Activation by intracellular ATP of a potassium channel in neurones from rat basomedial hypothalamus

I. C. M. Rowe, J. M. Treherne and M. L. J. Ashford*

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, UK

1. Cell-attached recordings from isolated glucose-sensitive hypothalamic neurones show that on removal of extracellular glucose there is an increased action current frequency concomitant with decreased single-channel activity. Conversely activation of single K^+ channels was observed when extracellular glucose was increased. Isolation of membrane patches into the inside-out configuration following cell-attached recording demonstrated the presence of an ATP-activated K^+ channel.

2. The ATP-activated K^+ channel was characterized by a mean single-channel conductance of 132 pS in symmetrical 140 mM KCl solutions. Single-channel open-state probability (P_o) was not calcium dependent, and the presence of calcium did not prevent activation of the channel by ATP.

3. Activation of the channel by ATP was concentration dependent and the P_o of the ATP-activated channel was unaffected by membrane voltage, regardless of the degree of activation elicited by ATP.

4. Open and closed time histograms were constructed from inside-out and cell-attached recordings and were consistent with a single open and two closed states. Channel openings were grouped in bursts. Application of ATP, in isolated patches, and glucose, in cell-attached patches, increased the burst duration and number of bursts per second and decreased the slow closed-state time constant. In neither case was there a significant change in the fast closed-state time constant nor the open-state time constant.

5. The non-hydrolysable ATP analogue adenylylimidodiphosphate (AMP(PNP)) and 'Mg^{2+}-free' ATP produced little change in the P_o of the ATP-activated K^+channel when applied to the intracellular surface of excised patches. These results suggest that activation of this channel is via an enzymic mechanism.

6. ADP, GTP and GDP also activated the channel in a Mg^{2+}-dependent manner. ADP and ATP activated the channel in an additive manner and neither GTP nor GDP inhibited channel activity induced by ATP.

7. It is concluded that the ATP-activated K^+ channel observed in isolated inside-out patches from hypothalamic neurones is the same as the channel activated by an increase in the concentration of extracellular glucose in cell-attached recordings from glucose-sensitive neurones.

The hypothalamus is one of the key areas of the central nervous system involved in the integration of metabolic, neural and hormonal signals resulting in the regulation of fuel metabolism (Niijima, 1989; Blundell, 1991). This management of carbohydrate homeostasis is brought about by both autonomic and endocrine pathways (Luiten, Ter Horst & Steffens, 1987) and results in short-term changes in hormone levels (e.g. insulin and glucagon), as well as long-term changes in feeding and appetite (Frohman, 1983; Morley & Levine, 1985). Amongst the areas within the hypothalamus that play a key role in the recognition and integration of these signals are the ventromedial nucleus (VMN) and the lateral hypothalamic area (LHA), which together comprise the basomedial hypothalamus.

* To whom correspondence should be addressed at the Department of Biomedical Sciences, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, UK.

Within these two centres are neurones which are responsive to changes in the extracellular concentration of glucose. Two populations of glucose-sensing neurones have been identified, glucose-receptive (GR) cells, which undergo depolarization and increase their action potential firing rate upon an increase in extracellular glucose concentration (Ono, Nishino, Fukuda, Sasaki, Muramoto & Oomura, 1982; Minami, Oomura & Sugimori, 1986; Boden & Hill, 1988) and glucose-sensitive (GS) cells, which hyperpolarize and decrease firing of action potentials to the same stimulus (Oomura, Ono, Ooyama & Wayner 1969; Oomura, Ooyama, Sugimori, Nakamura & Yamada, 1974).

The glucose-sensing neurones of the VMN are predominantly of the GR type and we have shown that the glucose-induced depolarization (and subsequent increase in action potential frequency) is mediated by ATP-sensitive K^+ (K_{ATP}) channels (Ashford, Boden & Treherne, 1990*a*). We have proposed that, as the concentration of extracellular glucose is increased, the intracellular concentration of ATP also rises and consequently inhibits a greater proportion of the K_{ATP} channel currents, which results in depolarization of the neurone and increased action potential firing. Thus, K_{ATP} channels mediate the glucose-sensing mechanism of these neurones in an analogous manner to that described for the pancreatic β-cell (Ashcroft, 1988; Ashford, 1990). Furthermore K_{ATP} channel activity of GR neurones (Ashford, Boden & Treherne, 1990*b*) and pancreatic β-cells (Sturgess, Ashford, Cook & Hales, 1985; Trube, Rorsman & Ohno-Shosaku, 1986) is blocked by the sulphonylurea tolbutamide.

The GS neurones are found predominantly in the LHA (although some are present in the VMN). The hyperpolarization induced by glucose is thought not to occur through a membrane conductance increase but as a result of the activation of the Na^+–K^+-ATPase (Oomura *et al.* 1974). However, a glucose-induced increase in membrane conductance has been reported for GS neurones in the nucleus tractus solitarii (Mizuno & Oomura, 1984). Furthermore the existence of a K^+ channel the activity of which is increased upon addition of ATP to the intracellular aspect of an excised membrane patch (ATP-activated K^+ channel) has been reported in cultured neurones of rat cerebral cortex (Ashford & Treherne, 1989). Therefore we have investigated the possibility that ATP-activated K^+ channels are present in the GS neurones of the basomedial hypothalamus and contribute to the glucose-sensing properties of these cells. Preliminary accounts of some of these data have been published in abstract form (Rowe, Treherne & Ashford, 1993; Rowe & Ashford, 1993).

METHODS

Drugs and solutions

All solutions were made using glass-distilled water and Analar grade chemicals. The artificial cerebrospinal fluid (ACSF) for rat hypothalamic slices contained (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 recording, the isolated neurones were washed thoroughly with normal external saline which consisted of (mM): NaCl, 135·0; KCl, 5·0; $CaCl_2$, 1·0; $MgCl_2$, 1·0; Hepes, 10·0 (pH adjusted to 7·2 with NaOH). For cell-attached recording the patch pipette was filled with the following solution (mM): KCl, 140·0; $CaCl_2$, 1·0; $MgCl_2$, 1·0 (or $CaCl_2$, 5·0; $MgCl_2$, 5·0); Hepes, 10·0 (pH adjusted to 7·2 with KOH) and the bathing solution was the normal external saline. The solution in the recording pipette used for inside-out patch studies either contained the same solution as for cell-attached recording (i.e. symmetrical 140 mM KCl) or normal external saline (i.e. approximately physiological ionic gradients). The bathing solution for inside-out patch recordings contained (mM): KCl, 140·0; $MgCl_2$, 1·0 (or $MgCl_2$, 0·005); $CaCl_2$, 0·9; EGTA, 1·0; Hepes, 10·0. The pH was adjusted to 7·2 with KOH and the free Ca^{2+} concentration was calculated to be 0·8 μM. In the presence of 1·5 mM ATP, these solutions resulted in concentrations of MgATP of 0·88 mM (1 mM Mg^{2+}) and 4·7 μM (0·005 mM Mg^{2+}). The modified bathing solution for 'Mg^{2+}-free' experiments was composed of (mM): KCl, 140·0; $CaCl_2$, 1·0; EDTA, 10·0; Hepes, 10·0; also resulting in 0·8 μM calculated free Ca^{2+}. The pH was adjusted to 7·2 with KOH. In experiments where high concentrations of nucleotides (>1 mM) were used the concentrations of the divalent cations and EGTA or EDTA were altered in order to compensate for their chelation by the nucleotides. These concentrations and those of the divalent cations were calculated using the metal ion/ligand binding program 'METLIG' (P. England & R. Denton, University of Bristol, UK).

ATP, ADP, GTP, GDP (Na^+ and K^+ salts, vanadium free), tolbutamide and glibenclamide were purchased from Sigma and the non-hydrolysable ATP analogue adenylylimidodiphosphate, AMP(PNP), from Boehringer Mannheim.

Isolation of hypothalamic neurones

Acutely dissociated neurones were prepared as described previously (Ashford *et al.* 1990*a*). In brief, male Wistar or Sprague–Dawley rats (100–300 g) fed *ad libitum* were stunned and decapitated and the hypothalamus dissected out. The ventromedial nuclei or lateral hypothalamic areas were isolated from 400 μm coronal slices. The isolated hypothalamic sections were incubated for 1–2 h in collagenase (0·5 mg ml^{-1}) and trypsin (1 mg ml^{-1}) in ACSF, bubbled with 95% O_2 and 5% CO_2, and dispersed by trituration into tissue culture dishes containing normal external saline. The neurones were allowed to settle and attach to the bottom of the dishes for 30 min prior to use.

Electrophysiological recordings and data analysis

Single-channel currents were recorded from cell-attached and inside-out membrane patches using standard patch-clamp recording procedures (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Recording pipettes, when filled with electrolyte, had resistances between 8 and 15 MΩ. Current recordings were obtained using a patch-clamp amplifier (EPC-7, List Electronic or Axopatch-2D, Axon Instuments) and recorded onto magnetic tape (Racal Store 4D or 4DS) with a bandwidth (−3 dB) of 1250 Hz or 5 kHz (Racal). The tapes were replayed at a later date, the data amplified and digitized at 10 or 25 kHz with a 12-bit analog-to-digital converter (Data Translation) and analysed on either an Elonex 386 or an Apricot Xen-i 286/45 computer using PAT 6.2 software (Patch-clamp analysis program 6.2, J. Dempster, University of Strathclyde, UK). The data were analysed for current amplitudes and open-state probability (P_o) as described

previously (Dempster, 1988; Kozlowski & Ashford, 1990). In multichannel patches, channel activity was determined by integration of the current signal (I) over 1–2 min at a constant voltage, hence a constant single-channel current amplitude (i), and the result expressed as the function $I = N_f P_o i$ where N_f is the number of functional channels and P_o, the open-state probability (Kozlowski & Ashford, 1990). P_o was calculated from the average current (I) as $P_o = I/(N_f i)$, where i is the single-channel current and N is the maximum number of channels seen in the patch when nucleotides are present and P_o is high. In recordings where only a single channel was present open and closed times were measured using a cursor set midway between the open and closed current levels. In addition to this automatic procedure the data used for the construction of the open and closed time histograms were also checked manually. The data for the open and closed time measurements were recorded at 5 kHz and digitized at 25 kHz resulting in a minimum time resolution of 200 μs. Open and closed time histograms formed from these data were fitted with one or more probability density functions (PDF) using a Levenberg–Marquardt, non-linear, least squares method (Dempster, 1988). Bursts of channel openings in isolated patches were defined as groups of openings separated by closed periods five times longer than the time constant for the brief closed times (i.e. 2–3 ms). These values were used both for the control and ATP data as the brief closed times were unaffected by ATP.

