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Lack of effect of potassium channel openers on ATP-modulated potassium channels recorded from rat ventromedial hypothalamic neurones

A.J. Sellers, *P.R. Boden & [1]M.L.J. Ashford

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QJ and *Park-Davis Research Unit, Department of Pharmacology, Tennis Court Road, Cambridge, CB2 1QJ

1 Single neuronal cells were freshly isolated from the ventromedial hypothalamic nuclei (VMHN) of the rat brain. Currents through ATP-modulated and large conductance (160 and 250 pS) calcium-activated potassium channels were recorded by the cell-attached and excised inside-out patch techniques.

2 BRL38227 (lemakalim; 30–90 μM) applied to the superfusing medium produced no change in firing rate of isolated glucose-receptive VMHN neurones in cell-attached recordings.

3 BRL38227, at concentrations of between 30–100 μM applied to the intracellular (cytoplasmic) aspect of inside-out patches, had no effect on the activity of ATP-sensitive K^+ channels in the absence of ATP or in the presence of a sub-maximal inhibitory concentration (3 mM) of ATP. Cromakalim, pinacidil, minoxidil sulphate and diazoxide also produced no effect under these conditions.

4 The potassium channel openers (KCO's) were tested on ATP-activated potassium channels recorded from a further subpopulation of VMHN neurones. Application of BRL38227 (up to and including 100 μM) to this channel in inside-out patches either in the absence of ATP or when activated by 5 mM ATP had no effect on channel activity. Identical results were obtained with cromakalim and pinacidil.

5 BRL38227 had no effect on either of the large conductance (250 pS and 160 pS) calcium-activated potassium channels in VMHN neurones.

6 Intracellular recordings were made from glucose-receptive VMHN neurones in rat brain slices. Cromakalim (50 μM) or diazoxide (60 μM) did not alter the firing rate or passive membrane properties of these neurones demonstrated to be sensitive to tolbutamide (0.1 mM).

7 These results show that the KCO's tested in this study have no effect either on VMHN neurones contained in brain slices or on the activity of any of the ATP-modulated potassium channels under isolated patch conditions associated with these neurones.

Keywords: Potassium channel openers; BRL38227; ATP-sensitive K^+ channels; Ca^{2+}-activated K^+ channels; hypothalamic neurones

Introduction

The heterogeneous class of drugs known collectively as the potassium channel openers (KCO's), have been widely researched as powerful smooth muscle relaxing agents with both hypotensive and bronchodilator activity *in vivo* (for review see Robertson & Steinberg, 1990). Recently it has been suggested that these compounds may be of some use in diseases of the central nervous system (Miller, 1990; Aronson, 1992). Cromakalim (BRL 34915) is an isomeric benzopyran molecule with activity residing mainly in the 3S-4R configuration (BRL 38227, [lemakalim]). In the CNS, cromakalim has been shown to be effective in reducing seizure activity in two models of epilepsy (Gandolfo *et al.*, 1989a,b), and has also been shown to affect neuronal excitability (Alzheimer *et al.*, 1988; Politi & Rogawski, 1991). Furthermore, potassium channel openers have been shown to block neurosecretion (Schmid-Antomarchi *et al.*, 1990). The target channel for these agents has been identified in some peripheral tissues. For instance, in cardiac muscle cromakalim has been shown to activate a glibenclamide-sensitive potassium current in isolated ventricular myocytes (Escande *et al.*, 1988; Sanguinetti *et al.*, 1988), and adenosine 5'-triphosphate (ATP)-sensitive K^+ channels isolated in membrane patches from the same cells have also been shown to be activated by cromakalim (Escande *et al.*, 1988) and pinacidil (Fan *et al.*, 1990). In skeletal muscle cells, potassium channel openers including cromakalim, pinacidil and RP 49356 have also been demonstrated to activate a sulphonylurea-sensitive potassium conductance (Quasthoff *et al.*, 1989). However, it is not as yet clear which type of potassium channel is affected by these drugs in the CNS (Alzheimer *et al.*, 1988) or smooth muscle (Weston, 1989). Although it has been little used in CNS preparations the direct approach of single channel recording offers the best opportunity to determine the target site (channel) of these compounds and perhaps indicate the possible mechanisms of action.

