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Tolbutamide blocks Ca^{2+}- and voltage-dependent K^+ currents of hippocampal Ca1 neurons

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Abstract

In current-clamp recordings with $KMeSO_4$ electrodes (either whole-cell or intracellular), though tolbutamide (0.5–1 mM) did not change the resting potential, it increased both input resistance (by 12 ± 3.8%) and spontaneous firing, and spikes were evoked by smaller depolarizing pulses. Tolbutamide reduced in a dose-dependent manner both components of post-burst afterhyperpolarizations: IC_{50} was 0.15 mM for medium afterhyperpolarizations and 0.33 mM for slow afterhyperpolarizations. In whole-cell recordings under voltage-clamp, 0.5–1 mM tolbutamide depressed slow outward currents by 65 ± 5.3%. The tolbutamide-sensitive current was Ca^{2+}-dependent – tolbutamide being ineffective in Mn^{2+}, low Ca^{2+}-containing medium – though tolbutamide did not significantly depress high voltage-activated Ca^{2+} currents. Tolbutamide reduced C-type outward currents by 45 ± 5.9% and M-type current inward relaxations by 41 ± 12.9%, as well as Q-type current inward relaxations by 22 ± 5.7%. Glyburide (10 μM) did not depress afterhyperpolarizations or outward currents, even in recordings with electrodes containing 1 mM guanosine diphosphate. We conclude that the most prominent effects of 0.5–1 mM tolbutamide on CA1 neurons are caused by suppression of Ca^{2+}- and voltage-dependent outward currents, including I_{AHP}, I_C and I_M.

Keywords: Sulfonylurea; Afterhyperpolarization; K^+ channel, Ca^{2+}-dependent

1. Introduction

The sulphonylureas tolbutamide and glyburide (glibenclamide) are well-known as blockers of ATP-sensitive K^+ channels (K_{ATP}) (Henquin and Meissner, 1982; DeWeille and Lazdunski, 1990; Ashford et al., 1990; Ashcroft and Ashcroft, 1992). Because of its very low affinity for the sulphonylurea receptor (> 1000-fold less than that of glyburide), in most tissues, including heart and brain, tolbutamide is typically applied in concentrations near 1 mM.

Like most drugs, tolbutamide is by no means a wholly selective agent: it is known that at concentrations of ≈ 1 mM, it inhibits cyclic AMP-mediated activation of protein kinase A in some gland cells (Brown et al., 1972; Kanamori et al., 1976); potentiates glycolysis in cardiac muscle (Kramer et al., 1983); suppresses a G-protein-activated Cl^- current in chromaffin cells (Doroshenko et al., 1991), as well as the cyclic AMP/ATP-activated Cl^- and ATP^- currents mediated by the cystic fibrosis transmembrane conductance regulator (Sheppard and Welsh, 1994; Schwiebert et al., 1995). According to a recent study on hippocampal slices (Crépel et al., 1993), both tolbutamide and glyburide depress the voltage-dependent D-type K^+ current (Storm, 1990).

Whether K_{ATP} channels are of functional significance in hippocampal neurons is still not certain. Although glyburide-binding sites are plentiful (Mourre et al., 1989; Tremblay et al., 1991) – both on presynaptic terminals and post-synaptic cells (Mourre et al., 1991) – this does not necessarily indicate the presence of K_{ATP} channels, in view of strong evidence that the relevant K^+ channels and sulphonylurea receptors are separate molecules (Ashford et al., 1994; Aguilar-Bryan et al., 1995; Inagaki et al., 1995). In any case, if functional K_{ATP} channels are indeed present, they are probably situated on presynaptic terminals – where they appear to diminish glutamate release during hypoxia (Mourre et al., 1989; Ben-Ari and Lazdunski, 1989; Ben-Ari, 1990) – rather than on post-synaptic cells. In isolated patches from comparable cortical cells, the

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incidence of K_{ATP} channels is extremely low (Ashford et al., 1990).

The unexpected finding (Godfraind and Krnjević, 1993) that tolbutamide blocks anoxic hyperpolarizations of CA1 neurons (Hansen et al., 1982; Krnjević, 1993) suggested that anoxic depletion of cellular ATP drives this hyperpolarization by opening K_{ATP} channels. Because of the lack of any comparable effect of glyburide, Godfraind and Krnjević (1993) concluded that if hypoxia indeed activates such channels in hippocampal cells, they must be of a non-classical type – perhaps being similar to some ATP-sensitive channels of hypothalamic glucoceptive neurons, whose unusual characteristics include susceptibility to block by tolbutamide but not glyburide (Ashford et al., 1990). However, other evidence (Krnjević and Xu, 1989; Krnjević, 1993; Erdemli and Krnjević, 1994a) suggests that anoxia activates a carbachol-sensitive Ca^{2+}-activated or Ca^{2+}-sensitive K^+ conductance, such as I_{AHP} (Lancaster and Adams, 1986; Storm, 1990) or I_M (Yu et al., 1994).

We therefore wondered whether tolbutamide may not also block I_{AHP} or I_M of CA1 neurons, as this could explain why it suppresses the anoxic hyperpolarization. Both tolbutamide and glyburide were therefore tested on the afterhyperpolarizations evoked by bursts of spikes (in current-clamp) and on voltage-activated currents (during voltage-clamp), using whole-cell patch or intracellular recording. Preliminary reports of some of the results have appeared (Erdemli and Krnjević, 1994b; Krnjević et al., 1994; Esplin et al., 1995).

2. Materials and methods

The brain was removed from young Sprague-Dawley rats (100–150 g) fully anaesthetized with halothane. The hippocampus was dissected out in ice-cold oxygenated saline, and 400 μm transverse slices were cut with a McIlwain tissue chopper (The Mickle Laboratory Gomshall, UK) or a Vibroslice (Campden Instruments, Loughborough, UK). They were kept for at least 1 h, at room temperature, in the standard artificial cerebrospinal fluid (ACSF), containing the following (in mM): 124 NaCl, 3.0 KCl, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose, aerated with carbogen (95% O_2 and 5% CO_2) to give a pH of 7.3.

Before the start of electrical recording, a slice was transferred to a Haas-type interface chamber (Medical Systems, Greenvale, NY, USA), where its upper surface was either directly ventilated by humidified carbogen – for conventional intracellular recordings – or submerged under 0.1–0.2 mm of flowing ACSF – for whole-cell patch electrode recordings. Both the ACSF and the aerating gas were warmed to 33 ± 0.5°C. The 'sharp' microelectrodes, filled with either 3 M KCl or 2 M K methyl sulphate ($KMeSO_4$), had a resistance of 60–120 MΩ. The patch electrodes (with 2.5–3 μm tips) were pulled from thin wall borosilicate glass (1.5 mm o.d., WP Instruments, New Haven, CT, USA). The standard filling solution contained 150 mM $KMeSO_4$ and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes). When afterhyperpolarizations were studied, K_2-ATP (2 mM) was added to the internal solution to prevent rundown – but not Mg^{2+}, to avoid a possible blockage of the relevant K^+ channels (Lancaster et al., 1991; Zhang et al., 1994). For recordings of outward currents, the internal solution contained K_2 ATP and $MgCl_2$ (both 1 mM). In some cases, to enhance any K_{ATP}-like currents, 1 mM guanosine diphosphate was substituted for ATP (Erdemli and Krnjević, 1994c). The pH was always adjusted to 7.2 with KOH. The osmolality of the internal solutions (measured with a Precision Systems osmometer (Natick, MA, USA) was in the range 287–300 mosm kg^{-1}. The patch electrodes initially had resistances of 2–3 MΩ; during whole-cell recordings, obtained in 'blind' insertions into the stratum pyramidale, the series resistance of useful electrodes remained below 15 MΩ.