Single-channel records used for illustrative purposes were replayed into a chart recorder (Gould, 2200) which had a nominal frequency response of 140 Hz. The potential across the membrane patch is described following the usual sign convention for membrane potential (i.e. inside negative). All experiments were carried out at room temperature (20–25 °C). All data in the text and tables are presented as mean values ± S.E.M. The statistical analysis of differences between data groups was assessed by Student's t test.

RESULTS

Responses of basomedial hypothalamic neurones to changes in extracellular glucose

Cell-attached patch-clamp recordings were obtained from acutely dissociated neurones taken from the VMN and LHA of male Sprague–Dawley rats (2–6 months old). Isolated GS neurones were found to have responses to changes in extracellular glucose concentration similar to those previously observed in brain slice preparations (Oomura *et al.* 1969, 1974). In cell-attached recordings, the GS neurones increased their frequency of firing of action currents when the extracellular glucose concentration was decreased from 10 to 0 mM (Fig. 1*A*). A marked decrease in single-channel activity was also observed associated with this change in firing frequency (Fig. 1*A*). Similar alterations to action current activity in response to changes in extracellular glucose concentration were recorded in four out of forty-one neurones from the VMN and seven out of twenty-three neurones from the LHA. In a further series of experiments, activation of single channels was observed when the extracellular glucose concentration was raised from 0 to 20 mM in eleven out of eighty-four neurones taken from the VMN and LHA (Fig. 1*B*). In the cell-attached configuration, the mean conductance of the channel activated by the rise in extracellular glucose was calculated to be 110 ± 12 pS ($n = 5$) when 140 mM KCl was present in the patch pipette and the reversal potential (E_{rev}) under these condition was 48 mV ($n = 5$). If the intracellular concentration of K^+ ions was close to the value of 93 mM previously reported in rat neurones (Jiang & Haddad, 1992) then the resting membrane potential of these neurones must lie close to the value of −58 mV previously recorded from GS neurones in the LHA using conventional intracellular microelectrodes (Shimizu & Oomura, 1986).

GR neurones, by way of contrast, made up 32% of the neuronal population of the VMN (13 out of 41) and 9% of the LHA (2 out of 23). Inside-out patch recordings indicated that these GR neurones contained K_{ATP} channels, which were inhibited by the application of ATP to the cytoplasmic surface of the patch, and Ca^{2+}-activated K^+ (K_{Ca}) channels, with mean single-channel conductances of 146 and 160 pS, respectively, in symmetrical potassium (Ashford *et al.* 1990*a*; Treherne & Ashford, 1991). The remaining 61% of neurones in the LHA and 58% of neurones in the VMN were not sensitive to changes in extracellular glucose concentration and contained, as the most prominent channel type in excised patches, K_{Ca} channels with a mean single-channel conductance of 250 pS in symmetrical KCl (Treherne & Ashford, 1991).

ATP activates a K^+ channel in excised membrane patches from GS neurones

On five occasions, following cell-attached recording, a patch was excised from an identified GS neurone and single-channel activity recorded in the inside-out configuration. In the absence of nucleotides few channel openings were observed (Fig. 1*C*). The channel was characterized by short-duration openings and larger current was activated by the application of ATP (1 mM) to the inner surface of the membrane (Fig. 1*C*). In a further series of experiments, using inside-out patches obtained from unidentified isolated hypothalamic neurones, the ATP-activated channel was observed in 45 out of 212 patches and was activated by 0·1–5 mM ATP; the majority of the remaining patches contained either K_{Ca} (160 or 250 pS) or K_{ATP} channels (also see below; other ATP-activated K^+ channels). Patches excised from GR neurones (identified by the excitation induced by an increase in glucose concentration) and unidentified neurones which contained K_{ATP} and/or 160 pS K_{Ca} channels did not exhibit any ATP-activated channel activity ($n = 62$). Therefore, the ATP-activated channel is present in a population of neurones distinct from the GR and glucose-insensitive neurones and is most probably associated with GS neurones.

In recordings from isolated patches under symmetrical 140 mM KCl conditions the ATP-activated channel displayed an approximately linear current–voltage

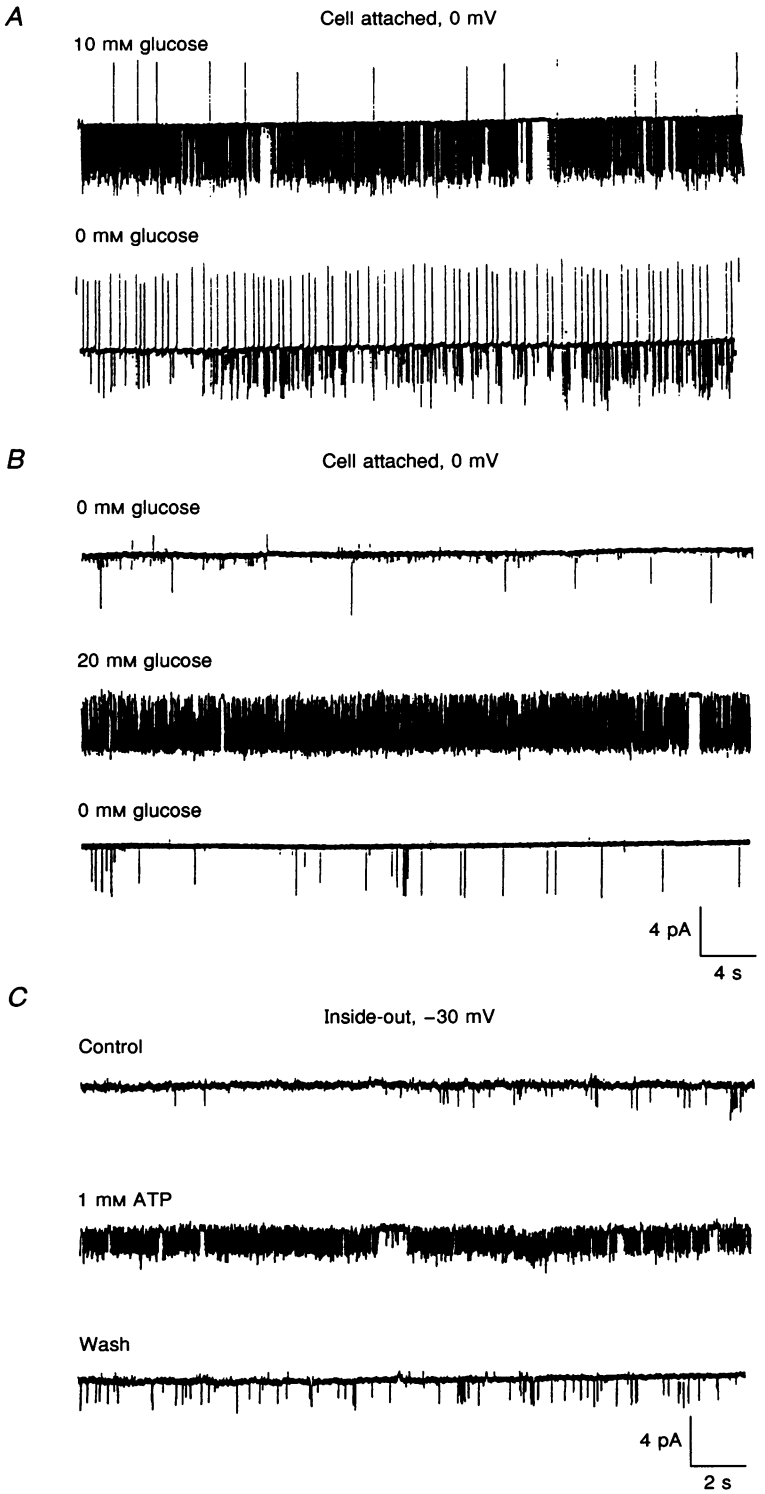

Figure 1. The activation of a K^+ channel by extracellular glucose and intracellular ATP

A, cell-attached recording from an acutely dissociated neurone from rat hypothalamus (pipette potential, 0 mV). The patch pipette was filled with the following solution (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10; pH adjusted to 7·2 with KOH. The bathing solution was normal external saline containing (mM): NaCl, 135; KCl, 5; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10; pH adjusted to 7·2 with NaOH. Top trace, recording in 10 mM glucose; upward deflections of the trace are action currents and downward deflections are channel openings ($P_o = 0·52$). Bottom trace, recording 10 min after superfusion of the same patch with a 0 mM glucose saline ($P_o = 0·09$). Note the increase in action potential frequency and decrease in channel activity

relationship (Fig. 2) and the mean single-channel conductance (over the range ±60 mV) was calculated to be 132 ± 16 pS ($n = 10$). Under an approximately physiological gradient (i.e. 135 mM NaCl, 5 mM KCl in the pipette and 140 mM KCl in the bath), single-channel currents recorded from inside-out patches exhibited pronounced outward rectification at positive potentials although the current–voltage relationship deviates from that produced by the Goldman–Hodgkin–Katz constant current equation (Goldman, 1943; Hodgkin & Katz, 1949) for this potassium gradient (Fig. 2). The extrapolated reversal potential under these recording conditions was −73 ± 3 mV ($n = 5$), which is close to the calculated value of − 84 mV for a potassium selective conductance.

Concentration-dependent activation by ATP

In excised inside-out patches the activation produced by ATP was found to be concentration dependent when more than one concentration was added to the same patch (Table 1). As different patches exhibited various degrees of activation to the same concentration of ATP there were large standard errors associated with the mean values of activation for any one concentration of ATP (Table 1). In patches where more than one channel was present, the results from binomial analysis suggested that ATP not only increased the open-state probability of the channel but also the number of channels available for activation, i.e. an increase in channel activity ($N_f P_o$).

The data, summarized in the tables and figures included in this paper, were obtained from patches in which the ATP-induced channel activity was reversed upon wash-out. An example of the time course of activation and the reversibility of the ATP effect is shown in Fig. 3. We have not attempted detailed analysis of the rate of the ATP activation as we used relatively slow flow rates, of 2–4 ml min^{-1}, when changing the bath solution and under these conditions could discern no significant difference in the rate of onset and offset of the channel activity. The increase in channel activity produced by ATP was maintained as long as ATP was present in the bath solution. These experiments also revealed that there was no consistent time-dependent change in channel activity after the initial concentration-dependent activation by ATP. Though some channel recordings displayed a reduction in activity with time (see Fig. 3) there was no predictable rundown or enhancement of $N_f P_o$ with time with patches exposed to ATP for up to 30 min. Finally, the concentration-dependent activation produced by ATP was reproducible in individual patches, in that the application of 1 mM ATP would induce a similar degree of activation at the end of an experiment as it did at the start (see e.g. Fig. 8). This allowed comparative studies to be made of the activation produced by a variety of nucleotides in the presence or absence of divalent cations and at various voltages.

