCl microelectrodes (50–80 MΩ). Current was injected through the recording electrode: bridge balance was checked repeatedly during the experiments. Capacitative transients were reduced to a minimum by negative capacity compensation. Membrane potential was estimated from the potential observed upon withdrawal of the electrode from the cell. Single-electrode voltage clamping (SEVC) was done with an Axoclamp 2 amplifier (Axon Instruments, Inc., Burlingame, CA, USA) operating in the discontinuous mode, usually at a frequency of 3–5 kHz, a gain of 25 nA mV^{-1} and the bandwidth upper limit set at 0·3–1 kHz. The voltage signal at the head stage amplifier was continuously monitored on a separate oscilloscope to ensure optimal voltage clamping. Responses were digitized and displayed on a Nicolet digital oscilloscope and a computer-driven chart recorder. Data are presented as means (±S.E.M.) and statistical significance was assessed using variance analysis (ANOVA test). The differences were considered significant when $P < 0·05$.

Solutions

For current-clamp recording, ACSF was the control solution. For single voltage-clamp experiments, three control solutions were used: (i) ACSF containing 1 μM; (ii) ACSF containing 1 μM TTX, 1 mM kynurenate and 10 μM bicuculline; (iii) ACSF containing 1 μM TTX, 1 mM kynurenate, 10 μM bicuculline, 10 mM TEA, 3 mM Cs^+ and 50 μM carbachol (CCh). The various drugs, dissolved at final concentration in ACSF, were applied through a three-way tap system. Sulfonylureas were prepared from dimethylsulphoxide (DMSO) stock solutions (10 mM glibenclamide, 10 mM gliquidinone and 0·5 mM tolbutamide). There was a delay of 20–30 s before the arrival of the new solution, at the slice. The high ratio of flow rate-to-bath volume ensured complete exchange within 1 min. The drugs used were purchased from Sigma (La Verpilliere, France; TTX, TEA, carbachol, Cs^+ and tolbutamide) or Tocris (Bristol; kynurenate and bicuculline); glibenclamide and gliquidone were gifts from Dr M. Ashford (Department of Pharmacology, University of Cambridge).

RESULTS

Stable, long-lasting intracellular recordings were made from thirty-four CA3 pyramidal neurons with action potentials greater than 80 mV and a mean input resistance of 55·5±1·1 MΩ. In voltage-clamp experiments, TTX (1 μM) was applied continuously to block the voltage-sensitive Na^+ currents and evoked synaptic activities.

Characteristics of I_D in CA3 neurons

In the presence of TTX (1 μM), hyperpolarizing pulses from a holding potential (V_h) near −50 mV (Fig. 1*A*) or depolarizing pulses from a V_h near −100 mV (Fig. 1*B*) induced a transient outward current. According to previous studies, the outward current consisted of two components: a fast inactivating I_A and a slow inactivating I_D (Storm, 1988). Because of its fast inactivation, I_A becomes vanishingly small within 50 ms (Gustafsson, Galvan, Grafe & Wigström, 1982; Storm, 1988). The slow

frequency response of our high-resistance microelectrodes, especially when inserted into submerged slices, precluded any accurate measurements of I_A (although an approximated estimated could be obtained graphically, see below). I_D is, however, a slowly inactivating current (time constant between 2 and 3 s) which is readily