Single channel recording techniques have identified three distinct potassium channels in neurones in the ventromedial hypothalamic nuclei (VMHN) the activity of which is modulated by intracellular levels of ATP. The ATP-sensitive K^+ channel is inhibited by increasing levels of intracellular ATP or by the sulphonylurea tolbutamide and is located in glucose receptive neurones where it contributes to resting membrane potential (Ashford *et al.*, 1990a,b). This channel closes under conditions of elevated extracellular glucose (Ashford *et al.*, 1990a) causing membrane depolarization in a manner analogous to that demonstrated in the pancreatic β-cell (Ashcroft *et al.*, 1988). An ATP-activated K^+-channel which in recordings from inside-out patches responds to elevated intracellular ATP levels with a large increase in channel activity, can be isolated from glucose sensing neurones which respond to elevated extracellular glucose with

[1] Author for correspondence.

a hyperpolarization possibly mediated by the opening of these channels (Rowe *et al.*, 1992). A third type of ATP-sensitive K^+ channel is sometimes observed in patches excised from VMHN neurones. This channel has been classed, according to its conductance (250 pS), as a maxi-calcium-activated potassium channel. However, this channel also shows sensitivity to changes in intracellular ATP (Treherne & Ashford, 1991). A separate large conductance (160 pS) Ca^{2+}-activated K^+-channel has also shown to be present in glucose-receptive neurones of the VMHN, and its activity is not sensitive to intracellular ATP levels (Treherne & Ashford, 1991).

As the ATP-K^+ channel is the target channel for cromakalim in cardiac muscle (Sanguinetti *et al.*, 1985) and various potassium channel openers can, at high concentrations, inhibit insulin release from the pancreatic β-cell by increasing ATP-sensitive potassium channel activity (Garrino *et al.*, 1989) the aim of the present experiments was to examine the effects of various potassium channel openers and this benzopyran molecule in particular on the membrane potential and spontaneous action potential firing rate of intact VMHN neurones and on the ATP-modulated potassium channels that can be recorded from isolated membrane patches.

Methods

Electrical recording and analysis

Coronal slices (350 μm thick) of hypothalamus were cut from brains of male Sprague-Dawley rats (80–200 g weight) with a Vibratome (Oxford Instruments). The slices were maintained at room temperature in artificial cerebrospinal fluid (ACSF) bubbled with 95% O_2 and 5% CO_2. For intracellular recording, the slices were transferred to a recording chamber where they were superfused with ACSF at 37°C. Electrodes were filled with 1 M potassium acetate and had d.c. resistances of 100–150 MΩ when measured in physiological saline. A period of 30 min was allowed for equilibration following impalement. Input resistances were derived from the slope of the current-voltage plot obtained by measuring the electronic potential during current injection. Pulses, of greater than 100 ms duration, were applied in order to ensure complete capacitance saturation of the membrane. All experiments were performed at 37°C. These recording procedures have been described in full elsewhere (Boden & Hill, 1988).

For single channel recording from VMHN neurones, cells were acutely dissociated from the VMHN isolated from rat hypothalamic slices. The nuclei were incubated with 0.5 mg ml^{-1} collagenase (Clostridiopaptidase A. Boehringer, Mannheim) and 1 mg ml^{-1} trypsin (Type XII, Sigma, Poole, Dorset) in ACSF at room temperature for 1–2 h and then triturated by the use of flame polished Pasteur pipettes. The dispersed cells were transferred onto Falcon 3001 dishes (35 mm) and left for 30 min to adhere. Single channel currents were recorded, at room temperature (21°C), from cell-attached and inside-out membrane patches by standard patch-clamp recording procedures (Hamill *et al.*, 1981). Current recordings were made with an Axopatch 2D patch clamp amplifier and stored on magnetic tape (Racal 4DS) for later reproduction of figures and analysis. The potential across the membrane is described with the usual sign convention for membrane potential (i.e. inside negative). The data were analysed for current amplitude and open-state probability P_{open} by computer (Apricot XEN-i 286/45) as described previously (Sturgess *et al.*, 1988; Kozlowski *et al.*, 1989). All data in the text are presented as mean values ± s.e.mean.