In a small number of patches, additional to the forty-five described above (11 out of 212) the effects of ATP did not match the general characteristics already outlined. In one group (7 patches), the application of ATP produced channel activation, as expected, but there was no reduction in activity upon wash-out of ATP, and re-application of ATP did not cause any further change in P_o. The remaining four patches were also activated by ATP, though this time in a reversible manner, but subsequent additions of ATP did not cause the expected increase in channel activity, even at high concentrations. These channels (i.e. 11 out of 212) had a mean conductance of 121 ± 14 pS ($n = 4$) in symmetrical KCl. The time course of the ATP-induced activation and the single-channel kinetics of the channels in these eleven patches were similar to those channels on which ATP activation was reversible and reproducible. We also observed in a small number of patches (6 out of 212) channels with smaller mean single-channel conductances (10–60 pS) which also were activated by the application of ATP to the intracellular surface of the membrane. The data obtained from these recordings have not been included as the channel conductances were significantly smaller than the 132 pS ATP-activated K^+ channel described thus far and their ionic and kinetic characteristics require further study.

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in the presence of the 0 mM glucose saline. *B*, cell-attached recording from an acutely dissociated neurone from rat hypothalamus (pipette potential, 0 mV). The patch pipette was filled with the following solution (mM): KCl, 140; $CaCl_2$, 5; $MgCl_2$, 5; Hepes, 10; pH adjusted to 7·2 with KOH. The bathing solution was normal external saline. Top trace, recording from a neurone that had been maintained in 0 mM glucose for 20 min ($P_o = 0{\cdot}008$). Note that there are only a few action currents. Middle trace, recording 3 min after application of 20 mM glucose ($P_o = 0{\cdot}66$). Bottom trace, reversal of the channel activation after return of the extracellular glucose concentration to 0 mM ($P_o = 0{\cdot}01$). *C*, inside-out patch recording from an identified GS neurone. The membrane patch was held at a potential of −30 mV. The electrode solution contained (mM) : KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10; pH 7·2. The bath (intracellular) solution contained (mM): KCl, 140; $MgCl_2$, 1; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Ca^{2+}, 0·8 μM). Top trace, control recording in the absence of ATP ($P_o = 0{\cdot}005$). Middle trace, single-channel current activity when 1 mM ATP was added to the recording bath and therefore to the intracellular membrane surface ($P_o = 0{\cdot}54$). Bottom trace, reversal of the activation after washing ($P_o = 0{\cdot}008$).

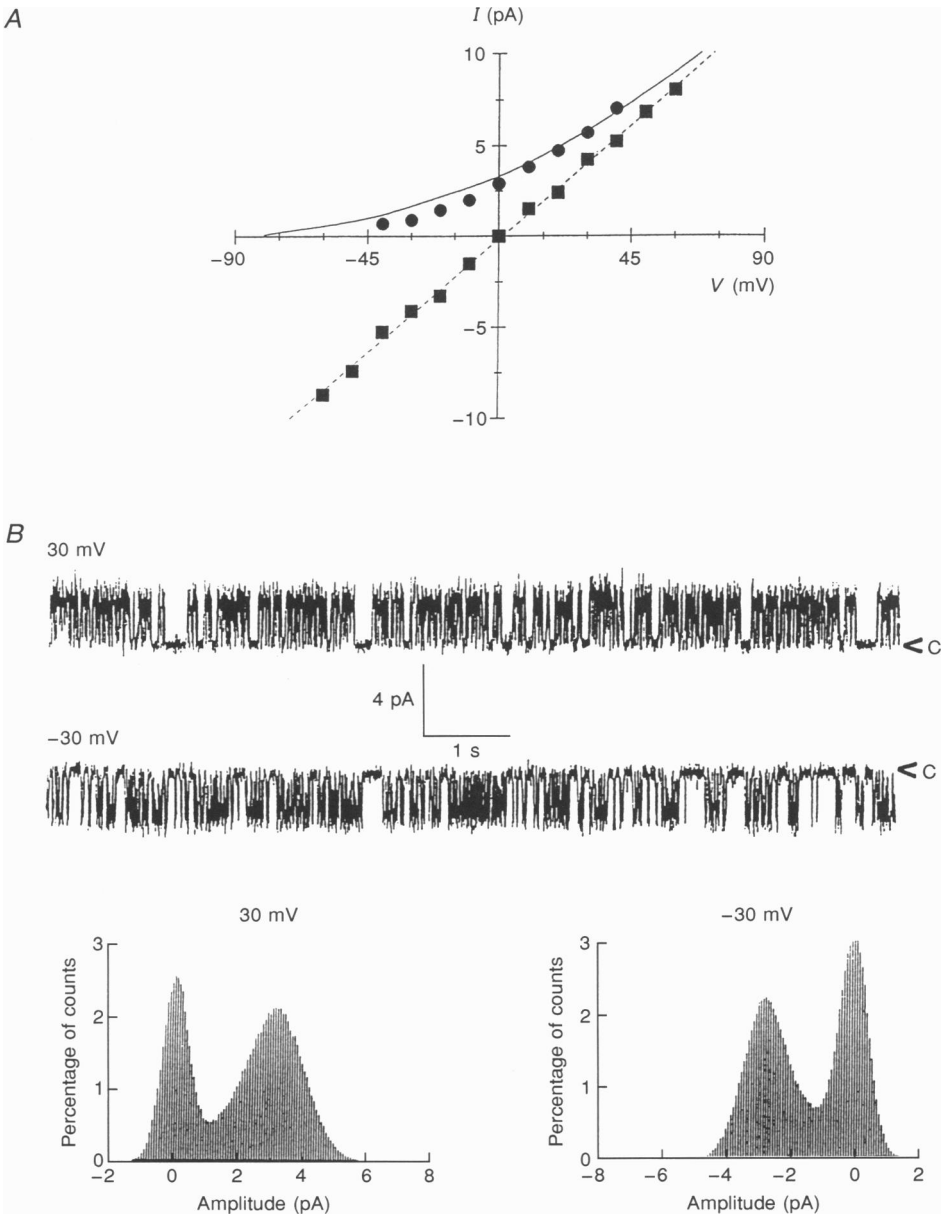

Figure 2. Current–voltage relationship for the ATP-activated K^+ channel

A, single-channel current–voltage (*I–V*) relationships for the ATP-activated potassium channel recorded from two inside-out membrane patches excised from acutely dissociated hypothalamic neurones. ■, data from an excised patch in symmetrical 140 mM KCl, with the pipette containing (mM): KCl, 140; $CaCl_2$, 5; $MgCl_2$, 5; Hepes, 10; pH adjusted to 7·2 with KOH. The bath (intracellular) solution containing (mM): KCl, 140; $MgCl_2$, 1; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Ca^{2+}, 0·8 μM). The conductance of this channel determined from the slope of the line fitted to the data by linear regression was 137 ± 2 pS. ●, data from an excised patch recorded under approximately physiological cation concentrations, with normal external saline used for cell-attached recordings in the pipette and with the bath (intracellular) solution identical to that used for recordings in symmetrical 140 mM KCl. The curve represents values obtained from the Goldman–Hodgkin–Katz equation for a P_K of $2{\cdot}5 \times 10^{-13}$ cm s^{-1}. *B*, single-channel activity obtained from an ATP-activated channel recorded from an inside-out patch from a hypothalamic neurone in symmetrical 140 mM KCl in the presence of 1 mM ATP. Examples of channel activity are shown at +30 and −30 mV. Below are the amplitude histograms produced from these recordings which show that separate open and closed states can be clearly distinguished for this channel. In this and subsequent figures 'C' indicates closed state.

The lack of effect of Ca^{2+} ions and voltage on the ATP-activated K^+ channel

A number of investigations have shown that K_{Ca} channels isolated from rat brain are modulated by phosphorylation (Chung, Reinhart, Martin, Brautigan & Levitan, 1991; Reinhart, Chung, Martin, Brautigan & Levitan, 1991). Therefore it was necessary to investigate the Ca^{2+} ion and voltage sensitivity of the ATP-activated channel to determine whether it was part of the K_{Ca} family. In inside-out patches the application of free Ca^{2+}, over the range 25 nM to 1 mM, to the inner surface of the membrane in the absence of ATP had no significant effect on channel activity (Table 2). For example, the open-state probability (P_o) for a single ATP-activated channel in the presence of 1 μM free Ca^{2+} was 0·016 in the absence of ATP and 0·018 when the Ca^{2+} concentration was raised to 1 mM. The addition of Ca^{2+} did not interfere with the activation of the channel as 1 mM ATP was still able to activate the channel even in the presence of 1 mM Ca^{2+} (Fig. 4). The application of Ca^{2+} ions was without effect at both hyperpolarizing and depolarizing membrane potentials.

When the current–voltage relationship for the ATP-activated channel was examined it was clear that the single-channel P_o was not greatly affected by the membrane potential of the patch (Fig. 2*B*). However, a possible modest influence of membrane voltage on the sensitivity of ATP activation of this channel was investigated in more detail. The open-state probabilities of the channels were examined over the range of −50 to +50 mV in the presence and absence of various concentrations of ATP. For example, in a patch containing a single ATP-activated channel, values for P_o of 0·016 and 0·021 were obtained at −40 and +40 mV, respectively, in the absence of ATP and with a Mg^{2+} concentration of 5 μM. When 1·5 mM ATP (i.e. 4·7 μM MgATP) was added to this patch channel activity was increased and the P_o rose to 0·15, and was not significantly affected by the holding potential (Fig. 5). The channel P_o