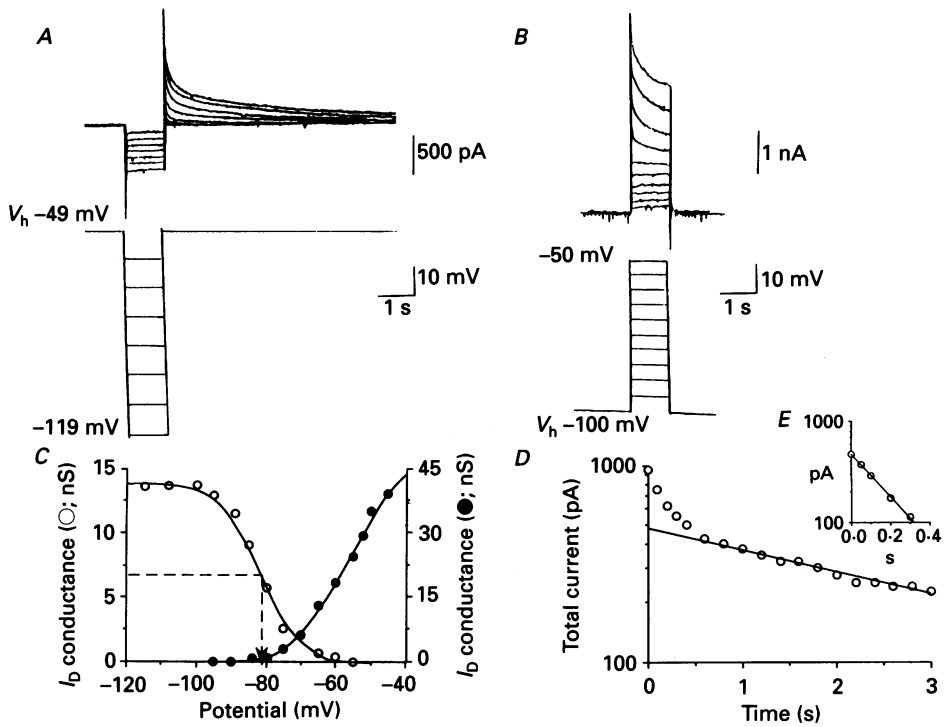

Fig. 1. Activation and inactivation characteristics of I_D, in CA3 hippocampal neurons. *A* and *B*, superimposed traces show outward currents: in *A* evoked by 1 s hyperpolarizing pulses (incremented in steps of 10 mV to a minimum of −119 mV, from a holding potential (V_h) of −49 mV), and in *B*, by 1 s depolarizing pulses (incremented in steps of 5 mV, to a maximum of −50 mV, from a V_h of −100 mV); the voltage steps (mV) are indicated below the traces. *C*, plots of activation (●) and inactivation (○) of I_D (measured 200 ms after the pulse onset or end) from the same data shown in *B* and *A*, respectively. The activation plot shows that the threshold for activation of I_D was near −80 mV and I_D rose steeply above −60 mV. The inactivation plot shows that I_D began to inactivate at potentials positive to −100 mV and was completely inactivated near −55 mV. The potential where I_D was half-inactivated was at −82 mV (arrow). *D*, semilogarithmic plot of the outward current evoked by a hyperpolarizing pulse to −109 mV (V_h −49 mV) revealed a fast and a slow component: the slow component was well fitted (straight line) by the equation: $Y = 474\exp(-t/3)$ (correlation coefficient, r = 0·96). *E*, the initial fast component, obtained by subtracting the slow component in *D* (extrapolated to its origin) from the total current, is shown on a faster time scale. This fast component was well fitted (straight line) by the equation: $Y = 475\exp(-t/0{\cdot}10)$ (r = 0·99).

clamped by the SEVC technique (Storm, 1988; McCormick, 1991; Neuman, Ben-Ari & Cherubini, 1991), as shown by voltage steps in Fig. 1*A* and *B*. We therefore routinely measured the amplitude of I_D, 200 ms after the end of the hyperpolarizing

pulses or the onset of the depolarizing pulses. When I_D was evoked by depolarizing pulses, the amplitude of the net outward current was calculated by assuming that under the experimental conditions the steady-state membrane conductance, associated with passive membrane properties, would be similar over the same range of membrane potential as outward currents were evoked. At each potential, net outward current was calculated as the difference between the leak current, that could be attributed to passive membrane properties and the total current generated. The leak current was obtained from a family of small depolarizing voltage steps (ranging between 5 and 25 mV) from a V_h near -100 mV (Fig. 1*B*) and was linearly extrapolated when voltage steps were larger than 25 mV.

In normal ACSF, we studied the voltage dependence of activation and inactivation of I_D by measuring its amplitude, in response to incremental hyperpolarizing or depolarizing pulses. Conductances of I_D (calculated on the basis of E_K (reversal potential for potassium) $= -90$ mV) or amplitudes of I_D were plotted as a function of corresponding hyperpolarizing pulses (inactivation plot, ○ in Fig. 1*C*) or depolarizing pulses (activation plot, ● in Fig. 1*C*). The voltage dependence of I_D in these CA3 neurons did not differ substantially from earlier observations in CA1 pyramidal cells (Storm, 1988). The activation threshold was near -75 mV and the current rose steeply above -65 mV. Because of the limitations of the SEVC technique, which prevented a complete characterization of the activation curve (as described previously by Storm, 1988 and McCormick, 1991), the potential where I_D is half-activated could not be determined. Inactivation appeared above -100 mV and was completed at -60 mV. The voltage for half-inactivation was $-81{\cdot}0 \pm 3{\cdot}7$ mV ($n = 15$).

As shown in Fig. 1*D*, the time course of inactivation of the total outward current evoked by hyperpolarizing pulses could not be fitted by a simple exponential function (as reported by Storm, 1990). Over the first 2–3 s, the total outward current (I_{tot}) could regularly be fitted by a double exponential function of the form: $I_{tot} = F\exp(-t/\tau_F) + S\exp(-t/\tau_S)$; where F and S are the maximum initial amplitudes and τ_F and τ_S the time constants of decay of the fast and slow components, respectively. The initial faster component, illustrated by the semilogarithmic plot in Fig. 1*E*, was obtained by subtracting from the total outward current, the slower component extrapolated to its origin. The time constant of decay for the slower component, τ_S, was $1{\cdot}6 \pm 0{\cdot}21$ s ($n = 19$), and for the faster component, τ_F, 150 ± 18 ms ($n = 19$). These results are consistent with previous descriptions (Storm, 1990), except that the initial phase was slower than would be expected for pure I_A (Gustafsson *et al.* 1982; Storm, 1988). Nevertheless, this extrapolated initial phase may be considered as an *approximate* index of I_A; as indicated by its lack of sensitivity to 30–40 μM 4-AP, which is known to selectively block I_D (Storm, 1988) and which, in the present experiments, always caused a major suppression of the slower component (Figs 2*B* and *D*, 3*C* and 5*A* and *C*).

Thus, bath applications of 30–40 μM 4-AP reduced the amplitude of the slower component by $64{\cdot}4 \pm 4{\cdot}7$% ($n = 13$, $P < 0{\cdot}001$), whereas the amplitude of the faster component became larger (by $64{\cdot}6 \pm 18{\cdot}9$%, $n = 5$, $P < 0{\cdot}03$) and its rate of decay accelerated (time constant down by $42{\cdot}4 \pm 5{\cdot}6$%, $n = 5$, $P < 0{\cdot}005$; Fig. 3*D*). In the presence of 4-AP, the mean value of τ_F was $100{\cdot}9 \pm 11$ ms ($n = 5$).