Solutions

The ACSF contained (in mM): NaCl 128.0, KCl 5.0, NaH_2PO_4 1.2, $CaCl_2$ 2.4, $MgCl_2$ 1.3, $NaHCO_3$ 26.0, D-glucose 10.0, pH 7.4. Before single channel recordings the cells were washed with normal physiological salt solution (PSS) consisting of (mM): NaCl 135.0, KCl 5.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, HEPES 10.0 pH 7.4 with NaOH. For cell-attached and inside-out recordings the patch pipette contained (mM): KCl 140.0, $CaCl_2$ 1.0, $MgCl_2$ 1.0, HEPES 10.0, pH 7.2 with KOH and the bathing solution was either the normal external PSS (cell-attached recordings) or an intracellular solution (inside-out recordings) containing (mM): KCl 140.0, $MgCl_2$ 1.0, $CaCl_2$ 0.9, EGTA 1.0, HEPES 10.0, pH 7.2 with KOH (free Ca^{2+} concentration of 0.8 μM). Free calcium concentrations were controlled and changed by use of EGTA, and determined by the 'METLIG' metal ion/ligand binding programme (P. England & R. Denton, University of Bristol).

Drugs

All potassium channel openers were made up in a 10^{-2} M stock solution in 70% ethanol. Diazoxide, minoxidil sulphate and tolbutamide were obtained from Sigma Chemicals, Poole, Dorset. For brain slice experiments cromakalim was made up as a 10^{-2} M stock in dried dimethylsulphoxide (DMSO) and diluted to the required concentration in ACSF. We acknowledge kind gifts of pinacidil from Leo Pharmaceuticals, Ballerup, Denmark and cromakalim and BRL38227 from SmithKline Beecham Pharmaceuticals, Welwyn, Herts.

Results

Inside-out patches were obtained from isolated VMHN neurones bathed in symmetrical potassium solutions. In approximately one third of patches obtained from the VMHN cells ($n = 28$) the ATP-K^+ channel was observed and identified by its conductance (146 pS) and sensitivity to ATP. The ATP-K^+ channel can be isolated from glucose-receptive neurones that respond to elevated extracellular glucose with a depolarization mediated by closure of these ATP-K^+ channels (Ashford *et al.*, 1990a). ATP caused a concentration-dependent reduction in channel activity with an IC_{50} of approximately 3 mM, with channel activity exhibiting no sensitivity to calcium (Ashford *et al.*, 1990a). To reduce the activity of the large conductance (160 pS) calcium-activated potassium channel observed in most patches, the free calcium concentration was reduced to very low (nM) levels. Figure 1 shows a typical trace showing activity of an ATP sensitive K^+ channel recorded from an inside-out patch isolated from a VMHN neurone. In the absence of ATP the channel had a P_{open} of 0.863. The addition of 3 mM ATP caused a reduction in channel activity to an open probability of 0.110. BRL38227 was then applied concomitant with 3 mM ATP at a concentration of 30 μM and caused no observable change in channel activity (P_{open}; 0.110). The effects of ATP were readily reversed on washing (P_{open}; 0.870). BRL38227 was also applied under identical conditions at a concentration of 100 μM in three further experiments and no activation of channel activity was observed. Likewise, no other potassium channel opener tested on ATP-K^+ channel activity recorded from inside-out VMHN membrane patches, inhibited submaximally by 3 mM ATP, produced any observable activation. The openers tested were cromakalim (100 μM, $n = 2$), pinacidil (30 μM, $n = 2$, and 100 μM, $n = 2$), minoxidil sulphate (100 μM, $n = 3$) and diazoxide (100 μM, $n = 2$). For example, the typical open state probability of the ATP-K^+ channel in such an experiment was; control 0.774, 3 mM ATP 0.250, 3 mM ATP + 100 μM pinacidil 0.246, wash 0.800. In order to determine whether the KCO's had an underlying effect on ATP-K^+ channel activity, they were applied to the isolated inside-out patch in the absence of ATP. For exam-