All recordings were done with an Axoclamp 2 amplifier (Axon Instruments, Burlingame, CA, USA), either in the current-clamp mode or the discontinuous single-electrode voltage-clamp mode, as a rule operating at a cycling frequency of 3 kHz, a gain of 25 nA/mV, and with the bandwidth upper limit set at 300 Hz; the usual precautions were taken to optimize the performance of the clamp circuit and electrode.

In current-clamp mode, the input resistance (R_N) of cells at resting potential (V_m) was monitored by injecting pulses of hyperpolarizing current (200 ms, 0.1–0.2 nA) and measuring the peak voltage responses. To activate afterhyperpolarizations, spike trains were evoked by 200 ms depolarizing pulses. Under voltage-clamp, the base-line holding current (I_H) was kept to a minimum by keeping the holding potential (V_H) near the resting level (≈ -55 mV). Voltage-dependent currents were elicited with 500 ms depolarizing and hyperpolarizing pulses. Both initial ('instantaneous') and final ('steady state') values of currents were measured. The input conductance (G_N) was calculated by fitting first-order regressions to the linear portion of 'instantaneous' current-voltage plots, in the region negative to V_H.

Whenever necessary, Ca^+-dependent currents were suppressed by phosphate-free ACSF containing 0.4 mM $CaCl_2$, 2.3 mM $MnCl_2$ and 11 mM $NaHCO_3$ (Mn^{2+}/low Ca^{2+} ACSF). To avoid rundown, Ca^{2+}-currents were recorded intracellularly – with 3 M CsCl electrodes – from slices in low Ca^{2+} (0.5 mM) and phosphate-free ACSF, containing 1.5 mM $BaCl_2$, 10 mM tetraethylammonium, 2 mM CsCl, and 1 μM tetrodotoxin (Quinhuangdao Trading, China).

In *all* experiments, indirect effects of neurotransmitters were minimized by adding kynurenic acid (1 mM), and bicuculline (10–20 μM) or picrotoxin (50 μM). The following drugs were applied in the superfusate: 0.05–1

mM tolbutamide (Research Biochemical and Sigma), from a 100 mM stock solution in 150 mM NaOH; and 10 μM glyburide (Hoechst), from a 10 mM stock solution in dimethyl sulphoxide (ICN Biomedicals, Aurora, OH, USA). Any possible effects of solvents for glyburide and tolbutamide were controlled by ensuring that all superfusates contained the same amount of dimethyl sulphoxide (0.10%) or NaOH (as appropriate). Most chemicals were purchased from Sigma (St Louis, MO, USA) – except where indicated.

Means ± S.E.M. are given throughout and their significance assessed by Student's *t*-test.

3. Results

3.1. Current-clamp whole-cell recordings

3.1.1. Effects of tolbutamide on resting membrane properties

In nine cells, with a mean input resistance (R_N) of 113 ± 9.5 MΩ and a resting potential (V_m) of −54 ± 3.1 mV, tolbutamide (0.5–1 mM) increased R_N by 12 ± 3.8% (for $n = 9$, $P < 0.025$), without changing V_m (−0.10 ± 0.82 mV). In eight of these nine cells, the amount of current required to evoke 6–7 spikes decreased (Fig. 1A, inset); overall, the current was reduced by 56 ± 11.6% (for $n = 9$, $P < 0.005$). In six cells, 'spontaneous' firing appeared.

3.1.2. Effects of tolbutamide on afterhyperpolarizations

In hippocampal cells, spike bursts are followed by an afterhyperpolarization having two components, both K^+ channel-mediated (Storm, 1990): an early, medium afterhyperpolarization and a much longer-lasting slow afterhyperpolarization. They are generated by different K^+ currents: the medium afterhyperpolarization by the voltage- and Ca^{2+}-dependent I_C, as well as the voltage-dependent (but Ca^{2+}-sensitive) I_M; and the slow afterhyperpolarization by the purely Ca^{2+}-dependent I_{AHP}. Thus, both are initiated mainly by Ca^{2+} influx during action potentials. In whole-cell recordings, such afterhyperpolarizations were consistently depressed by tolbutamide.

Of the anions commonly used for electrode-filling solutions, only methylsulphate permits stable recording of slow afterhyperpolarizations in CA1 neurons (Zhang et al., 1994). Therefore all our patch electrodes contained $KMeSO_4$. Spike trains were evoked by 200 ms depolarizing pulses of several intensities; medium afterhyperpolarizations were measured at the early peak and slow afterhyperpolarizations at 1 s after the end of the depolarizing pulse. The amplitudes of afterhyperpolarizations increased with the number of spikes in a train. In most cases, the relationship was approximately linear and could be fitted by very significant regressions, as illustrated by the plots in Fig. 1A: the control values of slopes were 1.3 ± 0.08 mV/spike ($r = 0.994$) for control medium afterhyperpolarizations (open circles) and 0.60 ± 0.094 mV/spike ($r = 0.965$) for slow afterhyperpolarizations. In this cell, 1 mM

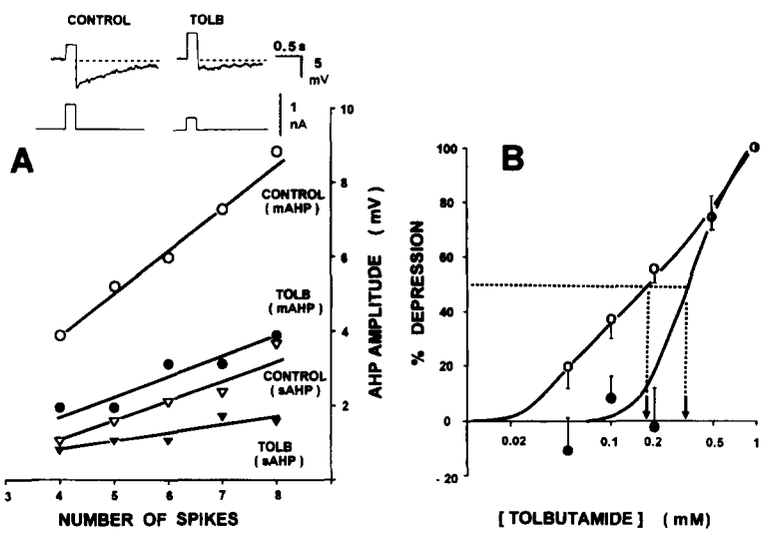

Fig. 1. In whole-cell recordings with $KMeSO_4$ electrodes, tolbutamide depresses after-hyperpolarizations in concentration-dependent manner. (A) Plots of data from a single CA1 neuron; open circles and triangles are control amplitudes of medium afterhyperpolarization (mAHP, measured at peak) and slow afterhyperpolarization (sAHP, measured at 1 s after end of depolarizing pulses that generated 4–8 spikes); closed circles and triangles are corresponding afterhyperpolarizations during tolbutamide (1.0 mM) bath application. Inset above: examples of afterhyperpolarizations after 7 spikes – evoked by current pulses shown below (spikes are not visible here, but were recorded separately at low gain); in presence of tolbutamide, same number of spikes were evoked by much smaller current. (B) Dose-response plots of tolbutamide-induced depression of afterhyperpolarizations: open and closed circles are respectively means (±S.E.M.) of 6–14 medium or slow afterhyperpolarizations (from 54 tests of tolbutamide on 11 cells). Curves of best-fit were obtained by fitting linear regressions to probit-transformed data. Both regressions had significant slopes with $r = 0.95$ ($P < 0.02$) for medium afterhyperpolarizations and $r = 0.89$ ($P < 0.05$) for slow afterhyperpolarizations; corresponding IC_{50}s are 0.15 and 0.33 mM (arrows). Recordings in this and *all* other figures were made in presence of kynurenate (1 mM) and bicuculline (10 μM) or picrotoxin (50 μM).