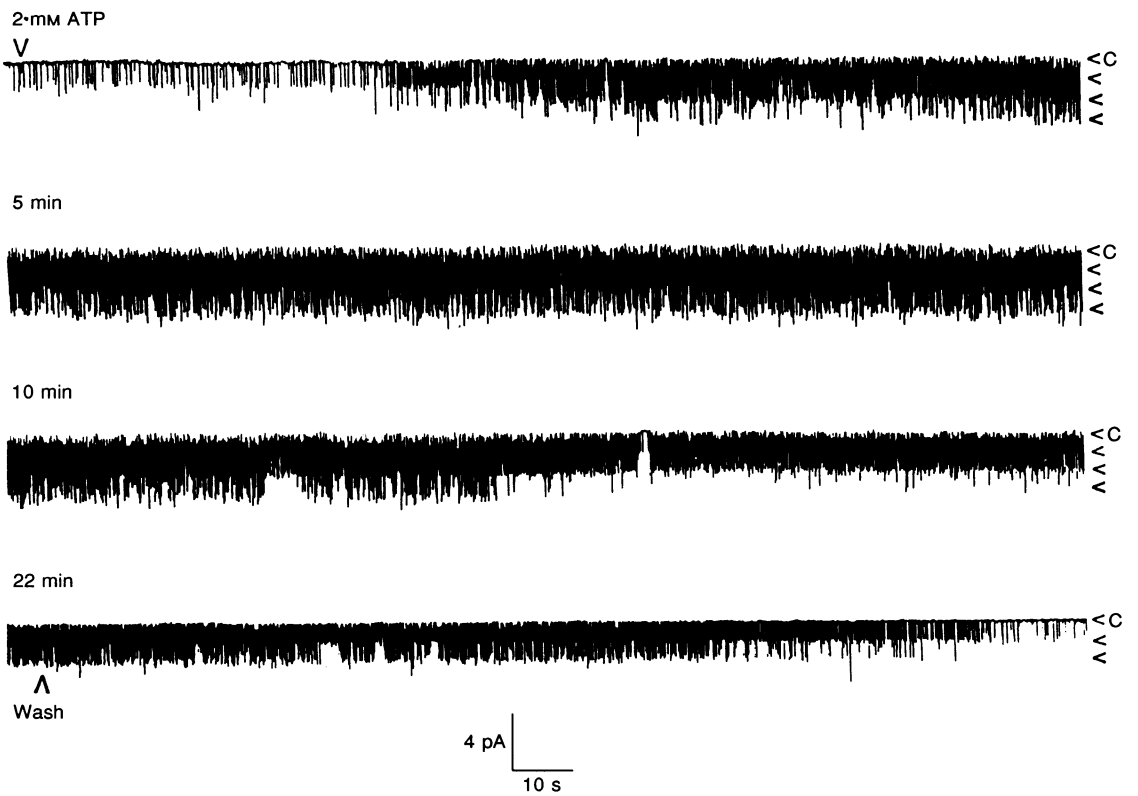

Figure 3. K^+ channel activation by ATP is sustained and reversible

An inside-out membrane patch recording from a dissociated neurone from rat hypothalamus held at a potential of −30 mV (these conditions pertain to all the following figures unless otherwise stated). The electrode solution contained (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10; pH adjusted to 7·2 with KOH. The bath (intracellular) solution contained (mM): KCl, 140; $MgCl_2$, 0·8; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH. The top trace shows the time course and level of channel activation produced by the addition of 2 mM ATP to the bath solution. The following discontinous traces show the channel activity after continuous exposure to 2 mM ATP for 5, 10 and 22 min. The bottom trace shows the reduction in channel activity upon removal of the ATP from the bath solution.

Table 1. Summary of the effects of ATP, 'Mg^{2+}-free' ATP and AMP(PNP) on potassium channel activity recorded from inside-out membrane patches excised from neurones acutely dissociated from rat hypothalamus

	Activation		
	Range	Mean ± S.E.M.	n
[ATP]			
0·5 mM	1·51–6·82	3·99 ± 0·83	6
1·0 mM	1·68–135·60	35·94 ± 19·27	8
3·0 mM	8·24–139·90	76·80 ± 21·33	7
5·0 mM	5·20–114·21	51·51 ± 22·82	5
['Mg^{2+}-free' ATP]			
3·0 mM	1·23–1·60	1·38 ± 0·09	5
[AMP(PNP)]			
3·0 mM	1·08–1·50	1·24 ± 0·07	6

Membrane patches were held at a potential of 0 mV under approximately physiological cation concentrations. The electrode was filled with normal external saline and the bath (intracellular) solution contained (mM): KCl, 140; $MgCl_2$, 1; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Ca^{2+}, 0·8 μM); except for solutions containing 'Mg^{2+}-free' ATP which were composed of (mM): KCl, 140; $CaCl_2$, 1; EDTA, 10; Hepes, 10; pH 7·2. The data are expressed as the level of channel activity ($N_f P_o$) in the presence of nucleotide divided by that in the absence of nucleotide. Both the mean values and the range of activation are shown.

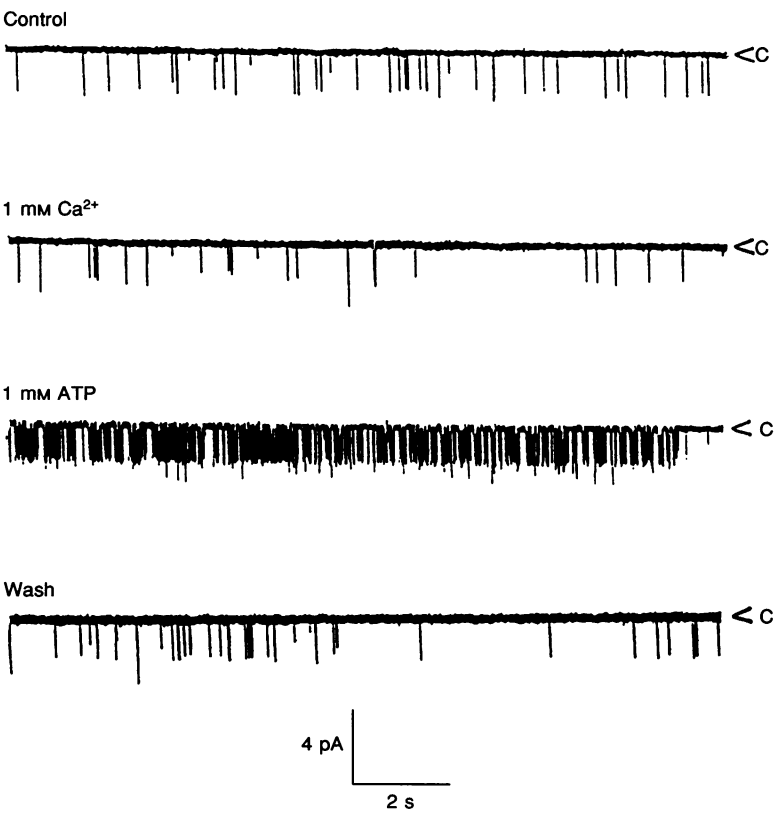

Figure 4. The ATP-activated K^+ channel is Ca^{2+} insensitive

Solutions are as described in Fig. 3. Top trace, control recording ($P_o = 0·008$). Upper middle trace, single-channel current activity when free Ca^{2+} was raised to 1 mM in the bathing solution ($P_o = 0·005$). Lower middle trace, activation of the channel by the addition of 1 mM ATP to the bathing solution in the presence of 1 mM free Ca^{2+} ($P_o = 0·490$). Bottom trace, reversal of the activation after washing ($P_o = 0·011$).

Table 2. Summary of the effects of changes in free Ca^{2+} ion concentration and membrane potential on ATP-activated K^+ channel activity recorded from inside-out membrane patches excised from rat hypothalamic neurones

Potential (mV)	−30	−30	−30	−30	+30	+30	+30
[ATP] (mM)	0	1	0	0	0	1	1
[Ca^{2+}] (μM)	0·8	0·8	0·025	1000	1000	1000	0·8
Mean activation	1·0	28 ± 12	1·2 ± 0·1	1·1 ± 0·1	1·4 ± 0·1	39 ± 14	36 ± 17

The patch pipettes contained (mM): KCl, 140; $MgCl_2$, 1; $CaCl_2$, 1; Hepes, 10; pH adjusted to 7·2 with KOH. The bath initially contained control solution (mM): KCl, 140; $MgCl_2$, 0·8; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Ca^{2+}, 0·8 μM). The membrane potential or free Ca^{2+} ion concentration was then altered and the channel activity recorded. The mean activation was calculated by dividing the channel activity ($N_f P_o$) obtained with changes in Ca^{2+}, potential and ATP by that found in the control solution. The free Mg^{2+} ion concentration was maintained at 0·8 mM throughout. Data represent the mean of 3 or 4 separate patches.

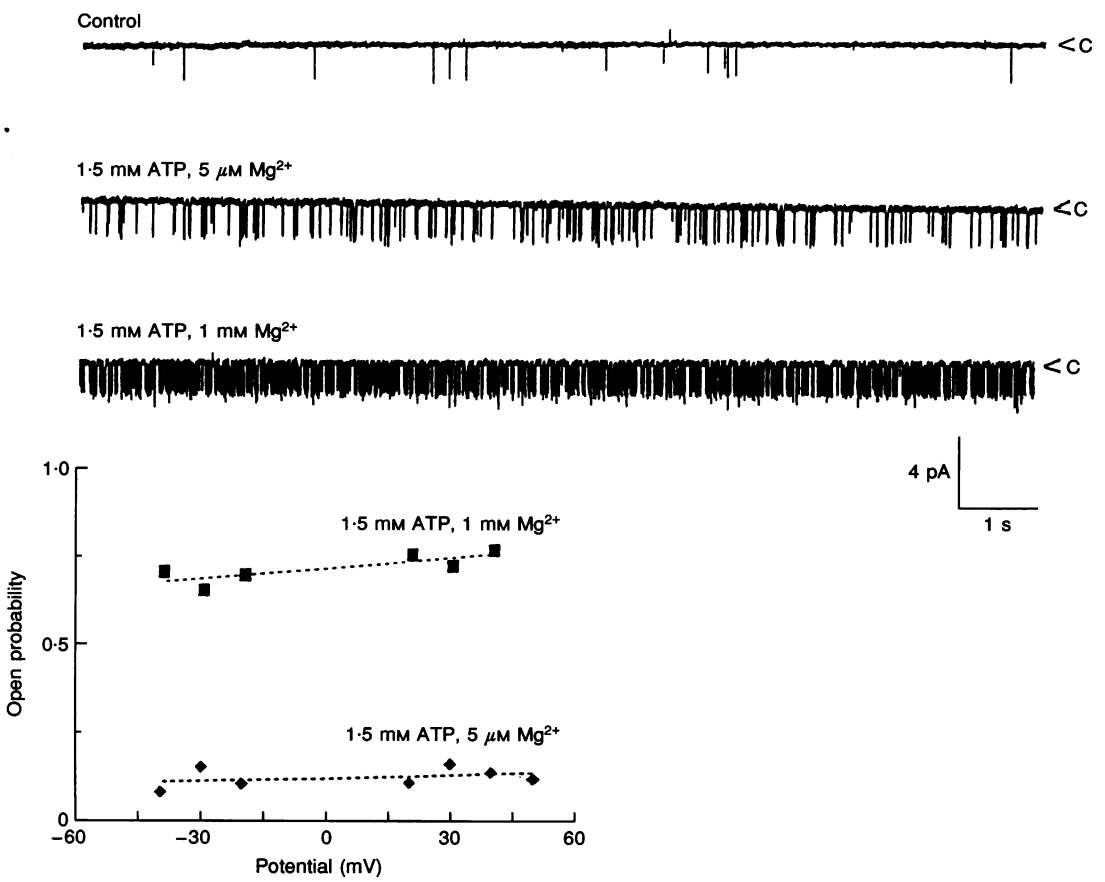

Figure 5. The effects of MgATP and voltage on the ATP-activated K^+ channel

A, the electrode solution contained (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10; pH adjusted to 7·2 with KOH. The bath (intracellular) solution contained (mM): KCl, 140; $MgCl_2$, 0·005; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Mg^{2+}, 5 μM). Top trace, control recording ($P_o = 0·003$). Middle trace, single-channel current activity when 1·5 mM ATP (MgATP, 4·7 μM) was added to the bathing solution ($P_o = 0·11$). Bottom trace, further activation of the channel by the addition of 1 mM Mg^{2+} to the bathing solution in the presence of 1·5 mM ATP (MgATP, 0·88 mM) ($P_o = 0·680$). *B*, graph of P_o against membrane potential obtained from the inside-out membrane patch shown above. ◆, the relationship between P_o and potential for the ATP-activated channel in the presence of 1·5 mM ATP and 5 μM Mg^{2+} (MgATP, 4·7 mM). ■, the relationship between P_o and potential for the same patch in the presence of 1·5 mM ATP and 1 mM Mg^{2+} (MgATP, 0·88 mM). The dashed lines are of linear regressions drawn through each set of points. P_o is not significantly voltage dependent in either case.