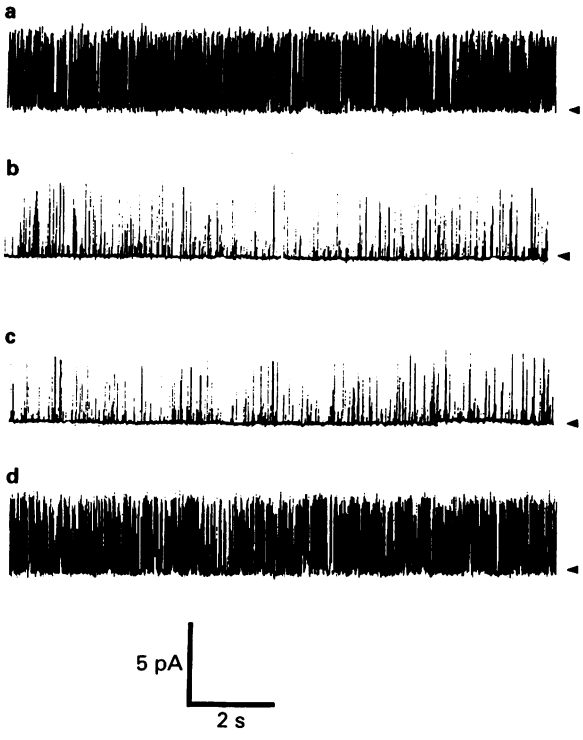

Figure 1 Single channel currents recorded from a single inside-out patch, excised from a VMHN neurone, held at a membrane potential of +30 mV. The recording pipette contained (in mM). KCl 140, $MgCl_2$ 1 and $CaCl_2$ 1, and the bath (in mM) KCl 140, $MgCl_2$ 1, EGTA 1 (with a free calcium concentration of <10 nM). The arrow denotes the channel in its closed state. Upward deflections indicate outward currents. (a) Channel activity in absence of ATP. (b) Application of 3 mM ATP to the bathing solution inhibits channel activity. (c) In the continued presence of ATP, BRL38227 at a concentration of 30 μM does not alter channel activity. (d) Wash demonstrates reversibility of effect of ATP. The values of P_{open} were as follows: control 0.863; 3 mM ATP 0.110; 3 μM BRL38227 + 3 mM ATP 0.110; wash 0.870.

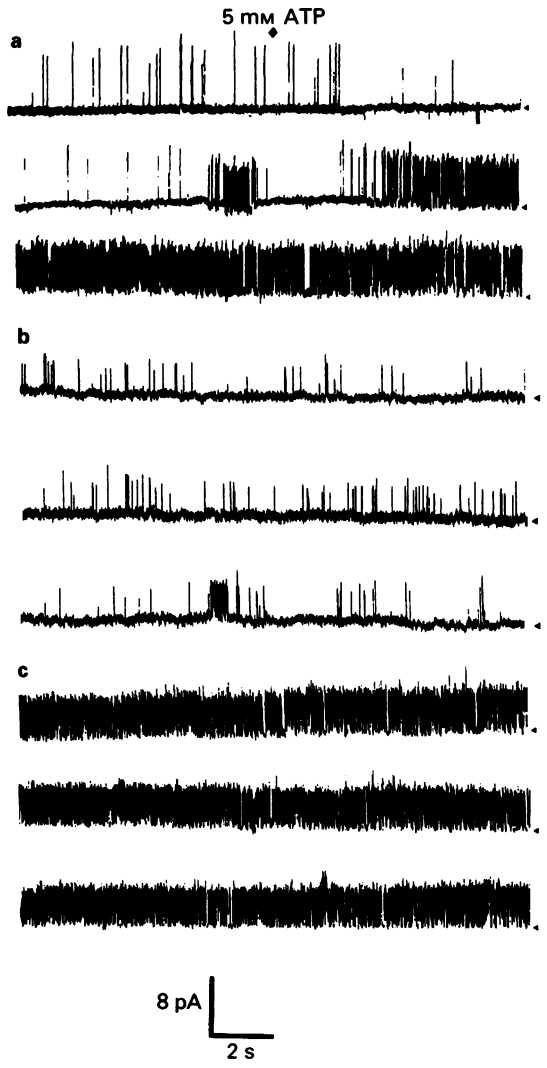

Figure 2 The lack of effect of BRL38227 on an ATP-activated K^+ channel recorded from an inside-out patch excised from a VMHN neurone. The patch potential was +40 mV, and the solutions were as follows (in mM): pipette, KCl 140, $MgCl_2$ 1, $CaCl_2$ 1; bath, KCl 140, $MgCl_2$ 1, free Ca^{2+} <10 nM. The channel closed state is denoted by the arrow. (a) Consecutive recordings showing activation of 5 mM ATP (start addition at diamond). P_{open} in absence of ATP, 0.030, after application of ATP, 0.680. Effects of ATP fully reversible on washing (not shown; P_{open} 0.03). (b) Effect on ATP-activated K^+ channel of BRL38227 (30 μM) in the absence of ATP. P_{open} values were; 0.020 control, 0.025 BRL38227, 0.025 wash. (c) Effect of BRL38227 on channel activated by 5 mM ATP. The values of the open probability were control 0.820; BRL38227 0.816; wash 0.819.

ple, there was no observable inhibition of ATP-K^+ channel activity induced by BRL38227 at either concentration tested (30 μM, $n = 3$ and 100 μM, $n = 3$). In a typical experiment the P_{open} was 0.668 in the absence of applied ATP, 0.666 in the presence of 100 μM BRL38227, and 0.666 after washout of the BRL38227.