Table 1
Depression of slopes of afterhyperpolarizations against number of spikes by 1 mM tolbutamide

	Control slope			Change in tolbutamide	
Anions (in electrode)	V_m (mV)	mAHP (mV/sp)	sAHP (mV/sp)	mAHP Δ(%)	sAHP Δ(%)
Whole-cell recordings					
KMeSO4 (5)	−55 ± 2.0	−0.86 ± 0.16 [b]	−0.40 ± 0.09 [a]	−77 ± 15 [b]	−88 ± 7.8 [c]
Intracellular recordings					
KMeSO4 (6)	−65 ± 4.3	−0.88 ± 0.10 [c]	−0.42 ± 0.04 [c]	−41 ± 10 [a]	−41 ± 13 [a]
KCl (7)	−65 ± 2.9	−0.66 ± 0.11 [c]	−0.32 ± 0.06 [b]	−13 ± 16	9 ± 23

Mean values of slopes obtained by fitting linear regressions to ≈ 5 data points, such as those illustrated in Figs. 1A and 2A. Number of cells for which slopes were calculated is in parentheses (at left). V_m, resting membrane potential; mAHP, medium afterhyperpolarization, measured at early peak; sAHP, slow afterhyperpolarization, at 1 s after end of depolarizing pulse. All paired comparisons were at same resting potential. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

tolbutamide sharply depressed the afterhyperpolarizations (cf. traces inset above), the best-fitting slopes being reduced to 0.51 ± 0.098 mV/spike ($r = 0.948$) for the medium afterhyperpolarizations and 0.22 ± 0.060 mV/spike ($r = 0.906$) for the slow afterhyperpolarizations. Thus tolbutamide reduced both components nearly equally, by 61 and 63%. At higher concentrations, the effects of tolbutamide were particularly marked and consistent (cf. Table 1).

Overall, tolbutamide's depressant action was clearly dose-dependent, as shown by the dose-response curves in Fig. 1B. These were calculated from the depressions of afterhyperpolarizations observed in 54 tests of tolbutamide, applied to 11 cells, at concentrations ranging from 0.05 to 1 mM. The respective IC_{50}'s were 0.15 mM for medium afterhyperpolarizations and 0.33 mM for slow afterhyperpolarizations. There was no discernible recovery of afterhyperpolarizations after washing for 20–60 min (3 cells).

3.2. Intracellular recordings (with 'sharp' electrodes)

Resting potentials were consistently lower (by ≈ 10 mV) than in whole-cell recordings (Table 1). Nevertheless, virtually identical afterhyperpolarizations were recorded with $KMeSO_4$ electrodes. But 1 mM tolbutamide was

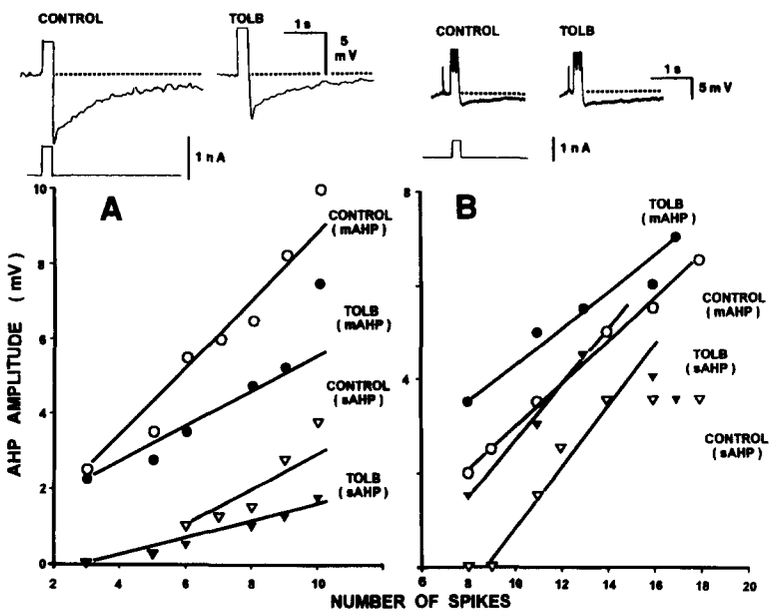

Fig. 2. Tolbutamide also depresses afterhyperpolarizations when these are recorded with $KMeSO_4$ but not KCl *intracellular* electrodes. 'Sharp' microelectrodes contained 2 M $KMeSO_4$ for the recording from the cell illustrated in (A), and 3 M KCl for the cell illustrated in (B). Open circles and triangles are control amplitudes of medium and slow afterhyperpolarization (mAHP and sAHP, respectively), generated by increasing numbers of spikes; closed circles and triangles are corresponding afterhyperpolarizations in presence of tolbutamide (1 mM, 30 min). Inset above are examples of single afterhyperpolarizations s evoked by depolarizing pulses generating 9 and 16 spikes (for A and B respectively), before and during tolbutamide application. Note sharp reduction of both slow and medium afterhyperpolarizations by tolbutamide in (A); whereas in (B), both slow and medium afterhyperpolarizations were somewhat enhanced by tolbutamide.

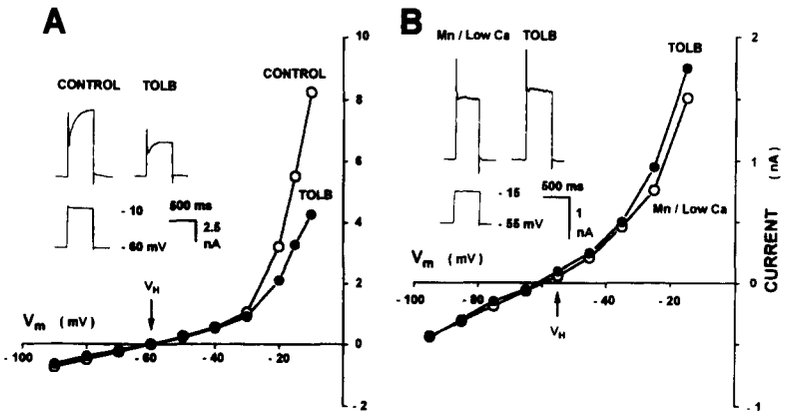

Fig. 3. Outward current depressed by tolbutamide is Ca^{2+}-dependent. Current-voltage plots were obtained with 500 ms pulses, during whole-cell recording from two different cells: (A) in regular ACSF (V_H −60 mV); (B) in Mn^{2+}/low Ca^{2+} ACSF (to eliminate Ca^{2+} and Ca^{2+}-dependent currents), with V_H −55 mV. Open circles are controls, closed circles data obtained after 15 min in 1 mM tolbutamide. Inset traces are examples of currents recorded at −10 mV (A) and −15 mV (B). Tolbutamide sharply reduced outward current in (A) but not (B).

somewhat less effective, both components being depressed by ≈ 40% (Fig. 2A and Table 1).

When recorded with KCl electrodes (at the same resting potential), afterhyperpolarizations and the corresponding slopes were consistently smaller (Table 1). The most striking difference, however, was the lack of any clear depression by tolbutamide, the afterhyperpolarizations showing either no change or some enhancement (Fig. 2B and Table 1).

3.3. Whole-cell voltage-clamp recordings

In agreement with the current-clamp data, in 5 cells clamped near resting potential (−53.0 ± 3.7 mV), tolbutamide (1 mM) consistently reduced the input conductance (G_N) (−29 ± 5.1%, from 19 ± 3.2 nS, $P < 0.01$), with no significant change in baseline current (−22 ± 16 pA).

3.3.1. Tolbutamide depresses voltage-dependent outward current (I_{out})

With V_H near −50 mV, depolarizing pulses to voltages > −30 mV evoked net outward currents, having a mean of 4.2 ± 1.3 nA at ≈ −10 mV ($n = 5$). Tolbutamide (1 mM) quite regularly depressed such outward currents over the full range of potentials (Fig. 3A): at ≈ −10 mV, by 65 ± 5.3% (for $n = 5$, $P < 0.001$). This effect was not reversed by a 30 min wash ($n = 2$).