was then raised to 0·65 by increasing the MgATP concentration to 0·88 mM, and altering the membrane potential once again failed to affect the channel's open-state probability (Fig. 5). Similar responses were seen in all patches examined in this manner ($n = 6$), clearly showing that the ATP-activated channel is voltage insensitive (see also Table 2).

Single-channel kinetics of the ATP activation

In a number of patches a single ATP-activated K^+ channel was present and therefore analysis of channel kinetics was possible. In these patches single-channel openings occurred in bursts, and similar activity was also observed in multi-channel patches. The application of ATP led to a large increase in the number of bursts per second from 3 to 45 bursts s^{-1} and a slight increase in burst duration (Table 3A). Due to the rapidity of the channels' fluctuations between open and closed states, recordings were made at 5 kHz and digitized at 25 kHz in an attempt to distinguish the kinetics. Analysis of open and closed time distributions obtained from the single-channel activity in the presence and absence of ATP revealed that under both conditions the kinetics can best be approximated using one open state and two closed states. The open-state distributions could be fitted with a single exponential and the fit was not significantly improved using two exponentials (Table 3A, Fig. 6). The closed-state distributions in contrast required the sum of two exponential terms to obtain an adequate fit (Table 3A, Fig. 6). The analysis shows that the ATP-activated channel is characterized by a single fast 'flickery'

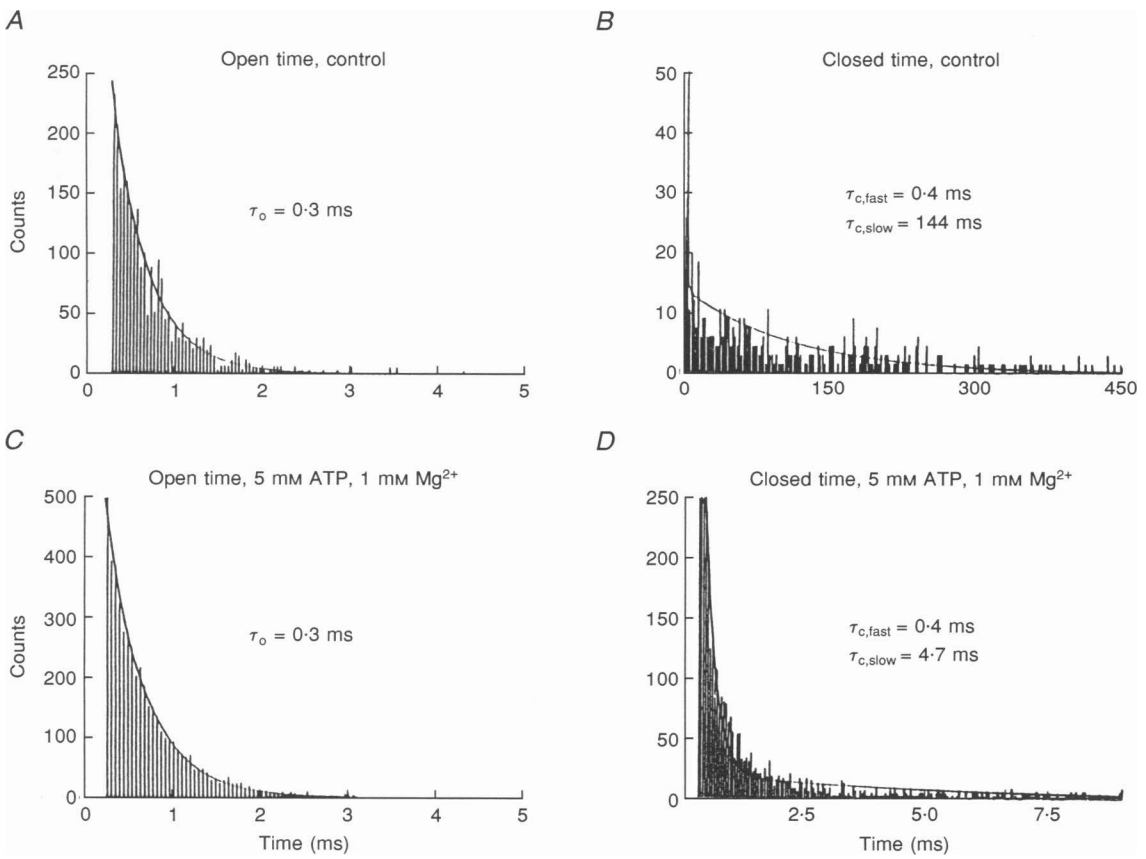

Figure 6. Single-channel kinetics

Open- and closed-state time distribution histograms. The data were produced from the analysis of the activity of a single channel in an inside-out patch held at a membrane potential of −30 mV. Open- and closed-state data are shown for the same ATP-activated channel in the absence of ATP (control; *A* and *B*) and in the presence of 1·5 mM ATP and 1 mM Mg^{2+} (0·88 mM MgATP; *C* and *D*). Exponential curves are fitted to each set of data and the time constants displayed. *A* and *C*, the open-state time constant (τ_o) was unaffected by the addition of 0·88 mM MgATP even though P_o rose from 0·01 to 0·61. However, in *B* and *D*, the closed state, best fitted by two exponentials, showed a reduction in the slower closed-state time constant ($\tau_{c,slow}$) which was reduced from 144 to 4·7 ms when MgATP was applied to the patch. The fast closed-state time constant ($\tau_{c,fast}$) was unaffected by MgATP and remained at 0·4 ms. The data were produced from the analysis of 9744 events in *C* and *D* and 1530 in the absence of ATP, shown in *A* and *B*. In the histograms of fast open and closed time distributions the data at short time periods (<0·2 ms) have been left out as there is poor resolution of events in this range. Note the different time axes in *B* and *D*.

Table 3. Summary of channel kinetics recorded from single ATP-activated channels in symmetrical K^+ in excised inside-out (A) and cell-attached (B) membrane patches

A. [MgATP] (mM)	P_o	τ_o (ms)	$\tau_{c,fast}$ (ms)	$\tau_{c,slow}$ (ms)	Open time (ms)	Closed time (ms)	Burst duration (ms)	Burst rate (bursts s^{-1})
0	0·02 ± 0·01*	0·33 ± 0·10	0·46 ± 0·14	82·26 ± 18·71*	0·49 ± 0·14	96·25 ± 14·65*	7·57 ± 1·97*	2·7 ± 0·6*
0·88	0·43 ± 0·11*	0·38 ± 0·9	0·35 ± 0·09	4·85 ± 1·48*	0·54 ± 0·09	3·41 ± 1·80*	12·18 ± 2·43*	45·4 ± 8·2*

B. [Glucose] (mM)	P_o	τ_o (ms)	$\tau_{c,fast}$ (ms)	$\tau_{c,slow}$ (ms)
0	0·008	0·5	0·8	87·0
20	0·66	0·7	0·8	1·6
0	0·01	0·8	0·6	76·0
10	0·54	0·6	0·9	3·7

In section A, the membrane potential was −30 mV. All the data presented were recorded using patch pipettes containing (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes 10 (pH 7·2) and bath solution containing (mM): KCl, 140; $MgCl_2$, 0·8; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10 (pH 7·2). Mean data taken from 6 separate patches which contained one ATP-activated channel. The data were produced from the analysis of 5000–10000 events in 0·88 mM MgATP, but only 1320–3200 in the absence of ATP. The table shows the mean open and closed times for individual channel events and the mean burst duration and open probability for the channel in the presence and absence of MgATP. Section B gives examples of 2 individual experiments. The pipette voltage was 0 mV for both. The patch pipette contained (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10 (pH 7·2) and the bathing solution was the normal external saline. The number of events was 1212 and 1560 for 0 glucose and 3755 and 5274 for 10 and 20 mM glucose, respectively. The table shows the open probability and best-fit values to the open and closed time histograms. Analysis of bursts was not undertaken as the values for $\tau_{c,fast}$ and $\tau_{c,slow}$ in the presence of glucose were too similar. These data show that associated with the ATP or glucose-induced increase in P_o there are only small changes in τ_o and $\tau_{c,fast}$, whereas there is a substantial decrease in $\tau_{c,slow}$. * $P < 0·05$.

open state, with a time constant (τ_o) of 0·3–0·4 ms, a fast 'flickery' closed state, with time constant ($\tau_{c,fast}$) of 0·3–0·5 ms, and a longer closed state with a long (>100 ms) time constant ($\tau_{c,slow}$).

Activation of the channel by increasing the concentration of MgATP from 0 to 0·88 mM raised P_o from 0·02 to 0·43 with no significant change in the single-channel mean open dwell time and the open-state time constant (τ_o). MgATP also did not produce a significant change in the fast closed-state time constant ($\tau_{c,fast}$). However, the rise in the MgATP concentration produced an increase in the number of bursts per second and a significant decrease in mean closed dwell times, while the long closed-state time constant ($\tau_{c,slow}$) fell dramatically, e.g. from 144 to 4·7 ms in an individual patch (shown in Fig. 6, Table 3A). In addition, the distribution of bursts appeared clustered and so it is possible that there is another unresolved closed state.