There is a separate population of neurones that can be isolated from the lateral hypothalamic area (LHA), and, to a lesser extent from the VMHN, which respond to an increase in extracellular glucose with a hyperpolarization and have thus been termed glucose-sensing neurones (Oomura *et al.*, 1974). Following cell-attached recording and excision of the patch into the inside-out configuration an ATP-activated potassium channel is observed from such cells (Rowe *et al.*, 1992). In the present series of experiments, this channel had a conductance of 137.4 ± 3.6 pS ($n = 10$) in symmetrical potassium solutions and could be activated in a concentration-dependent manner by ATP. Application of ATP caused a rapid and marked increase in channel open probability (Figure 2a) which was fully reversible on washing. This ATP-activated K^+ channel was identified (by its conductance and low P_{open} in the absence of ATP) in approximately 10% of patches isolated from VMHN neurones. In the absence of applied ATP the P_{open} was 0.031 ± 0.016 ($n = 10$). Figure 2b shows the lack of effect of BRL38227 at a concentration of 30 μM on channel activity with the patch membrane held at a potential of + 40mV in the un-activated state (i.e. 0 mM ATP). No change in channel activity was observed when BRL38227 was applied at this concentration ($n = 3$) or at a higher concentration (100 μM, $n = 2$). Other potassium channel openers tested (pinacidil and cromakalim, both at 100 μM with $n = 2$, data not shown) also failed to alter channel activity under these conditions. Figure 2c shows a typical example of BRL38227 when applied to an ATP-activated K^+ channel after activation by 5 mM ATP. In this case the membrane potential was also held at + 40mV, and the channel P_{open} increased from 0.030 to 0.820 in the presence of the ATP. BRL38227, at 30 μM had no inhibitory effect on the channel under these conditions. This was repeated on a further three separate inside-out patches with the same results. In order to show that BRL38227 had no effect on the sensitivity of the channel to ATP, the channel was activated by 5 mM ATP and this was then washed off, with a subsequent reversal of effect (P_{open} values; control 0.023, 5 mM ATP 0.760, wash 0.030). BRL38227 was then applied prior to re-application of ATP, and no alteration in the respon-

siveness of the channel to ATP was observed (P_{open} values; 30 μM BRL38227 0.028, 30 μM BRL38227 + 5 mM ATP 0.800).

Another potassium-selective channel that may be isolated from VMHN neurones is a large-conductance (250 pS) calcium-activated potassium channel the activity of which is also modulated by ATP (Treherne & Ashford, 1991). This channel is distinct from the 160 pS calcium-activated K^+ channel observed in VMHN neurones in that it is not thought to be present in glucose-receptive neurones and is not ATP-sensitive (Treherne & Ashford, 1991). Figure 3 shows the channel activity of this Ca^{2+}–K^+ channel in an inside-out patch held at a membrane potential of −30mV. Note the lack of effect of BRL38227 (60 μM), under conditions of free calcium levels of either 0.8 μM or <10 nM, which are associated with different levels of P_{open}. When the free calcium level was elevated to 0.2 mM it can be seen that there were, in fact, five 250 pS calcium activated K^+ channels in the patch of membrane. BRL38227, at a concentration of 30 μM ($n = 2$) was also applied to the 160 pS Ca^{2+}–K^+ channel in inside-out membrane patches isolated from glucoreceptive VMHN neurones without effect on channel activity.