In another 6 cells, also held near −50 mV, Ca^{2+}-dependent currents were eliminated with Mn^{2+}/low Ca^{2+} ACSF. Similar depolarizing pulses evoked smaller outward currents – 1.4 ± 0.2 nA near −15 mV – presumably consisting mainly of delayed rectifier current (I_{DR}) (Storm, 1990). In the presence of tolbutamide (1 mM), there was no significant depression of such currents. Thus, in Fig. 3B, the outward current was slightly *enhanced*; but the

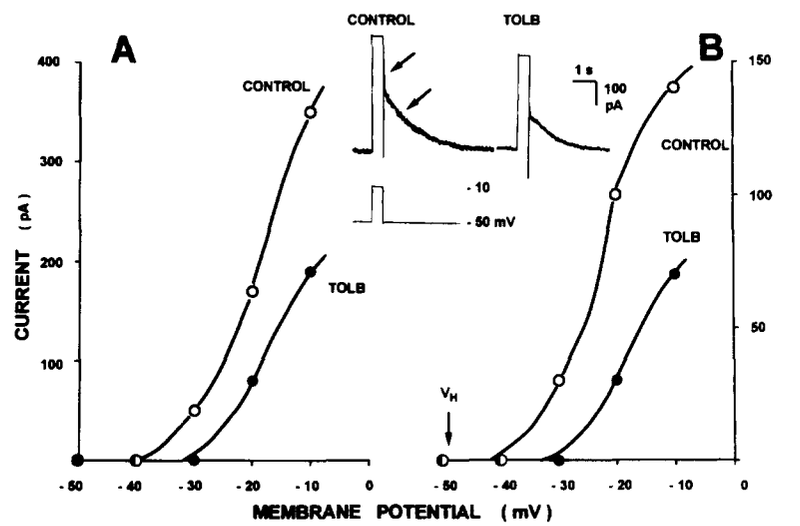

Fig. 4. Outward tail currents are also reduced by tolbutamide. Plots of tail currents observed in one cell following pulses to various potentials, from V_H −50 mV (whole-cell recording in presence of tetrodotoxin). (A) Fast component (first arrow in inset traces); (B) slow component, measured at 1 s (second arrow). Open circles are control data; closed circles, data obtained after 15 min in 1 mM tolbutamide.

overall change near −10 mV was a non-significant increase by 10.5 ± 6.4% ($n = 5$). There was also no significant reduction in G_N (−7.2 ± 4.5%, $n = 6$). Thus, the tolbutamide-sensitive I_{out} is probably a Ca^{2+}-activated or Ca^{2+}-sensitive current, such as I_{AHP}, I_C and/or I_M.

3.3.2. Tolbutamide also depresses outward tail currents

Large, slowly decaying outward tails followed the end of depolarizing pulses, as partly illustrated by inset traces in Fig. 4 (from a neuron held at −50 mV). According to previous studies (Lancaster and Adams, 1986; Storm, 1989, 1990; Lancaster et al., 1991), an initial fast component of the tail reflects deactivation of I_C and I_M (near −50 mV, I_A and I_{DR} would be inactivated); and a slowly decaying tail is generated by the long-lasting I_{AHP}. In our experience (see also Lancaster and Adams, 1986), there was seldom a clear distinction between fast and slow components of tail currents (Fig. 4). So we measured the slow tail (presumed to be mainly I_{AHP}) at 1 s after the end of the depolarizing pulse, and the fast component at the onset (this would include I_C and I_M, and perhaps some I_{AHP}).

In 5 cells clamped at ≈ −53 mV, the fast and slow components of the tails seen at the end of 40 mV pulses had mean amplitudes of 290 ± 62 pA and 87 ± 26.8 pA, respectively. They were equally depressed by tolbutamide (Fig. 4A,B), by 47 ± 10.6% and 57 ± 14.1% (for both $n = 5$ and $P < 0.025$).

3.3.3. Tolbutamide depresses I_C

To eliminate I_M and I_{AHP}, 4 cells were recorded in 20 μM carbachol and clamped at ≈ −55 mV; 40 mV pulses evoked a net I_{out} of 4.6 ± 1.2 nA. This current, made up of only I_C and I_{DR}, was much reduced by tolbutamide (by 45 ± 5.9%, $P < 0.005$) (Fig. 5). There was no recovery even after washing for 25 and 90 min ($n = 2$). Since tolbutamide had no effect on the Ca^{2+}-independent outward current in the other tests, we conclude that the tolbutamide-sensitive voltage-dependent outward current consists mainly of I_C.

3.3.4. Tolbutamide depresses also I_M

This muscarine-sensitive current is activated at potentials > −60 mV, with slow kinetics; being non-inactivating, in this range of V_m it contributes to the steady-state outward K^+ flux (Halliwell and Adams, 1982; Brown, 1988). Though not directly dependent on Ca^{2+}, it is sensitive to cytosolic [Ca^{2+}] (Tokimasa, 1985; Yu et al., 1994). To prevent its rundown (Pfaffinger, 1988; Yu et al., 1994), I_M was recorded with electrodes containing 2 mM ATP and 1 mM Mg^{2+}. In 7 cells, clamped at −30 mV, where I_M is fully activated, hyperpolarizing pulses produced slow inward relaxations (−54 ± 10.9 pA) (Fig. 6), which reflect the slow closing of M channels (Halliwell and Adams, 1982). In 6 of 7 cells, tolbutamide (1 mM) reduced the inward relaxations, for an overall depression by 41 ± 12.9% (for $n = 7$, $P < 0.025$) (Fig. 6). In 5 of these 6 cells (as in Fig. 6), there was also an inward shift in holding current (−126 ± 37 pA, $P \approx 0.025$), presumably caused mainly by suppression of ongoing I_M – which is maximally activated near −30 mV (Halliwell and Adams, 1982; Brown, 1988). In one cell, some remaining I_M was abolished by the addition of 20 μM carbachol (Fig. 6).

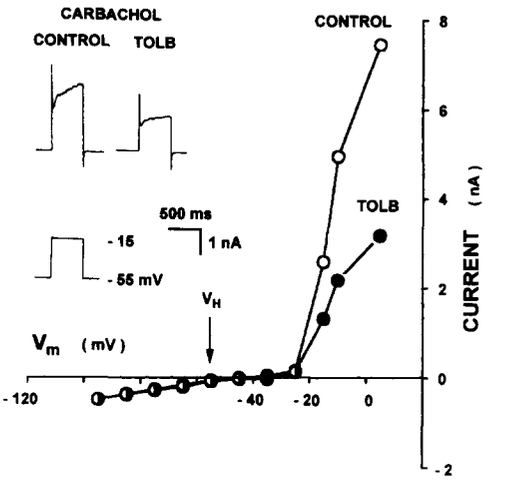

Fig. 5. Tolbutamide depresses Ca^{2+}- and voltage-dependent C current. I/V plots were obtained from single cell, with 500 ms pulses, during whole-cell recording at V_H −55 mV (arrow) in presence of 1 μM tetrodotoxin and 20 μM carbachol (to eliminate I_{AHP} and I_M): open circles are controls, closed circles, data obtained after 15 min in tolbutamide (1 mM). Inset traces are examples of currents evoked in same cell with 40 mV pulses.