In two cell-attached recordings a single channel was activated by the addition of 10 or 20 mM glucose to the extracellular saline (Fig. 1). Thus it was possible to make a limited comparison of the single-channel kinetics between the cell-attached and isolated patch configurations. Channel openings also occured in bursts and the channel open- and closed-state distributions were found to be similar to those observed in the inside-out configuration, with the open state best-fitted by a single exponential and the closed state by the sum of two exponentials (Table 3B). The increase in channel open-state probability was accompanied by no significant change in the open-state or fast closed-state time constant ($\tau_{c,fast}$) while the slow closed-state time constant ($\tau_{c,slow}$) was reduced markedly.

The activation of the channel by MgATP can therefore be described as a sequence of transitions between one open state and two closed states where the rate constants are voltage independent but may be influenced by the application of MgATP. The model may in the future have to be modified to include another closed state and possible inactivation or desensitization states

Mg^{2+}-dependent hydrolysis of ATP and channel activation

The examples used for analysis of voltage dependence and kinetics (Fig. 5 and Table 3) show quite clearly that MgATP is the more potent form of ATP as measured by its ability to increase the P_o of the ATP-activated channel. These results were confirmed by further experiments in which chelation of Mg^{2+} ions with EDTA markedly reduced the P_o produced by a given concentration of ATP ($n = 10$). For example, in one inside-out patch 3 mM ATP in the presence of 1 mM free Mg^{2+} increased P_o from a value of 0·005 to 0·538 while 3 mM ATP in the presence of 5 nM free Mg^{2+}

increased P_o to only 0·019 in the same patch (Fig. 7*A*). Mg^{2+} ions alone in the absence of ATP did not induce channel opening ($n = 4$) nor did they suppress channel activity as has been reported for the K_{ATP} channel in insulin-secreting cells (Kozlowski & Ashford, 1990). The non-hydrolysable analogue of ATP, AMP(PNP) at concentrations of 1–3 mM did not induce channel activation ($n = 10$), even in the presence of Mg^{2+} ions (Fig. 7*B*).

Effects of guanine and adenine nucleotides on ATP activation

Atrial muscarinic K^+ channels have been shown to be activated, in the absence of agonist, by the application of MgATP to the cytosolic surface of the membrane (Heidbüchel, Callewaert, Vereecke & Carmeliet, 1990, 1993). The activation is mediated by a nucleoside diphosphate kinase transphosphorylation of the muscarinic K^+ channel. The channel can be activated by both adenosine and guanine nucleotides, but guanine nucleotides inhibit the action of MgATP in the absence of acetylcholine. Therefore we examined the influence of GTP, ADP and GDP on the ATP-activated K^+ channel present in hypothalamic neurones. All three nucleotides activated this channel when applied to the intracellular surface of inside-out patches, in the absence of ATP ($n = 15$). The levels of activation produced by 2 mM GTP, ADP and GDP, with respect to that of 2 mM ATP, were 0·65 ± 0·12, 0·57 ± 0·13 and 0·36 ± 0·09 (Fig. 8). As with the ATP-

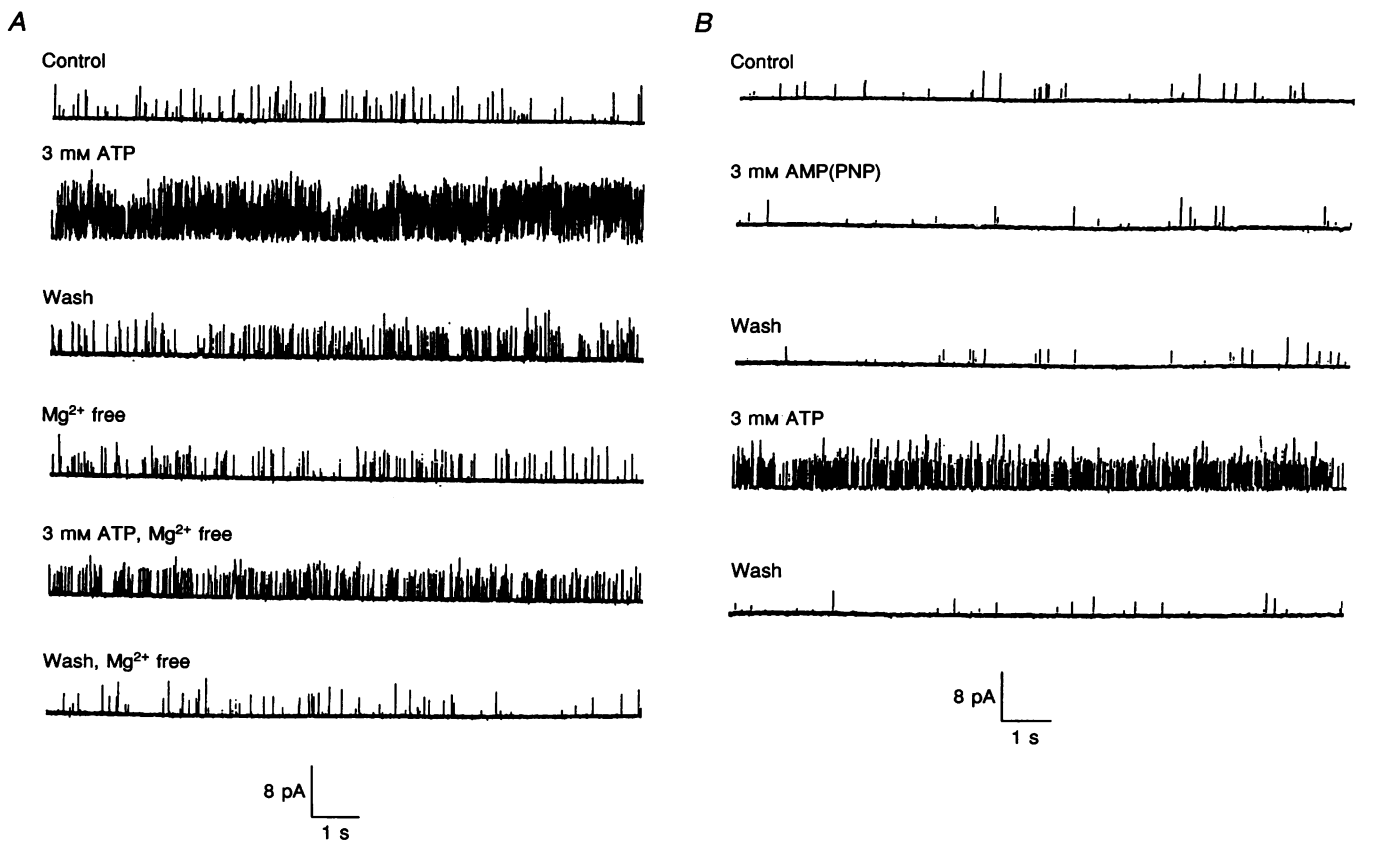

Figure 7. Mg^{2+}-free ATP and AMP(PNP) do not activate the channel

Inside-out membrane patch recordings from dissociated neurones from rat hypothalamus held at a potential of 0 mV. Electrodes were filled with normal external saline and the bath (intracellular) solution contained (mM): KCl, 140; $MgCl_2$, 1; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Ca^{2+}, 0·8 μM). *A*, the top trace shows a control recording in the absence of ATP ($P_o = 0·005$). The channel was activated when 3 mM ATP was added to the bath and therefore to the intracellular membrane surface ($P_o = 0·538$). Channel activation was reversed after the wash-out of the ATP ($P_o = 0·008$). Application of a Mg^{2+}-free solution ($P_o = 0·006$) and the addition of 3 mM 'Mg^{2+}-free' ATP produced a lower level of activation ($P_o = 0·019$) than had been the case in the presence of free Mg^{2+} ions. The ATP was then washed out of the bathing solution ($P_o = 0·003$). *B*, the figure shows (top trace) a control recording in the absence of ATP or AMP(PNP) ($P_o = 0·003$). The addition of 3 mM AMP(PNP) to the intracellular membrane surface failed to activate the channel ($P_o = 0·004$). The AMP(PNP) was then washed out of the bathing solution ($P_o = 0·002$). Subsequent addition of 3 mM ATP to the bathing solution induced channel activity ($P_o = 0·213$), which was reversed upon wash-out ($P_o = 0·001$).

induced activity, the effects of these nucleotides were both reversible and Mg^{2+} dependent. In contrast, AMP (0·1–6 mM) failed to produce channel activation ($n = 5$). However, unlike the muscarinic K^+ channel, neither GDP nor GTP, at concentrations of up to 2 mM, inhibited channel activity induced by the application of ATP to inside-out patches ($n = 6$) (Fig. 9). Furthermore activation of this channel by ADP is additive with that of ATP ($n = 4$). For example, addition of 1 mM ATP to a patch increased channel activity ($N_f P_o$) from 0·05 to 0·94 whereas 2 mM ADP alone increased activity to 0·55. Co-application of ATP (1 mM) and ADP (2 mM) resulted in a value for $N_f P_o$ of 1·59, i.e. their effects were additive.

The sulphonylureas tolbutamide (1 mM) and glibenclamide (1 μM) did not affect the open-state probability or the number of active channels either in the presence or absence of MgATP ($n = 4$ for each) (data not shown) nor did they influence channel activity in cell-attached recordings in the presence or absence of glucose ($n = 2$ for each) (data not shown).

DISCUSSION

Characteristics of the ATP-activated ion channel

The data presented here show that the application of ATP to the cytoplasmic surface of neuronal excised membrane patches activates a specific ion channel. The values obtained for the reversal potential in symmetrical and asymmetrical K^+ solutions (0 and −73 mV, respectively) indicate that this channel is strongly K^+ selective. The channel also exhibits outwardly rectifying properties in asymmetrical K^+ solutions, displays a linear current–voltage relation in symmetrical K^+ and is characterized by a large (130 pS) single-channel conductance. The evidence obtained using inside-out patches shows that the action of ATP is reversible and concentration dependent, and physiological concentrations of ATP (i.e. 1–3 mM) produced maximal channel activation. Therefore modulation of the ATP-activated K^+ channel could occur in the neurones studied under physiological conditions. As this action of ATP is dependent upon the presence of Mg^{2+} and cannot be mimicked by the non-hydrolysable analogue of ATP,