Recently, BRL38227 has been shown to modulate some metabolic processes including phosphoinositide turnover and calcium mobilization (Challis *et al.*, 1992; Bray *et al.*, 1991). In order to demonstrate that this compound not only had no effect on ATP-K^+ channels but also had no indirect effect on cellular activity via alteration of intracellular metabolic processes, the racemic mixture cromakalim (50 μM) was tested on glucose-receptive neurones by intracellular recording from intact rat VMHN slices maintained at 37°C. Figure 4 shows a typical pen recorder trace from a glucose-receptive neurone in a rat VMHN slice. The potassium channel opener had no effect on either the resting membrane parameters or spontaneous action potential firing rate of glucose-receptive neurones bathed in normal (10 mM glucose) ACSF (Figure 4a). Subsequent removal of glucose from the ACSF produced the expected hyperpolarization and decrease in input resistance, effects which could be reversed by reapplication of glucose (Figure 3b) or the sulphonylurea tolbutamide at a concentration of 100 μM (Figure 3c). Cromakalim had no effect on a further 4 neurones, all of which were shown to be glucose-receptive. Cromakalim (50 μM; $n = 3$) and diazoxide (60 μM; $n = 2$) were also without effect in experiments using glucose-receptive neurones in the presence of 10 mM glucose or which had been pretreated with tolbutamide (100 μM) in an attempt to ensure that the ATP-K^+ channels were mostly closed in the neuronal membrane. Furthermore, BRL38227 (30 μM, $n = 3$ and 90 μM, $n = 3$) applied to isolated glucose-receptive VMHN neurones in the cell-attached configuration (at 21°C) had no effect on the rate of firing of these neurones (data not shown).

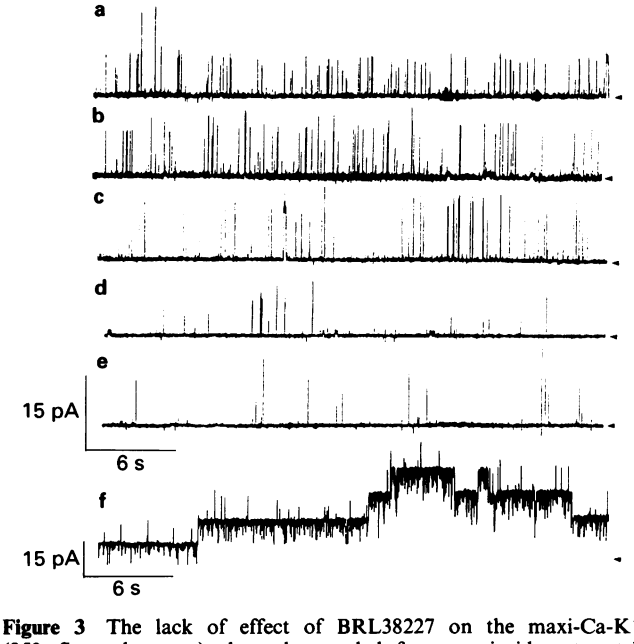

Figure 3 The lack of effect of BRL38227 on the maxi-Ca-K^+ (250 pS conductance) channel recorded from an inside-out patch excised from a VMHN neurone. Membrane potential was −30 mV in all traces, the arrow denotes the zero current level. (a) Control trace with free calcium concentration of 0.8 μM. (b) Addition of 60 μM BRL38227. (c) Washout of BRL38227. (d) Calcium concentration reduced to <10 nM free, with associated reduction in channel activity. (e) Application of BRL38227 (60 μM) at low calcium concentrations is also without effect on channel activity. (f) In absence of BRL38227, elevation of free calcium levels to 0.2 mM causes the simultaneous opening of five channels in the patch. Note the change in the ordinate scale. The values for the P_{open} were as follows; control 0.110; BRL38227 0.110; wash, 0.112; <10 nM calcium 0.010; BRL38227 0.010; 0.2 mM calcium 0.885.