3.3.5. Tolbutamide does not affect A current

In 5 cells held at ≤ −70 mV, depolarizing pulses elicited an early transient current (Gustafsson et al., 1982; Storm, 1990), with an amplitude of 145 ± 36 pA (at ≈ −40 mV). To minimize any contribution of I_C, these recordings were made in the presence of 1 mM tetraethylammonium (Lancaster and Adams, 1986; Storm, 1990). Tolbutamide

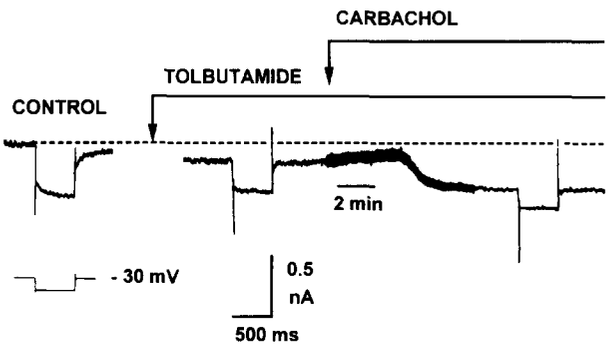

Fig. 6. Tolbutamide also depresses M current (whole-cell recording). With cell clamped at −30 mV, M-current inward relaxations were evoked by 10 mV, 200 ms hyperpolarizing pulses: they are displayed on accelerated traces obtained initially, then after 20 min in tolbutamide (1 mM), and finally after adding carbachol (20 μM). Tolbutamide induced an inward current (0.2 nA) and suppressed the inward relaxations. In carbachol, there was a further inward shift and fall in conductance. Dashed line is at initial current level (450 pA).

(1 mM) had no significant effect, either on its peak amplitude (17 ± 22.3%) or voltage dependence.

3.4. Effects of tolbutamide on voltage-dependent inward currents

3.4.1. High voltage-activated Ca^{2+} currents (I_{Ca})

Because afterhyperpolarizations are triggered by Ca^{2+} influx, they are indirectly blocked by antagonists of Ca^{2+}-channels (Lancaster and Adams, 1986; Madison and Nicoll, 1984; Storm, 1990). So we tested tolbutamide on Ca^{2+} currents, isolated as described in the Methods. HVA inward currents (mainly carried by Ba^{2+}) were evoked in 5 cells by 500 ms depolarizing steps from $V_H \approx -40$ mV. They had an early peak (-4.0 ± 0.65 nA), which rapidly decayed to a better sustained level (-2.1 ± 0.41 nA, as measured at the end of the 500 ms pulses). Tolbutamide (1 mM) had only a minor (non-significant) depressant effect, the initial peak I_{Ca} being reduced by 12.5 ± 5.8%, and the sustained I_{Ca} by 19.5 ± 9.6%. As can also be seen in Fig. 7, the voltage dependence of the I_{Ca} was not significantly changed. In 2 cells, such inward currents were subsequently eliminated by superfusion with a Mn^{2+}/low Ca^{2+} ACSF. Thus, the suppression of afterhyperpolarizations and Ca^{2+}-dependent outward currents by tolbutamide is probably not secondary to a block of I_{Ca}.

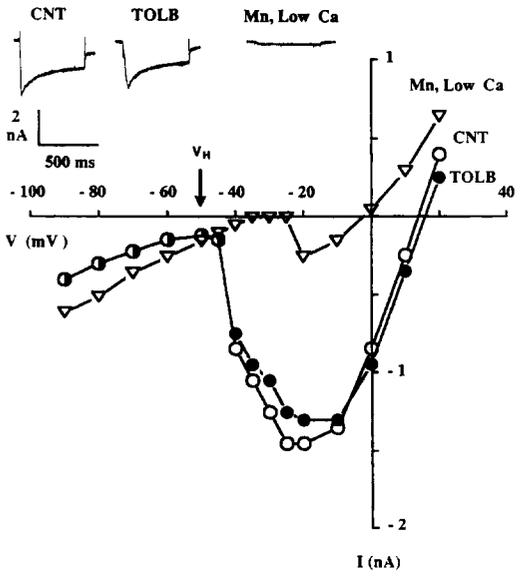

Fig. 7. Tolbutamide has no significant effect on high voltage-activated Ca^{2+} currents. Intracellular recording with CsCl electrode was from slice in PO_4^{2-} free-ACSF, containing reduced Ca^{2+} (0.5 mM), tetrodotoxin (1 μM), $BaCl_2$ (1.5 mM), CsCl (2 mM) and tetraethylammonium (10 mM). Cell was clamped at −50 mV (arrow). Open circles are control values of net sustained currents (measured at end of pulses); closed circles, sustained currents in 1 mM tolbutamide; open triangles, show that inward currents were nearly abolished after 10 min exposure to Mn^{2+}, low Ca^{2+} ACSF. Inset are examples of net currents evoked by 30 mV pulses: initial control (CNT); in presence of tolbutamide; and in Mn^{2+}, low Ca^{2+} ACSF. All data are from one cell.

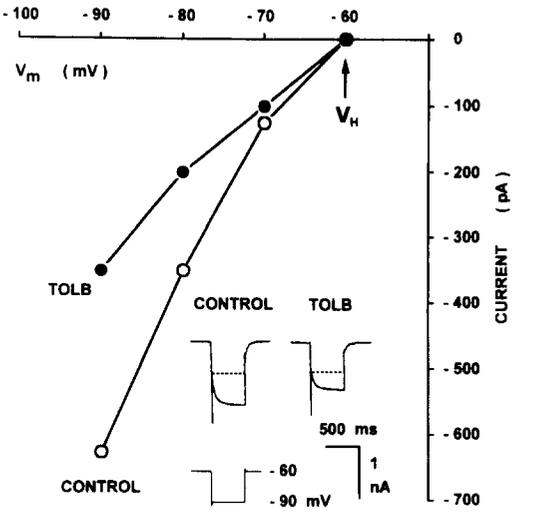

Fig. 8. Tolbutamide also reduces Q-type inward current. Whole-cell recording was at V_H −60 mV, in presence of tetrodotoxin. Slow inward relaxations during 500 ms hyperpolarizing pulses are plotted as function of voltage: open circles, controls; closed circles, in 1 mM tolbutamide. Inset traces are examples of currents evoked with 30 mV hyperpolarizing pulses; dotted lines indicate 'instantaneous' current levels from which inward relaxations were measured. All data are from a single neuron.

3.4.2. Q current (I_Q)

This Na/K-mediated inward rectifier is identified by its slow activation and resistance to Ba^{2+} (Halliwell and Adams, 1982; Brown et al., 1990; Maccaferri et al., 1993). The characteristic slow inward relaxation, evoked by hyperpolarizing pulses to ≈ −90 mV, had a mean amplitude of -340 ± 38 pA ($n = 14$). It was moderately (though significantly) depressed by tolbutamide ($-22.5 \pm 5.7\%$; for $n = 14$, $P < 0.005$) (Fig. 8). There was no recovery even after 15–20 min of wash ($n = 2$).

3.5. Glyburide (10 μM) does not depress afterhyperpolarizations or outward currents

In 8 cells, recorded under current-clamp with $KMeSO_4$-containing patch electrodes, glyburide (10 μM) produced a just significant increase in R_N, without a corresponding change in V_m (Table 2). In 7 of these cells, glyburide failed to depress either afterhyperpolarizations or the slopes of plots of afterhyperpolarizations vs. number of spikes (Table 2). Indeed, both medium and slow afterhyperpolarizations tended to increase somewhat, but only the latter perhaps significantly ($P = 0.05$) (Fig. 9 and Table 2).

When 1 mM tolbutamide was applied in the presence of glyburide ($n = 3$), it had its usual depressant effect, medium afterhyperpolarizations being reduced by 65 ± 20.1% and slow afterhyperpolarizations by 83 ± 16.7% (Fig. 9). In this respect, hippocampal neurons differ from hypothalamic glucoceptive cells, where glyburide prevents the block of K^+ channels by tolbutamide (Ashford et al., 1990).