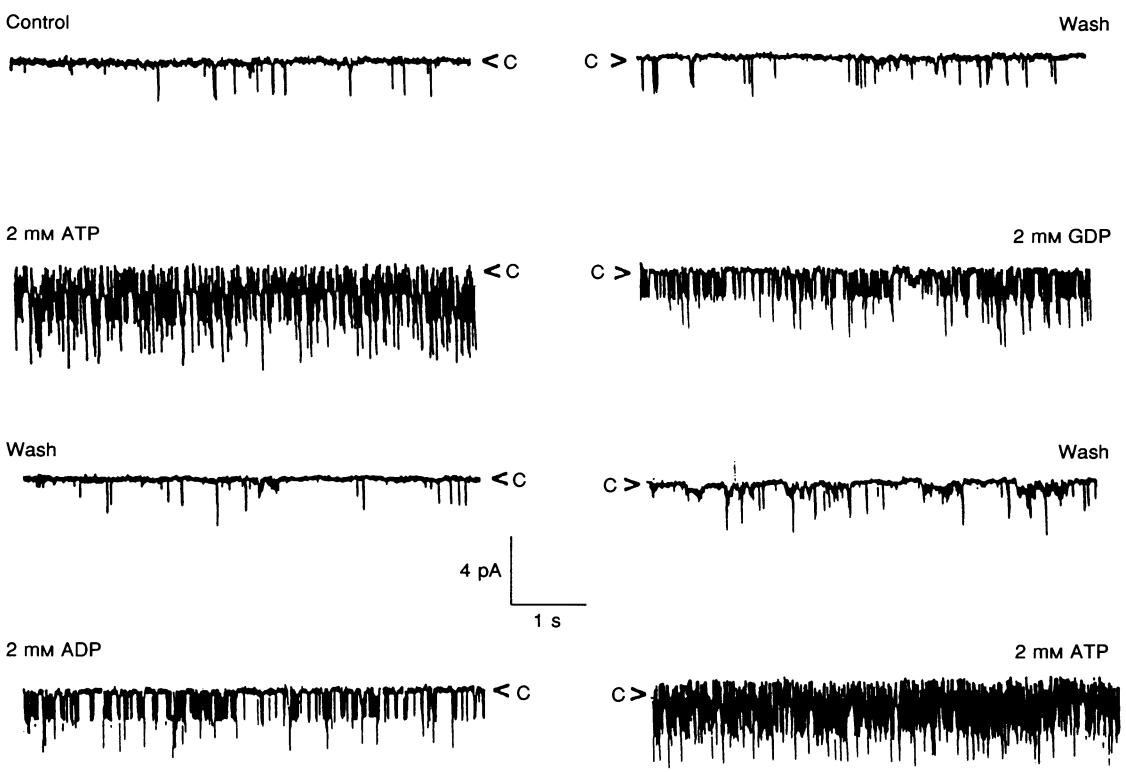

Figure 8. The effects of adenine and guanine nucleotides on the ATP-activated K^+ channel

The electrode solution contained (mM): KCl, 140; $CaCl_2$, 1; $MgCl_2$, 1; Hepes, 10; pH adjusted to 7·2 with KOH. The bath (intracellular) solution contained (mM): KCl, 140; $MgCl_2$, 0·8; $CaCl_2$, 0·9; EGTA, 1; Hepes, 10; pH adjusted to 7·2 with KOH (free Mg^{2+}, 0·8 mM). The figure shows the reversible activation of the channel by 2 mM ATP, ADP and GDP. Initial application of ATP increased P_o from 0·008 to 0·41, an action reversed upon wash-out ($P_o = 0·009$). ADP and GDP increased P_o to 0·23 and 0·25, respectively. These effects were reversible. The subsequent re-addition of ATP increased P_o to 0·47, close to the initial value of 0·41.

AMP(PNP), the most likely mechanism for activation is through the hydrolysis of ATP probably involving some phosphorylation process (Levitan, 1985; Walaas & Greengard, 1991). The considerable degree of variability in the level of activation produced by ATP from patch to patch is suggestive of an enzymic mechanism, and may represent the level of enzyme activity available in each membrane patch. The site of action has yet to be elucidated but it is reasonable to suppose the channel protein or a closely associated structure is the target, as has been suggested for other neuronal ion channels (Levitan, 1985; Chung *et al.* 1991; Rienhart *et al.* 1991).

Single-channel kinetic analysis shows that openings occur in bursts and that single openings tend to be of short duration (<1 ms). Upon application of ATP there is a marked increase in P_o. The activation is achieved by a large increase in the number of bursts, clustering of the bursts, and a small increase in the burst duration. ATP does not appear to stabilize single openings as there is no statistically significant change in mean open dwell times or in the open-state time constant. In many respects the K^+ channel kinetics recorded under cell-attached conditions are very similar to that obtained from inside-out patches. The increase in P_o elicited by an increase in the extracellular concentration of glucose is associated with no change in the open-state time constant or the fast closed-state time constant but with a substantial reduction of the slow closed-state time constant, effects also observed for the activation by ATP. The similarities in kinetics between the two recording modes before and after channel activation strengthen our belief that the channel activated by glucose in the intact cell is the ATP-activated channel observed in isolated patches. This is further supported by the similar values for mean single-channel conductance obtained in cell-attached patches (110 ± 12 pS) and excised patches (132 ± 16 pS). The lower value obtained in the intact cell may be due to intracellular factors. Thus we suggest that the ATP-activated channel, at least in part, underlies glucose sensing in these cells.

The inside-out patch data also indicate that ATP application recruits channels, as well as increasing P_o, and so has more than one action on this K^+ channel. It is also

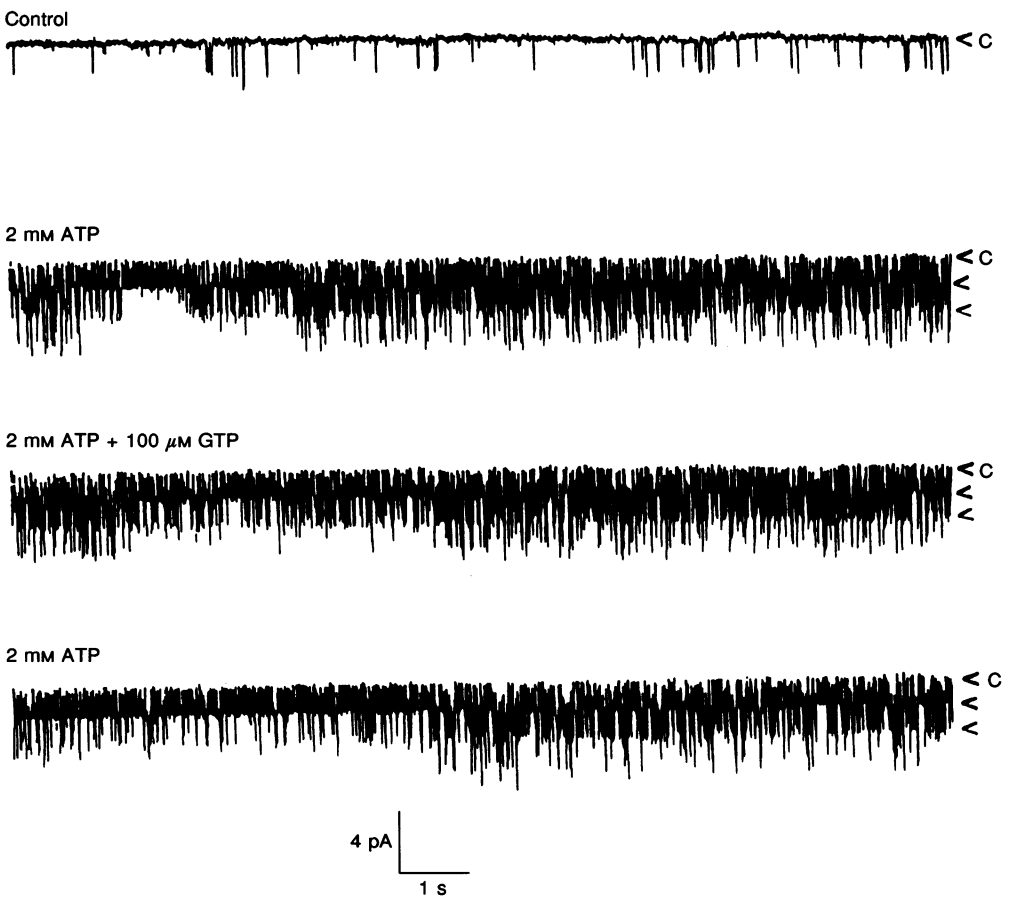

Figure 9. The ATP-activated K^+ channel is not inhibited by GTP

The solutions were as described in Fig. 8. The figure shows that the increase in channel activity produced by the addition of 2 mM ATP to the intracellular surface of the excised patch (P_o increased from 0·005 to 0·48) was not inhibited by the concomitant presence of 100 μM GTP ($P_o = 0·45$) nor upon removal of the GTP ($P_o = 0·42$). The channel activity returned to close to the control value (0·01) when ATP was finally washed out (not shown).

likely that Mg^{2+} influences channel activity in some way. The application of ATP at concentrations of 1–5 mM in the presence of 1 mM Mg^{2+} increases channel activity in a concentration-dependent manner, with a maximal effect at about 3 mM. However, the concentration of MgATP is constant at ATP concentrations of 1 mM and above under the conditions of our experiments. It may be the case that Mg^{2+} acts as a co-factor or catalyst and remains bound to the phosphorylation site while more than one molecule of ATP binds and is then hydrolysed to ADP.

Comparison with other ATP-modulated K^+ channels

A number of neuronal K_{Ca} channels which are also activated by intracellular ATP have been described in preparations of rat brain plasma membrane incorporated into planar lipid bilayers (Chung *et al.* 1991; Reinhart *et al.* 1991). The data show these channels to be modulated by the activity of cAMP-dependent protein kinase (PK-A), protein phosphatase 2A and protein phosphatase 1, while ATP hydrolysis also influenced channel activity. In these studies K_{Ca} channels acted as substrates for PK-A and phosphorylation altered the open probability in either direction while dephosphorylation reduced channel activity. ATP could modulate channel activity in some cases as endogenous protein kinase was present as part of the channel or closely linked with it in the membrane. However, the ATP-activated channel we have described is insensitive to both intracellular Ca^{2+} concentration and membrane potential, which indicates that it is a different type of K^+ channel from those seen in the bilayer experiments. Further investigation may reveal whether the channel in our study is also modulated by specific protein kinases and phosphatases and whether this type of regulatory complex is more generally important in controlling K^+ channel activity. However, the activation of the channel by GTP, GDP and ADP may indicate that hydrolysis is carried out by enyzmes such as ATPases which can use a wide range of substrates. Another possibility is that membrane-associated enzymes can use GTP and/or the diphosphates as substrates in the production of ATP as has been observed in the activation of K_{Ca} channels in rat cortical neurones (Lee, Rowe & Ashford, 1994).