Discussion

Three distinct potassium channels the activity of which may be modulated by intracellular ATP can be recorded from membrane patches isolated from neurones of the ventromedial hypothalamus, the ATP-sensitive K^+ channel, the ATP-activated K^+ channel and a large conductance (250 pS) calcium-activated K^+ channel. A fourth channel, a large-conductance (160 pS) calcium-activated K^+ channel is not sensitive to intracellular ATP. The ATP-K^+ channel from the VMHN has a clear physiological role in regulating the membrane potential of these cells according to the level of extracellular glucose. However, there are many differences between the two channels with regard to conductance (146 pS in the VMHN, approximately 60 pS in the β cell; Ashford *et al.*, 1990a; Ashcroft *et al.*, 1988), rectification properties (absent in the VMHN, present in the β-cell; Ashford *et al.*, 1990a; Ashcroft *et al.*, 1988) and the form of ATP more potent at inhibiting the channel (MgATP for the VMHN, ATP^{4-} for the β-cell; Rowe & Ashford, 1991; Ashcroft & Kakei, 1989). Pharmacologically, the most interesting difference so far reported is that the first generation sulphonylurea tolbutamide inhibits the channel indirectly in the VMHN (i.e. not in isolated membrane patches) whereas in the β-cell this inhibition may also be observed in isolated membrane patches (Ashford *et al.*, 1990b). As the sulphonylureas have been shown to antagonize the effects of the potassium channel openers in various peripheral tissues (heart; Fosset *et al.*, 1988; Ripoll *et al.*, 1990; pancreatic β-cells; Zünkler *et al.*, 1988; Garrino *et al.*, 1989; central neurones; Ben-Ari, 1989, Politi & Rogawski, 1991), sulphonylurea binding is often used as a means of identifying ATP-K^+ channels (and so, presumably, one of the sites of action of cromakalim) in the central nervous system (for examples Bernardi *et al.*, 1988; Miller *et al.*, 1991). The results shown in this paper demonstrate the lack of effect of potassium channel openers (BRL38227 in particular) on the ATP-sensitive K^+ channel from VMHN neurones, using either single channel or intact cell recording techniques. Previous work has shown that the sulphonylureas depolarize glucose-receptive neurones in the VMHN by a blockade of the ATP-K^+ channel (although indirectly; Ashford *et al.*, 1990b) indicating that the ATP-K^+ channel isolated from this brain region is fundamentally different from those found in the heart and pancreatic β-cell in its pharmacology, and that differences in the association between sulphonylurea receptor sites and ATP-K^+ channel exist between different tissues. Therefore it is possible that ambiguities may occur when using the sulphonylureas to identify brain ATP-K^+ channels by radioligand binding (for instance, the VMHN shows a relatively low specific tritiated glibenclamide binding and yet possesses ATP-K^+ channels at a high density; Treherne & Ashford, 1991).

The ATP-activated potassium channel represents a second

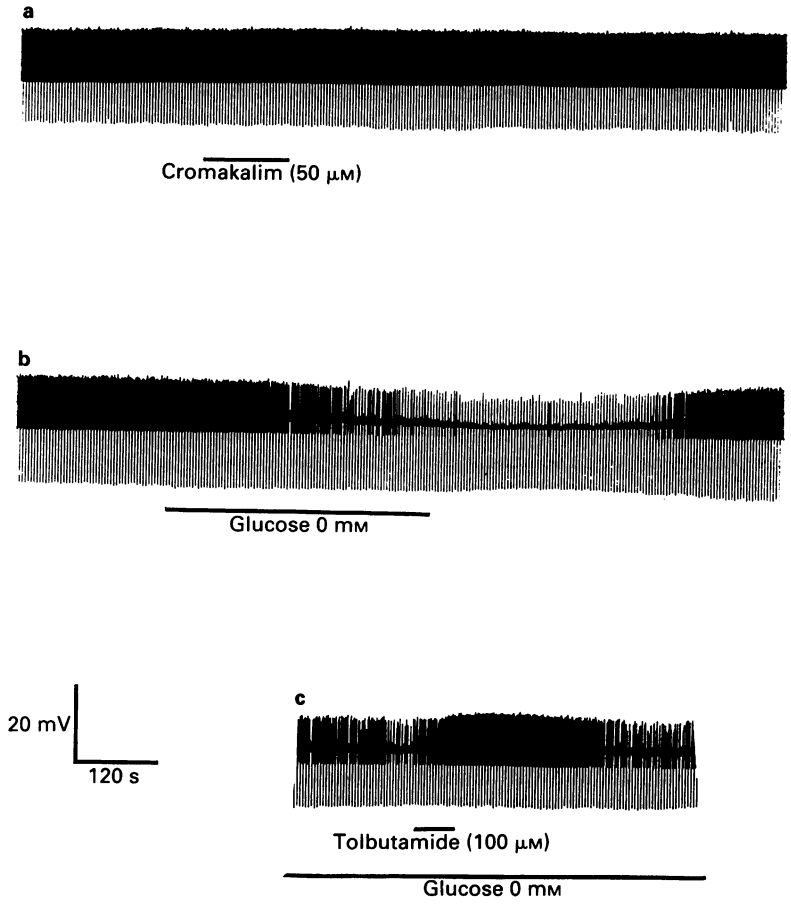

Figure 4 Lack of effect of cromakalim on membrane properties of a glucose-receptive neurone in the rat ventromedial hypothalamus. Resting potential of the neurone was −52 mV. (a) A 2 min application of cromakalim (50 μM) did not change the membrane potential or firing rate of the cell. (b) Removal of glucose from the perfusing ACSF for 5 min hyperpolarized the neurone concomitant with a decrease in input resistance and reduction in spontaneous action potential firing, all of which were reversed on return to normal (10 mM glucose) ACSF. (c) During a second period in the absence of glucose the neurone was challenged for 1 min with the sulphonylurea tolbutamide (100 μM) which produced a membrane depolarization and increase in action potential firing, effects which were fully reversed on return to drug-free ACSF.