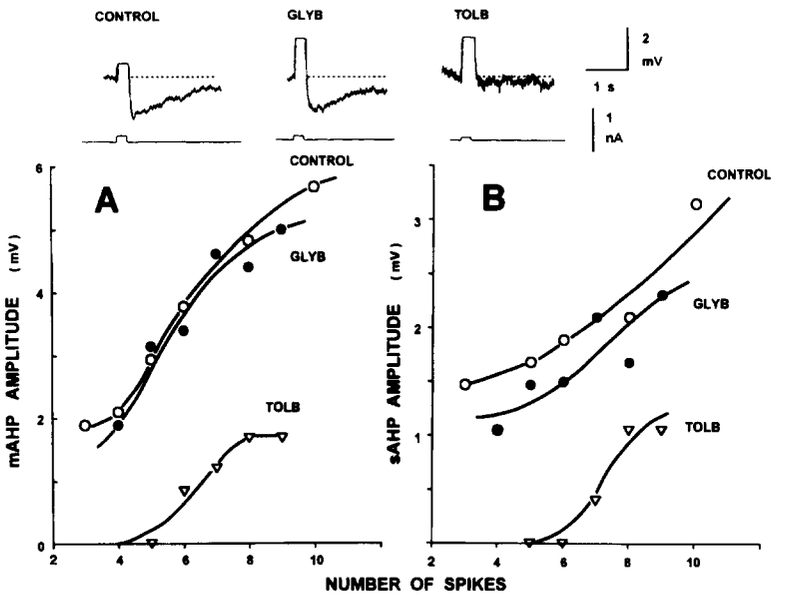

Fig. 9. Unlike tolbutamide, glyburide does not reduce afterhyperpolarizations. Whole-cell recording was throughout in presence of 0.10% dimethyl sulphoxide. (A,B) Plots of amplitudes of medium and slow afterhyperpolarizations from same cell: open circles are control values; closed circles, data obtained in presence of glyburide (10 μM), and then after adding 1 mM tolbutamide (open triangles). Inset traces are examples of afterhyperpolarizations seen after 6 spikes (below are corresponding current pulses). Membrane potential was initially -55 mV, and was held at this level.

In agreement with the current-clamp data, in 5 other cells under voltage-clamp ($V_H \approx -55$ mV), glyburide reduced G_N (by $20 \pm 7.2\%$, $P = 0.05$) without changing the base-line current (10 ± 24 pA). Outward currents evoked by pulses to ≈ -15 mV (3.2 ± 1.7 nA) were also unaffected by glyburide ($7.6 \pm 19.2\%$) (Fig. 10A). A further application of tolbutamide to one of these cells reduced the outward current by 52%.

We recently reported (Erdemli and Krnjević, 1994c) that intracellular guanosine diphosphate appears to enhance outward currents and a cromakalim-sensitive current in CA1 neurons (cromakalim activates K_{ATP} channels in some cells, Ashcroft and Ashcroft, 1992) and that this effect could be blocked by glyburide. So we also tested glyburide while recording with patch electrodes containing 1 mM guanosine diphosphate. Under these conditions,

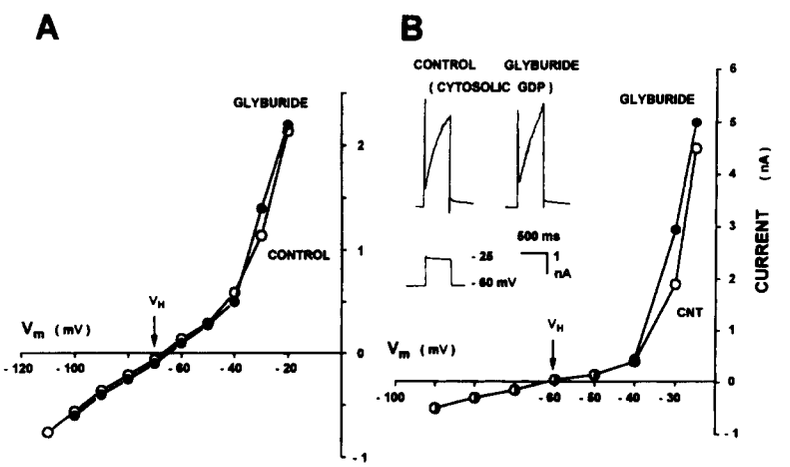

Fig. 10. Glyburide does not depress outward current, even when recorded with guanosine diphosphate (GDP)-containing patch electrode. Current-voltage plots were obtained from two cells, in presence of tetrodotoxin and dimethyl sulphoxide (0.10%, throughout). (A) Data recorded with $KMeSO_4$- and Hepes-containing electrode (V_H -70 mV); (B) from second cell, with similar electrode containing also 1 mM guanosine diphosphate (V_H -60 mV). Open circles are controls; closed circles, after 15 min in 10 μM glyburide. Inset in (B) are examples of currents recorded before and during glyburide application.

Table 2
Effects of glyburide (10 μM) on afterhyperpolarization and resting membrane properties

	Control	in glyburide (change)	N
V_m (mV)	−58 ± 3.1	−0.9 ± 1.5 mV	8
R_N (MΩ)	94 ± 12	26 ± 11% [a]	8
mAHP			
Amplitude (mV)	−4.6 ± 0.85	21 ± 13%	7
Slope (mV/spike)	−0.63 ± 0.05	6.8 ± 12%	7
sAHP			
Amplitude (mV)	−1.8 ± 0.27	66 ± 27% [a]	7
Slope (mV/spike)	−0.25 ± 0.03	97 ± 53%	7

Whole-cell recordings with $KMeSO_4$ electrodes: V_m, resting membrane potential; R_N, input resistance; mAHP, medium afterhyperpolarization; sAHP, slow afterhyperpolarization; N, number of cells; [a] $P = 0.05$.

outward currents evoked by pulses to ≈ −20 mV (2.3 ± 0.32 nA) showed no consistent change (−9.2 ± 32.0%) – as illustrated in Fig. 10B.

4. Discussion

The present finding that 0.5–1 mM tolbutamide blocks several voltage- and Ca^{2+}-activated (or Ca^{2+}-sensitive) currents in CA1 neurons, including I_M, I_C and I_{AHP}, as well as I_Q, is further evidence that, at these concentrations, tolbutamide is not just a specific K_{ATP} blocker. This is in agreement with previous reports of a variety of other actions of tolbutamide (listed in the Introduction) – including depression of the D-type K^+ currrent (Crépel et al., 1993), which may also be Ca^{2+}-dependent (Hamon et al., 1994). Albeit a much higher concentration than is effective on insulin-secreting cells, 0.5–1 mM tolbutamide is routinely applied in tests on many tissues, including cardiac muscle (Belles et al., 1987) and central neurons (Ashford et al., 1990; Jiang et al., 1994). The present results are therefore of quite wide relevance.

4.1. Effects of tolbutamide on resting membrane properties

Previous studies on several tissues, including brain, have reported that tolbutamide causes a depolarization and increase in input resistance as a result of block of K_{ATP} (Ashford et al., 1990; Henquin and Meissner, 1982). In the present experiments, tolbutamide increased R_N (or decreased G_N) without any consistent change in either V_m or I_H. Tolbutamide's effect on resting G_N can be explained by the suppression of K^+ currents likely to be active near resting V_m, such as I_D, I_M and perhaps I_C and I_{AHP}. Bearing in mind that $KMeSO_4$ does not favour K_{ATP} activity (McKillen et al., 1994), it is unlikely that blockage of K_{ATP} contributed in a major way to this effect. The absence of any consistent depolarization (or inward shift under voltage-clamp) is presumably due to the concurrent depression of ongoing *inward* I_Q (Maccaferri et al., 1993; Krnjević and Xu, 1996).

4.2. Tolbutamide reduces voltage-activated Ca^{2+}-dependent conductances

This is strongly indicated by three findings: first, tolbutamide very substantially depresses both the medium and the slow afterhyperpolarizations; second, it causes a similar depression of sustained outward currents (and tail currents) evoked under voltage-clamp by depolarizing pulses; third, it is ineffective in a Mn^{2+}/low Ca^{2+} medium that eliminates Ca^{2+}-dependent currents. Accordingly, tests on isolated currents showed that I_C, I_{AHP}, I_M and I_Q – all either Ca^{2+}-activated or Ca^{2+}-sensitive (Hagiwara and Irisawa, 1989; Lancaster and Adams, 1986; Storm, 1990; Yu et al., 1994) – were reduced by tolbutamide; whereas I_{DR} and I_A were not.