The apparent increase in the initial amplitude, F, can be explained as follows: normally an overlapping contribution of I_D slows down the initial rate of decay of the fast component; and extrapolation of the corresponding line to its origin reduces the apparent value of F. When most of the slowly decaying I_D is removed by

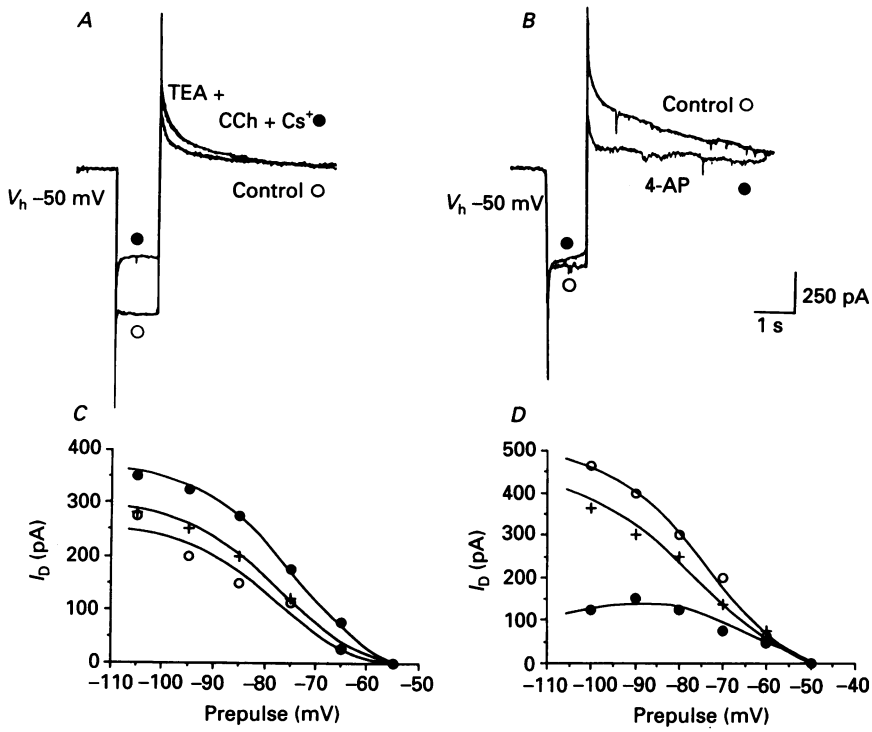

Fig. 2. Pharmacological properties of I_D. *A*, superimposed traces of transient outward current evoked by hyperpolarizing pulses (1 s duration) to −95 mV, from V_h −50 mV, in control ACSF (containing 1 mM kynurenate and 10 μM bicuculline) and during application of 10 mM TEA, 3 mM Cs^+, 50 μM carbachol (CCh) (together with 1 mM kynurenate and 10 μM bicuculline). Note that bath application of potassium blockers slightly increased the total outward current. *B*, superimposed traces of the outward current evoked as in *A*, in control ACSF and during bath application of 4-AP (40 μM), which clearly reduced the slowly inactivating outward current. *C*, voltage dependence of inactivation of the slowly inactivating outward current in control ACSF (containing 1 mM kynurenate and 10 μM bicuculline; ○), in the presence of 10 mM TEA, 3 mM Cs^+ and 50 μM CCh (together with 1 mM kynurenate and 10 μM bicuculline; ●) and after wash (+). As in control ACSF, inactivation was complete near −55 mV in the presence of TEA, Cs^+ and CCh. *D*, same type of curve as in *C*, in control ACSF (○), in the presence of 40 μM 4-AP (●) and after wash (+).

4-AP, the remaining fast component gives a better indication of the true τ_F, and extrapolation to the origin yields a higher value of F. In these experiments, applications of 4-AP (30–40 μM) did not significantly change the input conductance (2·1 ± 5·8 %, $n = 13$) or the holding current (−33·75 ± 82 pA, $n = 13$).

I_D was observed in the presence of a variety of K^+ channels blockers; we tested the

effects of TEA (10 mM), Cs^+ (3 mM) and carbachol (50 μM), which block several potassium currents, including the delayed rectifier K^+ current (I_K), the fast Ca^{2+}-dependent K^+ current (I_C), the inward anomalous rectifier K^+ current (I_Q), the H K^+ current (I_M) and the slow Ca^{2+}-dependent K^+ current (I_{AHP}) (Halliwell, 1990;