link between internal cellular metabolism and neuronal excitability in VMHN neurones. The role of this channel is not clear at present although it is tempting to speculate that it provides a glucose-sensing mechanism. This channel has also been identified, albeit at low densities, in the cortex (Ashford & Treherne, 1989). Although the physiology of the channel has not been characterized fully it is clearly shown from the present study that BRL38227, cromakalim, and pinacidil had no effect on this channel.

The large conductance (250 pS) calcium-activated potassium channel that can be identified in non-glucose-receptive neurones from the VMHN may also provide a link between cell metabolism and neuronal excitability. A similar channel, also sensitive to calcium and ATP, and of a similar conductance, was reported to be isolated from a vascular smooth muscle preparation (Gelband *et al.*, 1989). However, Klöckner & Isenberg (1992), have shown that the ATP sensitivity was due to chelation of calcium by the ATP, and that the channel showed no sensitivity to ATP when the calcium was buffered more effectively. The Ca-K^+ channel from the VMHN is sensitive to ATP because ATP causes an inhibition in channel activity in conditions of high calcium where chelation would not reduce the free calcium concentration to below maximally stimulating levels (Treherne & Ashford, 1991). ATP sensitivity has also been demonstrated for calcium-activated potassium channels isolated from the *Amphiuma* renal distal tubule (Hunter & Giebisch, 1988) and from respiratory epithelial cells (Kunzelmann *et al.*, 1989). Thus, there is evidence for a role for the calcium-activated K^+ channels in responding to elevated extracellular glucose. The channel in the VMHN showed no sensitivity to BRL38227 but it remains to be seen if similar channels sensitive to both calcium and ATP exist elsewhere in the brain. The other (160 pS) Ca^{2+}-K^+ channel that may be recorded from neurones is found almost exclusively in glucose-receptive cells. This channel is insensitive to ATP or tolbutamide applied to the cytoplasmic aspect of the patch isolated in inside-out patches (Treherne & Ashford, 1991) and from the present study we have shown that it is also insensitive to BRL38227.

BRL38227 applied to isolated inside-out membrane patches has not been shown to activate any of the channels modulated by intracellular nucleotides found in the VMHN. However, there are reports that BRL38227 can alter intracellular calcium levels or IP_3 release, thus altering cell excitability through mechanisms other than potassium channel opening. For instance, Bray *et al.* (1991) reported that in calcium-free conditions, BRL38227 inhibited the release of calcium from, or the refilling of, calcium stores within smooth muscle cells. Challiss *et al.* (1992) have also reported that BRL38227 can inhibit histamine-induced IP_3 release from airway smooth muscle. However, in cell-attached recordings from isolated neurones, and, more importantly, intracellular studies performed in ventromedial hypothalamic slice preparations maintained at 37°C, cromakalim had no effect on the activity of glucose-receptive neurones regardless

of whether the majority of the ATP-sensitive K^+ channels were open or closed. This demonstrates that this potassium channel opener has no effect on intact neurones which have not been subjected to the isolation procedures used in the single channel recording experiments.

In conclusion, BRL38227 has been shown not to activate any of the nucleotide-sensitive potassium channels in the VMHN. However, cromakalim has been reported to have effects in a number of other CNS preparations (Tricklebank *et al.*, 1988; Gandolfo *et al.*, 1989a,b; Schmid-Antomarchi *et al.*, 1990; Politi *et al.*, 1991). There are a number of possibilities that may explain this dichotomy. For example, there may be subtypes of ATP-sensitive K^+ channel throughout the CNS, with different biophysical properties, and perhaps different physiological functions, and only a certain type is activated by BRL38227; or, alternatively, BRL38227 may act in the CNS on a channel that is insensitive to intracellular nucleotides but sensitive to sulphonylureas. What is clear from the present study is that the sulphonylurea-sensitive channel in the VMHN is not activated by BRL38227 or any of the other potassium channel openers tested, indicating important differences in the pharmacology of ATP-sensitive K^+ channels between peripheral tissues and the CNS.

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