The afterhyperpolarizations of hippocampal cells are believed to have two components: the medium, lasting 50–100 ms and the slow, lasting more than 1 s. The medium afterhyperpolarization (or the early outward tail current under voltage-clamp) is mediated mainly by I_M, I_C and inactivation of I_Q (Lancaster and Adams, 1986; Storm, 1989; Maccaferri et al., 1993). Depression of these currents would satisfactorily explain why medium afterhyperpolarization were suppressed by tolbutamide. Similarly, the reduction of the slow tail current (I_{AHP}) is obviously in keeping with the depression of slow afterhyperpolarizations.

A simple explanation for the Ca^{2+} dependence of currents depressed by tolbutamide would be that tolbutamide blocks Ca^{2+} currents. But this was evidently not the case. Another possibility is that tolbutamide acts more-or-less directly on mechanisms of cytosolic $[Ca^{2+}]$ homeostasis or at the site where Ca^{2+} triggers or modulates G_K. There is good evidence that tolbutamide suppresses several cyclic AMP-mediated phenomena – such as lypolysis (Brown et al., 1972), activation of kinase A (Kanamori et al., 1976) and cystic fibrosis transmembrane conductance regulator Cl^- currents (Sheppard and Welsh, 1994). A block of protein kinase A, however, would not explain the suppression of afterhyperpolarizations, which are strongly *depressed* by cyclic AMP (Madison and Nicoll, 1986); but if cyclic AMP and protein kinase A play an important role in $[Ca^{2+}]_i$ homeostasis by stimulating Ca^{2+} release from mitochondria (Borle, 1974), tolbutamide could markedly lower $[Ca^{2+}]_i$. Alternatively, tolbutamide may inactivate a Ca^{2+}-dependent protein kinase that modulates the tolbutamide-sensitive currents. These kinds of actions are unlikely to be a competitive type of block, and therefore may explain why tolbutamide's effects were not readily reversible by washing.

The irreversible effects of tolbutamide inevitably suggest a possible rundown, even when recording with

$KMeSO_4$ and ATP-containing patch electrodes (Zhang et al., 1994). However, comparable applications of glyburide were ineffective. Moreover, afterhyperpolarizations recorded with $KMeSO_4$-containing 'sharp' electrodes, albeit much less prone to rundown, were also depressed by tolbutamide. So its inhibitory effect cannot be simply due to rundown of afterhyperpolarizations during patch recording.

Various components of pipette-filling solutions can have marked and often unexpected effects on membranes currents (Kay, 1992). In our experiments, afterhyperpolarizations were somewhat smaller and tolbutamide was ineffective in recordings with KCl electrodes. It has long been known that high intracellular [Cl^-] is not conducive to stable intracellular recording from cortical neurons (Kelly et al., 1969). It appears that Cl^- depresses several K^+ currents (Lenz et al., 1994; Zhang et al., 1994), perhaps by increasing the sensitivity of G-proteins to guanosine triphosphate (Nakajima et al., 1992). A variety of plausible mechanisms have been proposed, such as changes in protein conformation and cytoskeletal structure, but none supported by compelling evidence. There is a curious (and significant) contrast in this respect between I_{AHP} and K_{ATP}: although Cl^- greatly accelerates the rundown of both types of channels, $MeSO_4^-$, but not gluconate$^-$, prevents I_{AHP} rundown (Zhang et al., 1994); whereas gluconate$^-$, but not $MeSO_4^-$, prevents rundown of K_{ATP} (at least in muscle, McKillen et al., 1994).

Like tolbutamide, glyburide lowers G_N – probably by reducing I_D (Crépel et al., 1993). However, because glyburide does not depress afterhyperpolarizations (or outward currents), at these concentrations, it is a more selective agent. In the light of the present results, the fact that tolbutamide (but not glyburide) can suppress the anoxic hyperpolarization of CA1 neurons suggests that this hyperpolarization could be mediated mainly by I_{AHP}, activated by a rise in cytosolic Ca^{2+}, at least partly due to Ca^{2+} release from an IP_3-sensitive store (Belousov et al., 1995). Although these results point to Ca^{2+}-dependent K^+ channels as the predominant site of tolbutamide's action, we cannot exclude the interesting possibility that these K^+ channels are sensitive to both ATP and Ca^{2+}, like certain channels observed in hypothalamic (Treherne and Ashford, 1991) and nigral neurons (Jiang et al., 1994).

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References

Aguilar-Bryan, L., C.G. Nichols, S.W. Wechsler, J.P. Clement IV, A.E. Boyd III, G. Gonzalez, H. Herrera-Sosa, K. Nguy, J. Bryan and D.A. Nelson, 1995, Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion, Science 268, 423.

Ashcroft, S.J.H. and F.M. Ashcroft, 1992, The sulphonylurea receptor, Biochim. Biophys. Acta 1175, 45.

Ashford, M.L.J., P.R. Boden and J.M. Treherne, 1990, Tolbutamide excites rat glucoreceptive ventromedial hypothallamic neurons by indirect inhibition of ATP-K^+ channels, Br. J. Pharmacol. 101, 531.

Ashford, M.L.J., C.T. Bond, T.A. Blair and J.P. Adelman, 1994, Cloning and functional expression of a rat heart K_{ATP} channel, Nature 370, 456.

Belles, B., J. Hescheler and G. Trube, 1987, Changes of membrane currents in cardiac cells induced by long whole-cell recordings and tolbutamide, Pflügers Arch. 409, 582.

Belousov, A.B., J.-M. Godfraind and K. Krnjević, 1995, Internal Ca^{2+} stores involved in anoxic responses of rat hippocampal neurones, J. Physiol. 486, 547.

Ben-Ari, Y., 1990, Galanin and glibenclamide modulate the anoxic release of glutamate in rat CA3 hippocampal neurons, Eur. J. Neurosci. 2, 62.

Ben-Ari, Y. and M. Lazdunski, 1989, Galanin protects hippocampal neurons from the functional effects of anoxia, Eur. J. Pharmacol. 165, 331.

Borle, A.B., 1974, Cyclic AMP stimulation of calcium efflux from kidney, liver and heart mitochondria, J. Memb. Biol. 16, 221.

Brown, D.A., 1988, M-currents, in: Ion Channels, Vol. 1, ed. T. Narahashi (Plenum, New York) p. 55.

Brown, D.A., B.H. Gähwiler, W.H. Griffith and J.V. Halliwell, 1990, Membrane currents in hippocampal neurons, Prog. Brain Res. 83, 141.

Brown, J.D., A.A. Steele, D.B. Stone and F.A. Steele, 1972, The effect of tolbutamide on lipolysis and cyclic AMP concentration in white fat cells, Endocrinology 90, 47.

Crépel, V., K. Krnjević, and Y. Ben-Ari, 1993, Sulphonylureas reduce the slowly-inactivating D-type outward current in rat hippocampal neurones, J. Physiol. 466, 39.

DeWeille, J.R. and M. Lazdunski, 1990, Regulation of the ATP-sensitive potassium channel, in: Ion Channels, Vol. 2, ed. T. Narahashi (Plenum Press, New York) p. 205.

Doroshenko, P., R. Penner and E. Neher, 1991, Novel chloride conductance in the membrane of bovine chromaffin cells activated by intracellular GTPγS, J. Physiol. 436, 711.

Erdemli, G. and K. Krnjević, 1994a, Effects of anoxia on CA1 hippocampal neurons recorded by whole-cell patch clamping, Proc. Can. Fed. Biol. Soc. p. 123.

Erdemli, G. and K. Krnjević, 1994b, Tolbutamide suppresses slow and medium afterhyperpolarization in hippocampal slices, NeuroReport 5, 2145.