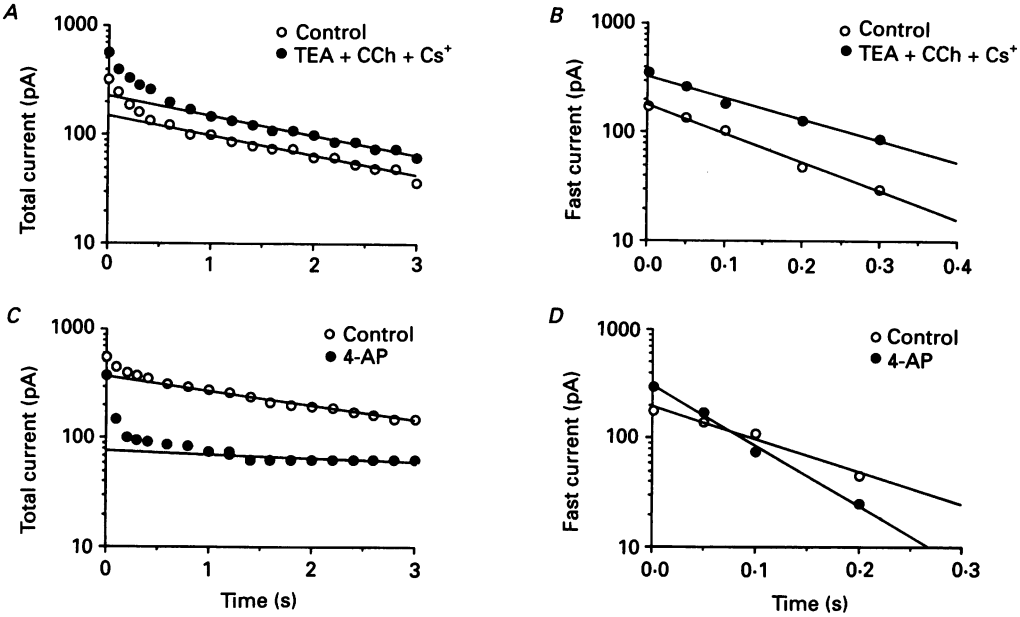

Fig. 3. Effect of K^+ channel blockers on the time course of decay of the fast and slow component of the outward currents. *A*, semilogarithmic plots of the outward currents evoked by a 1 s hyperpolarizing pulse to −105 mV (from V_h −50 mV) revealed, as in Fig. 1, fast and slow components. The slow components were well fitted (straight line) by the following equations: $Y = 151\exp(-t/2{\cdot}4)$ ($r = 0{\cdot}96$) in control ACSF (containing 1 mM kynurenate and 10 μM bicuculline; ○), and $226\exp(-t/2{\cdot}55)$ ($r = 0{\cdot}98$) in the presence of 10 mM TEA, 3 mM Cs^+, 50 μM CCh (together with 1 mM kynurenate and 10 μM bicuculline; ●). Although application of these drugs somewhat increased the amplitude of I_D, they did not change its time course. *B*, the equations fitting the decay time of the initial fast component (obtained as in Fig. 1) were $Y = 181\exp(-t/0{\cdot}163)$ ($r = 0{\cdot}99$) in control ACSF (containing 1 mM kynurenate and 10 μM bicuculline; ○), and $326\exp(-t/0{\cdot}2)$ ($r = 0{\cdot}98$) during application of 10 mM TEA, 3 mM Cs^+, 50 μM CCh (together with 1 mM kynurenate and 10 μM bicuculline; ●). *C*, semilogarithmic plots of the outward currents, evoked as in *A*, before (○) and during application of 40 μM 4-AP (●). The equation describing the decay of the slow component, in control ACSF was $Y = 366\exp(-t/3{\cdot}2)$ ($r = 0{\cdot}98$). In the presence of 4-AP, the slowly inactivating current was nearly abolished and it was not possible to accurately fit a slow component. *D*, the fast component was well fitted by the equations $Y = 193\exp(-t/0{\cdot}15)$ ($r = 0{\cdot}97$) in control ACSF (○), and $302\exp(-t/0{\cdot}080)$ ($r = 0{\cdot}99$) in the presence of 4-AP (●), respectively. Note that the bath application of 4-AP appeared to accelerate the decay of the fast component.

Storm 1990). Because application of a high concentration of TEA would dramatically increase the on-going synaptic activities (Paulsen, Rasstad & Storm, 1990), 1 mM kynurenate (non-NMDA receptor antagonist) and 10 μM bicuculline ($GABA_A$

receptor antagonist) were added to the control bath solution to clear recordings from synaptic noise. Bath applications of tetraethylammonium (TEA, 10 mM), Cs^+ (3 mM) and carbachol (50 μM) (together with 1 mM kynurenate and 10 μM bicuculline) sharply diminished the input conductance (by $31{\cdot}4 \pm 7{\cdot}3\%$, $P < 0{\cdot}007$; Fig. 2*A*) and induced an inward current (between -50 and -100 pA at a V_h near -50 mV), but did not reduce I_D. Indeed, as shown in Fig. 2*A* and *C*, the application of potassium channel blockers (TEA, Cs^+ and carbachol) often caused some increase in I_D (overall non-significant, by $16{\cdot}6 \pm 18{\cdot}9\%$, $n = 7$), without any change of its voltage dependence (Fig. 2*C*) or its time course of inactivation (Fig. 3*A*). The enhancement of I_D may be explained by the fact that blocking I_K, I_C, I_Q, I_M and I_{AHP} by TEA, Cs^+ and carbachol may reveal a part of I_D masked by one of these other currents. Bath applications of 4-AP (30–40 μM), in the presence of the potassium channel blockers, as well as kynurenate and bicuculline, reduced I_D (Fig. 5*A* and *C*) to the same extent as in control ACSF (by $61{\cdot}8 \pm 4{\cdot}7\%$, $n = 4$).

Effects of sulphonylureas on I_D

The dimethyl sulphoxide (DMSO) solution was used to dissolve the sulphonylureas (glibenclamide, gliquidone and tolbutamide, see Methods). DMSO, dissolved at final concentration (1%) in ACSF, had no effect on I_D ($n = 4$).

Effects of glibenclamide on I_D

Glibenclamide (10 μM) clearly diminished the slowly inactivating component (I_D) of the outward current, in sixteen of the nineteen cells tested. As illustrated in Fig. 4*A* and *B*, this depressant effect, which was largely reversible after a wash of 20–30 min, was not associated with marked changes in the voltage dependence (Fig. 4*B*) and the time course of inactivation (Fig. 4*C*). The overall mean depression of I_D was by $31{\cdot}2 \pm 5{\cdot}6\%$ ($n = 16$, $P < 0{\cdot}001$). There was no significant change in half-activation potential ($+0{\cdot}4 \pm 1{\cdot}6$ mV, $n = 7$) or time constant of inactivation of the slow component (τ_S; $-2{\cdot}9 \pm 9{\cdot}8\%$, $n = 6$). There was also no consistent depression of the initial fast component of outward current (judging by plots such as those in Fig. 4*D*), suggesting that I_A is not affected by glibenclamide (the mean change of τ_F in 10 tests was $-8{\cdot}6 \pm 17\%$).