ATP has previously been shown to activate atrial muscarinic K^+ channels in the absence of both acetylcholine and guanine nucleotides. The activation involves a phosphorylation reaction, probably a transphosphorylation by a nucleoside diphosphate kinase from ATP to G_k-protein-bound GDP (Heidbüchel *et al.* 1990, 1993). It is unlikely that the ATP-activated K^+ channel observed in this study is activated in this manner for a number of reasons. Firstly, the data obtained using cell-attached patches clearly show that the ATP-activated channel is stimulated by the addition of glucose. At these concentrations of glucose the neurone would contain up to micromolar concentrations of GTP and GDP within the range required to inhibit the ATP effects in atrial cells (Heidbüchel *et al.* 1990). Secondly neither GDP nor GTP inhibit the activation of channel activity produced by ATP in isolated patches; indeed GTP also activates this channel but is less effective than ATP. Thirdly application of acetylcholine to these cells results in excitation through activation of muscarinic receptors (Oomura, Nakamura, Sugimori & Yamada, 1975) and not the inhibition expected upon K^+ channel activation. Thus the 132 pS ATP-activated channel in the hypothalamic neurones differs markedly in its activation properties from the muscarinic-activated K^+ channel observed in atrial cells. Agonist-activated K^+ channels with similar enzyme systems and G-protein interactions to those descibed for the muscarinic K^+ channel are probably present in the hypothalamic neurones studied. The lower conductance (10–60 pS) channels briefly mentioned in the present study, which were activated by ATP, may make up such a population of agonist-linked K^+ channels and are worthy of further investigation.

Distribution of the ATP-activated K^+ channels

One of the most interesting features of this investigation has been the distribution of the ATP-activated channel in the basomedial hypothalamus. The cell-attached recordings followed by patch excision clearly demonstrate the channel to be present in GS neurones. The GS neurones were found predominantly in the LHA making up 42% of the neurones present there. In contrast only 8% of VMN neurones were glucose sensitive or contained ATP-activated channels. This distribution of GS neurones is similar to that described by other investigators using intracellular recordings from brain slices (Oomura *et al.* 1969, 1974), and any variation may be due to the smaller sample we have used. Furthermore, the ATP-activated channel was never observed in GR neurones or in isolated patches containing K_{ATP} or K_{Ca} channels, which suggests that it is specific to a group of hypothalamic neurones rather than being common to all.

Physiological role for the ATP-activated channel

Glucose-sensing neurones in the hypothalamus play a vital role in the central control of metabolism (Niijima, 1989). The mechanisms by which the central glucose sensors are able to monitor extracellular glucose concentration or by which they are modulated are therefore of great interest. In particular, glucose-sensitive neurones are thought to play an important role in appetite regulation and ATP-activated potassium channels may be an important mediator of their sensitivity to glucose. GS neurones hyperpolarize and decrease their frequency of firing of action potentials when the extracellular glucose concentration is raised (Oomura *et al.* 1969, 1974). It has been proposed that this hyperpolarization results from the activation of the Na^+–K^+-ATPase by intracellular ATP, since it can be inhibited by ouabain (Oomura *et al.* 1969, 1974). Our data are consistent with an alternative or complementary mechanism being involved. We suggest that, as the extracellular glucose

concentration increases and the intracellular ATP concentration rises, this results in the activation of an ATP-regulated K^+ channel. The resulting increase in K^+ conductance would hyperpolarize glucose-sensitive neurones and thus may contribute to their role in appetite regulation. However, the changes in K^+ channel activity we have recorded in the GS neurones in the presence of ATP may be of general importance in modulation of neuronal excitability.

If ATP-activated potassium channels are important in appetite regulation they would be potential sites of action for pharmacological compounds. Unlike K_{ATP} channels (Sturgess *et al.* 1985; Ashford, Boden & Treherne, 1990*c*), ATP-activated potassium channels are unaffected by sulphonylureas. Therefore it is feasible that ATP-activated K^+ channel activity can be manipulated selectively by drugs which do not influence K_{ATP} channels.

Ashcroft, F. M. (1988). Adenosine 5′-triphosphate-sensitive potassium channels. *Annual Review of Neuroscience* **11**, 97–118.

Ashford, M. L. J. (1990). Potassium channels and modulation of insulin secretion. In *Potassium Channels*, ed. Cook, N. S., pp. 300–319. Halstead Press, Chichester, UK.

Ashford, M. L. J., Boden, P. R. & Treherne, J. M. (1990*a*). Glucose-induced excitation of rat hypothalamic neurones is mediated by ATP-sensitive K^+ channels. *Pflügers Archiv* **415**, 479–483.

Ashford, M. L. J., Boden, P. R. & Treherne, J. M. (1990*b*). Sulphonylurea excitation of dissociated glucose-responsive hypothalamic neurones in the rat. *Journal of Physiology* **420**, 93*P*.

Ashford, M. L. J., Boden, P. R. & Treherne, J. M. (1990*c*). Tolbutamide excites glucoreceptive ventromedial hypothalamic neurones by indirect inhibition of ATP-K^+ channels. *British Journal of Pharmacology* **101**, 531–540.

Ashford, M. L. J. & Treherne, J. M. (1989). Adenosine triphosphate increases the activity of a potassium channel recorded from neonatal rat cultured neurones. *Journal of Physiology* **409**, 54*P*.

Blundell, J. (1991). Pharmacological approaches to appetite suppression. *Trends in Pharmacological Sciences* **12**, 147–157.

Boden, P. R. & Hill, R. G. (1988). Effects of cholecystokinin and related peptides on neuronal activity in the ventromedial nucleus of the rat hypothalamus. *British Journal of Pharmacology* **94**, 246–252.

Chung, S., Reinhart, P. H., Martin, B. L., Brautigan, M. & Levitan, I. B. (1991). Protein kinase activity closely associated with a reconstituted calcium-activated potassium channel. *Science* **253**, 560–562.

Dempster, J. (1988). Computer analysis of electrophysiological signals. In *Microcomputers in Physiology: a Practical Approach*, ed. Fraser, P. J., pp. 51–93. IRL Press, Oxford.

Frohman, L. A. (1983). CNS peptides and glucoregulation. *Annual Review of Physiology* **45**, 95–107.

Goldman, D. E. (1943). Potential, impedance and rectification in membranes. *Journal of General Physiology* **27**, 37–60.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

Heidbüchel, H., Callewaert, G., Vereecke, J. & Carmeliet, E. (1990). ATP-dependent activation of atrial muscarinic K^+ channels in the absence of agonist and G-nucleotides. *Pflügers Archiv* **416**, 213–215.

Heidbüchel, H., Callewaert, G., Vereecke, J. & Carmeliet, E. (1993). Acetylcholine-mediated K^+ channel activity in guinea-pig atrial cells is supported by nucleoside diphosphate kinase. *Pflügers Archiv* **422**, 316–324.

Hodgkin, A. L. & Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *Journal of Physiology* **108**, 37–77.

Jiang, C. & Haddad, G. (1991). Effect of anoxia on intracellular and extracellular potassium activity in hypoglossal neurons *in vitro*. *Journal of Neurophysiology* **61**, 103–111.

Kozlowski, R. Z. & Ashford, M. L. J. (1990). ATP-sensitive K^+ channel run-down is Mg^{2+} dependent. *Proceedings of the Royal Society* B **240**, 397–410.

Lee, K., Rowe, I. C. M. & Ashford, M. L. J. (1994). Adenosine and cytidine nucleotides activate K_{Ca} channels in rat isolated cortical neurones. *Journal of Physiology* **477.P**, 83*P*.

Levitan, I. B. (1985). Phosphorylation of ion channels. *Journal of Membrane Biology* **87**, 177–190.

Luiten, P. G. M., Ter Horst, G. J. & Steffens, A. B. (1987). The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. *Progress in Neurobiology* **28**, 1–54.

Minami, T., Oomura, Y. & Sugimori, M. (1986). Electrophysiological properties and glucose responsiveness of guinea-pig ventromedial hypothalamic neurones *in vitro*. *Journal of Physiology* **380**, 127–143.

Mizuno, Y. & Oomura, Y. (1984). Glucose responding neurones in the nucleus tractus solitarius of the rat: *In vitro* study. *Brain Research* **307**, 109–116.

Morley, J. E. & Levine, A. S. (1985). The pharmacology of eating behavior. *Annual Review of Pharmacology and Toxicology* **25**, 127–146.

Niijima, A. (1989). Nervous regulation of metabolism. *Progress in Neurobiology* **33**, 135–147.

Ono, T., Nishino, H., Fukuda, M., Sasaki, K., Muramoto, K. L. & Oomura, Y. (1982). Glucoresponsive neurones in rat ventromedial hypothalamic tissue slices *in vitro*. *Brain Research* **232**, 494–499.

Oomura, Y., Nakamura, T., Sugimori, M. & Yamada, Y. (1975). Effect of free fatty acid on the rat lateral hypothalamic neurons. *Physiology and Behavior* **14**, 483–486.

Oomura, Y., Ono, T., Ooyama, H. & Wayner, M. J. (1969). Glucose and osmosensitive neurones in the rat hypothalamus. *Nature* **222**, 282–284.

Oomura, Y., Ooyama, H., Sugimori, M., Nakamura, T. & Yamada, Y. (1974). Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature* **247**, 284–286.

Reinhart, P. H., Chung, S., Martin, B. L., Brautigan, D. L. & Levitan, I. B. (1991). Modulation of calcium-activated potassium channels from rat brain by protein kinase A and phosphatase 2A. *Journal of Neuroscience* **11**, 1627–1635.

Rowe, I. C. M. & Ashford M. L. J. (1993). The effects of adenosine and guanine nucleotides on the ATP-activated K^+ channel in rat isolated hypothalamic neurones. *Journal of Physiology* **467**, 268*P*.

ROWE, I. C. M., TREHERNE, J. M. & ASHFORD, M. L. J. (1993). The effects of adenosine nucleotides on the ATP-activated K^+ channel in rat isolated hypothalamic neurones. *Journal of Physiology* **459**, 54*P*.

SHIMIZU, N. & OOMURA, Y. (1986). Calcitonin-induced anorexia in rats: Evidence for its inhibitory action on lateral hypothalamic chemosensitive neurons. *Brain Research* **367**, 128–140.

STURGESS, N. C., ASHFORD, M. L. J., COOK, D. L. & HALES, N. C. (1985). The sulphonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* **44**, 474–475.

TREHERNE, J. M. & ASHFORD, M. L. J. (1991). Calcium-activated potassium channels in rat dissociated ventromedial hypothalamic neurones. *Journal of Neuroendocrinology* **3**, 323–329.

TRUBE, G., RORSMAN, P. & OHNO-SHOSAKU, T. (1986) Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic b-cells. *Pflügers Archiv* **407**, 493–499.

WALAAS, S. I. & GREENGARD, P. (1991). Protein phosphorylation and neuronal function. *Pharmacological Reviews* **43**, 300–334.

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Authors' present addresses

I. C. M. Rowe: Department of Biomedical Sciences, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, UK.

J. M. Treherne: Discovery Biology Department, Pfizer Central Research, Pfizer Ltd, Sandwich, Kent CT13 9NJ, UK.

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