Erdemli, G. and K. Krnjević, 1994c, Guanosine diphosphate is required for activation of a glyburide, ATP and cromakalim-sensitive outward current in rat hippocampal neurons, NeuroReport 5, 1362.

Esplin, B., G. Erdemli and K. Krnjević, 1995, Tolbutamide suppresses several voltage-dependent K currents of CA1 hippocampal neurons, Soc. Neurosci. Abstr. 21, 1832.

Godfraind, J.M. and K. Krnjević, 1993, Tolbutamide suppresses anoxic outward current of hippocampal neurons, Neurosci. Lett. 162, 101.

Gustafsson B., M. Galvan, P. Grafe and H. Wigström, 1982, A transient outward current in a mammalian central neurone blocked by 4-aminopyridine, Nature 299, 252.

Hagiwara, N. and H. Irisawa, 1989, Modulation by intracellular Ca^{2+} of the hyperpolarization-activated inward current in rabbit single sino-atrial node cells. J. Physiol. 409, 121.

Halliwell, J.V. and P.R. Adams, 1982, Voltage-clamp analysis of muscarinic excitation in hippocampal neurons, Brain Res. 250, 71.

Hamon, B., E. Audinat and F. Crépel, 1994, Calcium-dependent, slowly inactivating K^+ currents in cultured neurons of rat dissociated cortex, J. Physiol. 477, 95P.

Hansen, A.J., J. Hounsgaard and H. Jahnsen, 1982, Anoxia increases

potassium conductance in hippocampal nerve cells, Acta Physiol. Scand. 115, 301.

Henquin, J.C. and H.P. Meissner, 1982, Opposite effects of tolbutamide and diazoxide on $^{86}Rb^+$ fluxes and membrane potential in pancreatic β cells, Biochem. Pharmacol. 31, 1407.

Inagaki, N., T. Gonoi, J.P. Clement IV, N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino and J. Bryan, 1995, Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor, Science 270, 1166.

Jiang, C., F.J. Sigworth, G.G. Haddad, 1994, Oxygen deprivation activates an ATP-inhibitable K^+ channel in substantia nigra neurons, J. Neurosci. 14, 5590.

Kanamori, T., T. Hayakawa and T. Nagatsu, 1976, Characterization of protein kinases from bovine parotid glands. The effect of tolbutamide and its derivative on these partially purified enzymes, Biochim. Biophys. Acta 429, 147.

Kay, A.R., 1992, An intracellular medium formulary, J. Neurosci. Meth. 44, 91.

Kelly, J.S., K. Krnjević, M.E. Morris and G.K.W. Yim, 1969, Anionic permeability of cortical neurones, Exp. Brain Res. 7, 11.

Kramer, J.H., W.G. Lampson and S.W. Schaffer, 1983, Effect of tolbutamide on myocardial energy metabolism, Am. J. Physiol. 245, H 313.

Krnjević, K., 1993, Membrane current activation and inactivation during hypoxia in hippocampal neurons, in: Surviving Hypoxia, ed. P.W. Hochachka, P.L. Lutz, T. Sick, M. Rosenthal and G. Van der Thillard (CRC Press, Boca Raton, FL) p. 365.

Krnjević, K. and Y.Z. Xu, 1989, Dantrolene suppresses the hyperpolarization or outward current observed during anoxia in hippocampal neurons, Can. J. Physiol. Pharmacol. 67, 1602.

Krnjević, K. and Y.Z. Xu, 1996, Dantrolene depolarizes hippocampal neurons in slices from rats, Can. J. Physiol. Pharmacol. 74, in press.

Krnjević, K., G. Erdemli and A.T. Tan, 1994, Anoxic hyperpolarization of hippocampal neurons is probably mediated by activation of slow-AHP K channels, Soc. Neurosci. Abstr. 20, 223.

Lancaster, B. and P.R. Adams, 1986, Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons J. Neurophysiol. 55, 1268.

Lancaster, B., R.A. Nicoll and D.J. Perkel, 1991, Calcium activates two types of potassium channels in rat hippocampal neurons in culture, J. Neurosci. 11, 23.

Lenz, R.A., T.A. Pitler, P.J. Yarowsky and B.E. Alger, 1994, Intracellular Cl^- diminishes G-protein-activated K^+ conductances in rat hippocampal neurons, Soc. Neurosci. Abstr. 20, 1519.

Maccaferri, C., M. Mangoni, A. Lazzari and D. DiFrancesco, 1993, Properties of the hyperpolarization-activated current in rat hippocampal CA1 pyramidal cells, J. Neurophysiol. 69, 2129.

Madison, D.V. and R.A. Nicoll, 1984, Control of repetitive discharge of rat CA1 pyramidal neurons in vitro, J. Physiol. 354, 319.

Madison, D.V. and R.A. Nicoll, 1986, Cyclic adenosine 3,5'-monophosphate mediates β-receptor actions of noradrenaline in rat hippocampal pyramidal cells, J. Physiol. 372, 245.

McKillen, H.C., N.W. Davies, P.R. Stanfield and N.B. Standen, 1994, The effect of intracellular anions on ATP-dependent potassium channels of rat skeletal muscle, J. Physiol.479, 341.

Mourre, C., Y. Ben-Ari, H. Bernardi, M. Fosset, M. and M. Lazdunski, 1989, Antidiabetic sulfonylureas: localization of binding sites in the brain and effects on the hyperpolarization induced by anoxia in hippocampal slices, Brain Res. 486, 159.

Mourre, C., C. Widmann and M. Lazdunski, 1991, Specific hippocampal lesions indicate the presence of sulfonylurea binding sites associated to ATP-sensitive K^+ channels both post-synaptically and on mossy fibers, Brain Res. 540, 340.

Nakajima, T., T. Sugimoto and Y. Kurachi, 1992, Effects of anions on the G protein-mediated activation of the muscarinic K^+ channel in the cardiac atrial cell membrane, J. Gen. Physiol. 99, 665.

Pfaffinger, P., 1988, Muscarine and t-LHRH suppress M-current by activating an IAP-sensitive G protein, J. Neurosci. 8, 3343.

Schwiebert, E.M., M.E. Egan, T.H. Hwang, S.B. Fulmer, S.A. Allen, G.R. Cutting and W.B. Guggino, 1995, CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP, Cell 81, 1063.

Sheppard, D.N. and M.J. Welsh, 1994, Effect of ATP-sensitive channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents, J. Gen. Physiol. 100, 573.

Storm, J.F., 1989, An after-hyperpolarization of medium duration in rat hippocampal pyramidal cells, J. Physiol. 409, 171.

Storm, J.F., 1990, Potassium currents in hippocampal pyramidal cells, Prog. Brain Res. 83, 161.

Tokimasa, T., 1985, Intracellular Ca^{2+}-ions inactivate K^+-current in bullfrog sympathetic neurons, Brain Res. 337, 386.

Treherne, J.M. and M.L.J. Ashford, 1991, The regional distribution of sulphonylurea binding sites in rat brain, Neuroscience 40, 523.

Tremblay, E., S. Zini and Y. Ben-Ari, 1991, Autoradiographic study of the cellular localization of [^{3}H] glibenclamide binding sites in the rat hippocampus, Neurosci. Lett. 127, 21.

Yu, S.P., M.D. O'Malley and P.R. Adams, 1994, Regulation of M current by intracellular calcium in bullfrog sympathetic ganglion neurons, J. Neurosci. 14, 3487.

Zhang, L., J.L. Weiner, T.A. Valiante, A.A. Velumian, P.L. Watson, S.S. Jahromi, S. Schertzer, P. Pennefather and P.L. Carlen, 1994, Whole-cell recording of the Ca^{2+}-dependent slow afterhyperpolarization in hippocampal neurones: effects of internally applied anions, Pflügers Arch. 426, 247.