Similar depressions of I_D were observed when the recordings were performed in the presence of the potassium channel blockers (10 mM TEA, 3 mM Cs^+, 50 μM CCh), together with 1 mM kynurenate and 10 μM bicuculline. As shown in Fig. 5, there was a reduction in I_D amplitude by approximately 38% (in 3 cells 32, 45 and 37·5%), without modification of its voltage dependence: the activation of I_D (evoked by depolarizing pulses, from a V_h of -105 mV) started near -75 mV and rose steeply above -70 mV both in the control run and during the application of glibenclamide. These effects were partly reversible after 30 min of wash.

Glibenclamide depressed I_D in a dose-dependent manner. Thus, in the presence of the potassium channel blockers, kynurenate and bicuculline, glibenclamide gave its maximal effect at 10 μM: 3 μM depressed I_D by approximately 5% (in 3 cells 11·5, 4·5 and 0%), 10 μM depressed I_D by approximately 38% (see above) and 30 μM depressed I_D, by the same extent as 10 μM i.e. by approximately 36·5% (in 3 cells

33·5, 46·5 and 29·5 %). The range of concentration found to depress I_D is in agreement with the concentration commonly used to inhibit K_{ATP} channels (5–30 μM) in cardiac and smooth muscles (Buckingham, Hamilton, Howlett, Mootoo & Wilson, 1989; Standen, Quayle, Davies, Brayden & Huang, 1989; Wilson, 1989; Tseng & Hoffman,

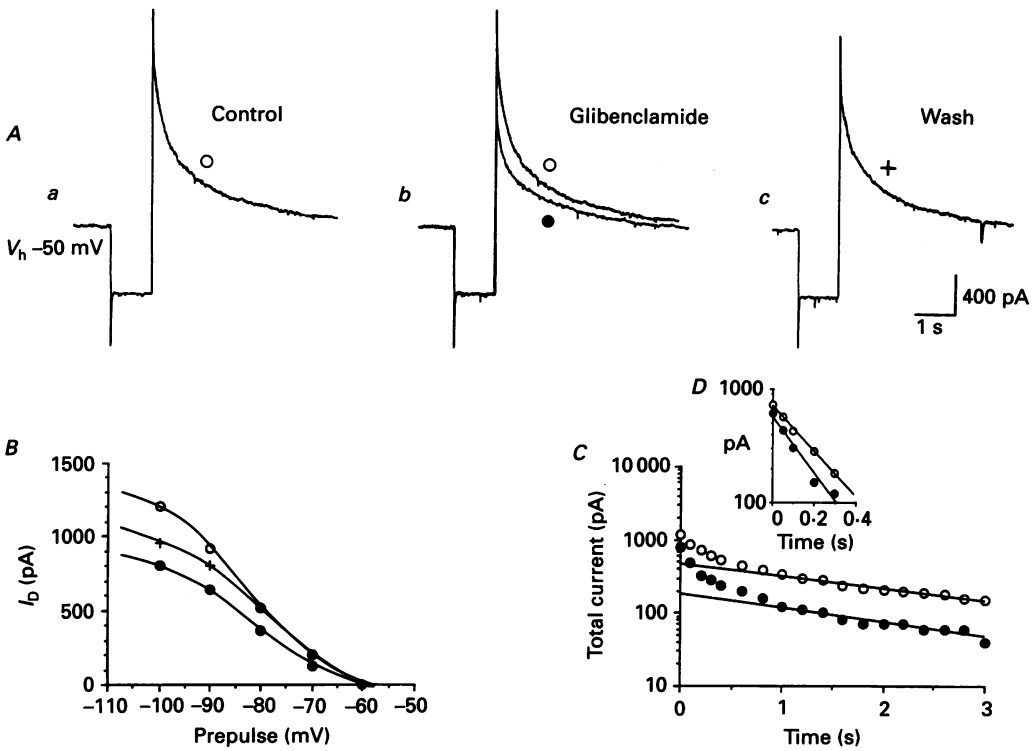

Fig. 4. Effects of glibenclamide on I_D. *A*, outward currents (evoked by a 1 s hyperpolarizing pulse to −100 mV (V_h −50 mV); *a*, in control ACSF (○); *b*, in the presence of 10 μM glibenclamide (●); and *c*, after the wash (+). In *b*, the superimposed control (○) and glibenclamide (●) traces show that glibenclamide reduced the outward current. *B*, voltage dependence of inactivation of the slowly inactivating outward current in control ACSF (○), in the presence of 10 μM glibenclamide (●) and after wash (+). As in control ACSF, half-inactivation was near −82 mV in the presence of glibenclamide. *C* and *D*, plots of the slow and fast components in control ACSF (○) and in the presence of glibenclamide (●). The slow component was well fitted (straight lines) by the following equations: $Y = 468\exp(-t/2{\cdot}6)$ ($r = 0{\cdot}97$) in control ACSF (○) and $168{\cdot}9\exp(-t/2{\cdot}2)$ ($r = 0{\cdot}95$) in the presence of glibenclamide (●). For the fast initial component the equations were $Y = 715\exp(-t/0{\cdot}21)$ ($r = 0{\cdot}98$) in control ACSF (○) and $568\exp(-t/0{\cdot}18)$ ($r = 0{\cdot}95$) in the presence of glibenclamide (●). Glibenclamide did not substantially modify the time course of the slow and fast components but reduced their initial amplitudes by 64 and 21 % respectively.

1990; Zini *et al.* 1991*b*), as well as in the CNS (Grigg & Anderson, 1989; Ben-Ari, 1989, 1990; Amoroso *et al.* 1990; Häusser, De Weille & Lazdunski, 1991; Politi & Rogawski 1991; Jiang *et al.* 1992; see Discussion).

Effects of gliquidone and tolbutamide on I_D

In thirteen tests of gliquidone (10 μM), the depressions of I_D were even more pronounced than those seen with glibenclamide. A particularly striking example is given by the traces of Fig. 6*A* and the corresponding activation curve in 6*B*.

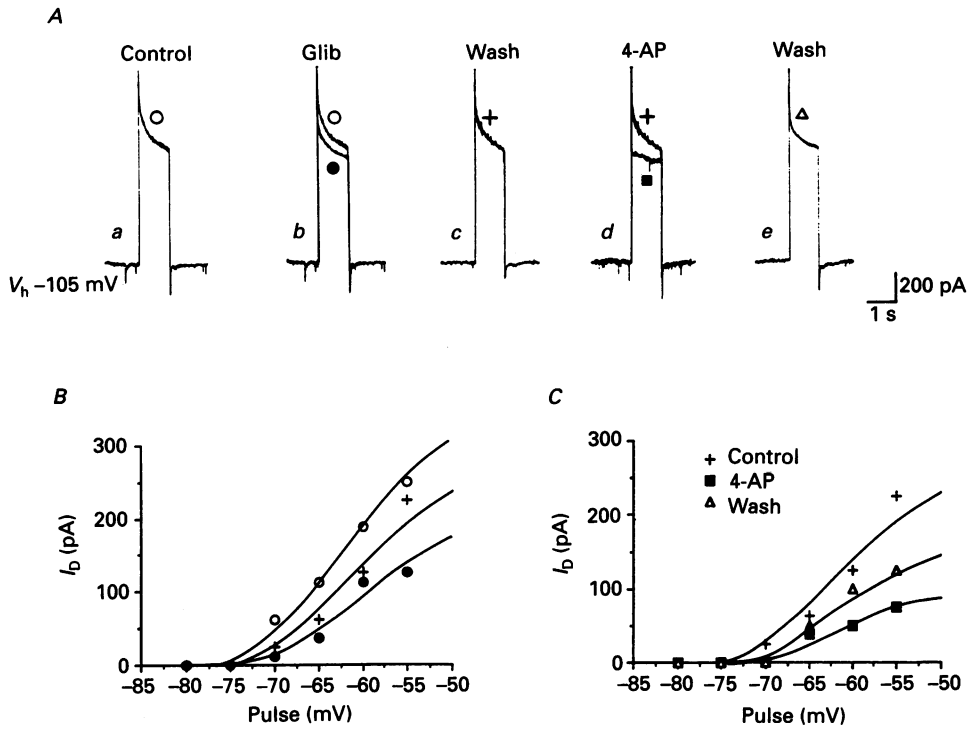

Fig. 5. Glibenclamide and 4-AP reduced I_D in presence of high concentrations of TEA, Cs^+ and carbachol. *A*, in this figure the control solution consisted of ACSF containing 10 mM TEA, 3 mM Cs^+ and 50 μM carbachol (CCh), 1 mM kynurenate and 10 μM bicuculline. The outward current was evoked by 1 s depolarizing pulses to −55 mV (V_h −105 mV); *a*, in control conditions (○); *b*, during application of 30 μM glibenclamide (Glib; ●); and *c*, after wash (+). In *b*, the superimposed control (○) and glibenclamide (●) traces clearly show that, in presence of TEA, Cs^+, CCh, kynurenate and bicuculline, glibenclamide reduced the outward current. After the wash-out of glibenclamide (*c*), 40 μM 4-AP was applied (*d*); the superimposed post-glibenclamide control (+) and 4-AP (■) traces show that, in the presence of TEA, Cs^+, CCh, kynurenate and bicuculline, 4-AP strongly suppressed the slowly decaying outward current, as in standard ACSF. The effect of 4-AP was partly reversed by wash-out (△; *e*). *B*, voltage dependence of activation of the slowly inactivating outward current in control condition (○), during application of glibenclamide (●) and after wash (+). In the control condition, the threshold of activation in presence of glibenclamide was near −75 mV. *C*, voltage dependence of activation of I_D, in post-glibenclamide control (+), during application of 4-AP (●) and after the wash-out of 4-AP (△).

Gliquidone depressed the amplitude of I_D by 42·6 ± 7·9 % ($n = 13$, $P < 0{\cdot}002$). These effects were usually reversed after 20–30 min of wash. They were not accompanied by any consistent change in voltage dependence (Fig. 5*B*). The potential where I_D

was half-inactivated varied by only 1·5 ± 3 mV ($n = 6$). Gliquidone induced a small inward current shift, but did not modify the input conductance (cf. Table 1). Fully comparable reductions (by 32·8 ± 11·3 %) were observed in three tests of gliquidone (10 μM) on cells recorded in the presence of the potassium channel blockers (with kynurenate and bicuculline).

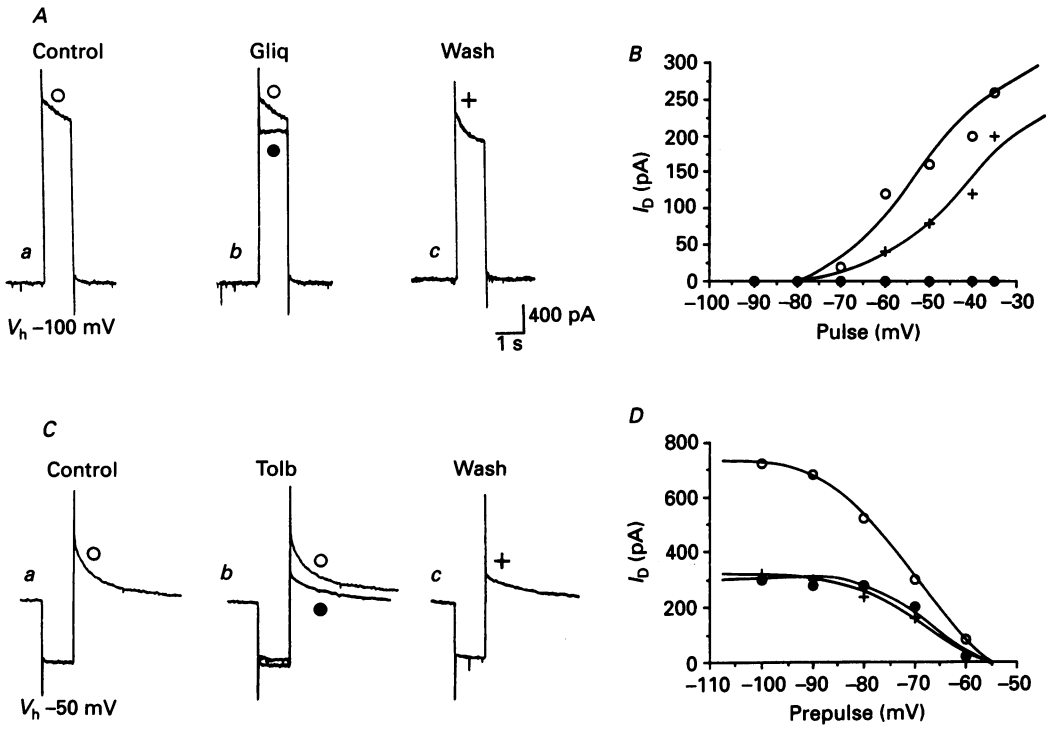

Fig. 6. Effect of gliquidone and tolbutamide on I_D. *A*, slowly inactivating outward current evoked by a 1 s single depolarizing pulse to −50 mV (V_h −100 mV): *a*, in control ACSF (○); *b*, after application of 10 μM gliquidone (Gliq; ●), and *c*, after wash (+). *B*, voltage dependence of activation of the slowly inactivating outward current (obtained by subtracting the leak current from the total current) in control ACSF (○), in the presence of 10 μM gliquidone (●) and after wash (+). Note the complete inhibition of I_D by 10 μM gliquidone. *C*, slowly inactivating outward current evoked by a single hyperpolarizing pulse to −100 mV (V_h −50 mV): *a*, in control (○); *b*, after application of 500 μM tolbutamide (Tolb; ●), and *c*, after wash (+). *D*, voltage dependence of inactivation of the slowly inactivating outward current in control ACSF (○), in the presence of 500 μM tolbutamide (●) and after wash (+). There was a small shift in half-inactivation potential from −72 to −67 mV in the presence of tolbutamide. Note that the effect of tolbutamide did not disappear after 1 h of wash-out.

A similar depression of I_D was observed in eight cells tested with tolbutamide (500 μM). As can be seen in Fig. 6*C* and *D*, I_D was reduced by 39·1 ± 12·8 % ($n = 8$, $P < 0·01$), without a significant change in the half-inactivation potential (0·66 ± 0·38 mV; $n = 4$) or the time constant of inactivation (τ_F was reduced by

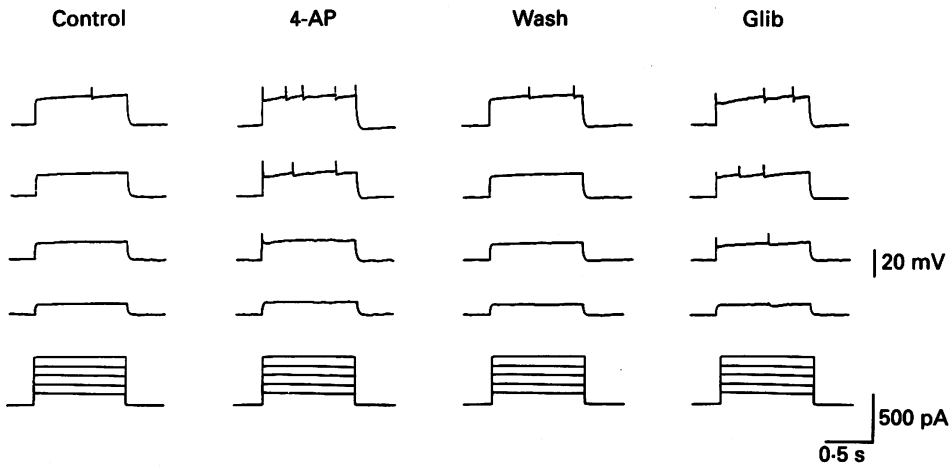

Fig. 7. Effect of glibenclamide and 4-AP on neuronal firing evoked by long depolarizing pulses. Current-clamp recordings show the response of a CA3 pyramidal neuron to a family of depolarizing current pulses (1 s duration) evoked from a potential of −85 mV (action potentials are truncated). When the cell was depolarized enough to generate action potentials, in the control there was a typical long delay before the first action potential. At a dose of 40 μM, which is known to block I_D, 4-AP eliminated this delay. The effect was reversed by 15 min of wash and was reproduced by application of 10 μM glibenclamide (Glib).

TABLE 1. Effects of sulfonylureas on amplitude of I_D, input conductance and holding current, observed during single-electrode voltage clamping of CA3 pyramidal neurons

	Number of tests	Change in I_D (%)	Change in input conductance (%)	Change in holding current (pA)
Glibenclamide (10 μM)	16	−31·2±5·6**	8·0±6·5	−86·7±45·6
Gliquidone (10 μM)	13	−42·6±7·9**	2·2±8·3	−123·0±44·1*
Tolbutamide (500 μM)	8	−39·1±12·8*	4·4±8·3	−11·0±67·8

Values are expressed as mean±S.E.M.
* Mean $P \leqslant 0{\cdot}05$ and ** mean $P \leqslant 0{\cdot}005$.

16·7±20%, $n = 6$). The effect of tolbutamide was not readily reversible: only in one case was a small recovery observed, even after 1 h of wash. It was not associated with any consistent changes in input conductance or holding current (cf. Table 1).

Effects of glibenclamide on the firing characteristics of CA3 pyramidal cells

I_D was originally so named because it tends to delay for some seconds neuronal firing evoked by a long depolarizing pulse (Storm, 1988). The fact that, at the low concentrations sufficient to block I_D, 4-AP abolished this delay (as shown in Fig. 7) provided further evidence for the functional involvement of I_D in controlling cell firing. If glibenclamide reduces I_D, it should have a qualitatively similar effect. Tests on four CA3 pyramidal cells, with current-clamp recordings, showed that glibenclamide (10 μM) increased the frequency of firing evoked by long depolarizing

pulses (500 ms in duration), and reduced the latency of firing onset. Examples of these effects are displayed in Fig. 7, which shows the membrane depolarizations evoked by incremental current pulses, before (i.e. after the 30 min wash of 40 μM 4-AP) and during the application of glibenclamide (10 μM).

DISCUSSION

The main conclusion of the present study is that sulphonylureas depress the slowly inactivating current, I_D, in CA3 pyramidal neurons.

Properties of an I_D-like current in CA3 pyramidal neurons

The physiological and pharmacological properties of the outward current that we have examined are similar to those of the I_D observed in CA1 pyramidal cells (Storm, 1990). This slowly inactivating current can be evoked either by depolarizing pulses of 40–60 mV, from a V_h near -100 mV, or by hyperpolarizing pulses of 40–60 mV, from a V_h near -50 mV. It is highly sensitive to 4-AP and insensitive to the potassium channel blockers TEA, Cs^+ and carbachol. Clearly, this current possesses all the characteristics of I_D but differs considerably from the currents mediated by K_{ATP} channels, as described for peripheral tissues (Rorsman & Trube, 1990) or central neurons (Trussel & Jackson, 1987; Ashford *et al.* 1988, 1990; Politi & Rogawski, 1991). Thus, in contrast to I_D, which is strongly voltage dependent, highly sensitive to 4-AP and insensitive to Ba^{2+}, Cs^+ or TEA (Storm, 1988), the activity of K_{ATP} channels is not (or only weakly) voltage dependent (Rorsman & Trube, 1990); it is readily blocked by Ba^{2+} (Kakei & Noma, 1984; Quayle, Standen & Stanfield, 1988), partly by TEA (Kakei, Noma & Shibasaki, 1985; Spruce, Standen & Stanfield, 1987) and is insensitive to 4-AP (Cook & Hales, 1984; Kakei *et al.* 1985). Experiments using a low concentration of 4-AP and several potassium channel blockers also provide compelling evidence that this current is not mediated through another voltage- or Ca^{2+}-dependent K^+ channel (Halliwell, 1990; Storm, 1990). Previous experiments also indicated an I_D-like component in the CA3 pyramidal cells, judging by its slow time course and its sensitivity to 4-AP (Gustafsson *et al.* 1982; Neuman *et al.* 1991).

Sulphonylureas depress I_D

Glibenclamide, gliquidone and tolbutamide depressed the slowly inactivating K^+ current, I_D, without altering its voltage dependence and inactivation kinetics. Current-clamp experiments showed that glibenclamide, as 4-AP, nearly abolished the delay of the firing evoked by long depolarizing steps, confirming that glibenclamide depresses I_D, since it has been shown that it is principally I_D which delays the firing of pyramidal neurons (Storm, 1988). Glibenclamide depressed I_D, in a dose-dependent manner, and this effect on I_D was achieved with micromolar concentrations of glibenclamide. Although, high- and low-affinity glibenclamide binding sites are present in the hippocampus and other brain regions (Gopalakrishnan *et al.* 1991; Zini *et al.* 1991*a*), our observations suggest that the effects of glibenclamide on I_D are mediated by low-affinity binding sites. Earlier studies, performed with glibenclamide in slices or cultures, also suggest that the efficacy of

glibenclamide to modulate K_{ATP} channels is better correlated with low-affinity binding sites; micromolar concentrations (5–30 μM) are commonly used to inhibit K_{ATP} channels in CNS (Grigg & Anderson, 1989; Ben-Ari, 1989, 1990; Amoroso *et al.* 1990; Häusser *et al.* 1991; Politi & Rogawski, 1991; Jiang *et al.* 1992). We also compared the efficacy of sulphonylureas, from the first (tolbutamide) and second (glibenclamide and gliquidone) generation, and found an excellent agreement with their respective efficacy to block K_{ATP}, in the CNS (Amoroso *et al.* 1990) and peripheral systems (Schmid-Antomarchi *et al.* 1987*a*). We conclude that sulphonylureas are not selective blockers of K_{ATP} channels, in the hippocampus.

Interestingly, several recent studies also indicate that sulphonylureas act on currents which are not of the K_{ATP} types. Thus (i) in the substantia nigra, tolbutamide reverses the hyperpolarization evoked by quinpirole (a D_2 receptor agonist) and baclofen (the $GABA_B$ receptor agonist) (Roeper, Hainsworth & Ashcroft, 1990); (ii) in human neuroblastoma cell line, glibenclamide inhibits voltage-gated K^+ currents, which are not mediated by K_{ATP} channels (Reeve, Vaughan & Peers, 1992). Therefore, in contrast to β-cells (Rorsman & Trube, 1986; Trube, Rorsman & Ohno-Shosaku, 1986; Sturgess, Kozlowski, Carrington, Hales & Ashford, 1988), in the CNS, the activity of sulphonylureas is not restricted to K_{ATP} channels.

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