Characterization of an ATP-modulated large conductance Ca^{2+}-activated K^+ channel present in rat cortical neurones

K. Lee, I. C. M. Rowe and M. L. J. Ashford*

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

1. Single channel current recordings were used to study the characteristics of a large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel present in neurones acutely dissociated from the rat motor cortex. Application of ATP to the intracellular surface of excised inside-out patches produced a large, concentration-dependent increase in BK_{Ca} channel activity.

2. This ATP-mediated activation was dependent upon the presence of Mg^{2+} in the intracellular bathing solution and was diminished by the phosphatases 2,3-butanedione monoxime (BDM) or alkaline phosphatase and by the protein kinase inhibitors staurosporine, H-7 and PKI.

3. ADP stimulated BK_{Ca} channel activity in a Mg^{2+}-dependent manner, an action also inhibited by the concomitant application of PKI or BDM. The effect of ADP was reduced by application of hexokinase and glucose or by application of the adenylate kinase inhibitor Ap_5A.

4. Of other nucleotides tested, only CTP consistently activated BK_{Ca} channel activity.

5. Using the cell-attached configuration, bath application of forskolin or dibutyryl cAMP stimulated BK_{Ca} channel activity.

6. It is concluded that BK_{Ca} channel activity in the rat motor cortex is subject to modulation by the activity of a closely associated kinase. The ability of cAMP activators to stimulate BK_{Ca} channel activity in the intact cell suggests that this system may be of physiological importance.

Large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels have been identified in many cell types including neurones, smooth and skeletal muscle, kidney tubules, and both endocrine and exocrine glands (Latorre, Oberhauser, Labarca & Alvarez, 1989). Although the precise role of these channels is not clear, they have been implicated in a number of important physiological processes such as the regulation of secretion from endocrine and exocrine glands, the co-ordination of membrane excitability in neurones and the control of K^+ ion movement across epithelia.

The regulation of BK_{Ca} channel activity is complex, with both a rise in intracellular Ca^{2+} and membrane depolarization producing an increase in channel activity. Additionally, it has recently been shown that conditions which favour protein phosphorylation can also enhance BK_{Ca} channel activity in a number of tissues (Sadoshima, Akaike, Kanaide & Nakamura, 1988; Kume, Takei, Tokuno & Tomita, 1989). However, the most widely studied example of BK_{Ca} channel regulation by phosphorylation has been obtained using synaptosomal membranes from the rat brain incorporated into lipid bilayers (Farley & Rudy, 1988).

Using this preparation, investigators have demonstrated the presence of at least two types of neuronal BK_{Ca} channel; type I BK_{Ca} channels demonstrate rapid gating kinetics and are charybdotoxin sensitive, whilst type II BK_{Ca} channels exhibit much slower gating kinetics and are insensitive to the external application of charybdotoxin (Reinhart, Chung & Levitan, 1989). Furthermore, the activity of these two channel types appears to be differentially regulated by the catalytic subunit of protein kinase A (PKA). The type I channel undergoes an increase in activity in response to the application of this kinase whilst the type II channel is downregulated (Reinhart, Chung, Martin, Brautigan & Levitan, 1991).

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

In the present study a nucleotide-modulated BK_{Ca} channel has been identified in neurones acutely dissociated from rat motor cortex and its characteristics have been examined and compared with those of BK_{Ca} channels previously reported in the rat CNS. Some of these results have been communicated to the Physiological Society (Lee, Rowe & Ashford, 1994*a*,*b*).

METHODS

Drugs and solutions

All solutions were made using glass-distilled water and Analar grade chemicals. The artificial cerebrospinal fluid (ACSF) for rat cortical slices contained (mM): 128·0 NaCl, 5·0 KCl, 1·2 NaH_2PO_4, 2·4 $CaCl_2$, 1·3 $MgCl_2$, 26·0 $NaHCO_3$ and 10·0 D-glucose; pH 7·4. Before single channel recording, the cells were washed thoroughly with normal external saline (solution A) which consisted of (mM): 135·0 NaCl, 5·0 KCl, 1·0 $CaCl_2$, 1·0 $MgCl_2$ and 10·0 Hepes; the pH was adjusted to 7·2 with NaOH. For cell-attached recording the patch pipette was filled with the following solution (solution B; mM): 140·0 KCl, 1·0 $CaCl_2$, 1·0 $MgCl_2$ and 10·0 Hepes; pH adjusted to 7·2 with KOH; and the bathing solution was solution A. The solution in the recording pipette used for inside-out patch studies either contained the same solution as for cell-attached recording (solution B, i.e. symmetrical 140 mM KCl), or normal external saline (solution A, i.e. approximately physiological ionic gradients). The bathing solution for inside-out patch recordings (solution C) contained (mM): 140·0 KCl, 0·8 $CaCl_2$, 1·0 $MgCl_2$, 1·0 EGTA and 10·0 Hepes; the pH was adjusted to 7·2 with KOH and the free Ca^{2+} and Mg^{2+} concentrations were 1·0 μM and 1 mM, respectively. Experiments to determine the BK_{Ca} channel pH sensitivity were performed with Mes buffer for solutions of pH less than 7·2 and Tris buffer for solutions of pH greater than 7·2. In experiments to ascertain the Ca^{2+} sensitivity of the channel, the concentrations of $CaCl_2$ and $MgCl_2$ were varied in order to produce the required change in the concentration of Ca^{2+} whilst keeping the concentration of Mg^{2+} constant. Similarly, in experiments where nucleotides were used, the concentrations of $CaCl_2$ and $MgCl_2$ were varied in order to compensate for their chelation by the nucleotides. The concentrations of free ions in each solution were calculated using the binding constants determined by Fabiato & Fabiato (1979). To confirm that none of the added nucleotides at their maximal concentrations affected the level of free Ca^{2+}, solutions were examined fluorimetrically using free fura-2 acid as previously documented (Thomas & Delaville, 1991).

All nucleotides, drugs and other chemicals with the exception of okadaic acid, 1-(5-isoquinolinesulphonyl)-2-methylpiperazine (H-7), P^1,P^5-di(adenosine-5′)pentaphosphate (Ap_5A) and charybdotoxin were purchased from Sigma. Okadaic acid, charybdotoxin and H-7 were obtained from Calbiochem (Nottingham, UK) whilst Ap_5A was obtained from Boehringer Mannheim. Solutions were exchanged during recordings by superfusing the whole bath using a gravity feed system. Complete solution exchange was achieved within 30 s to 1 min.

Isolation of cortical neurones

Acutely dissociated neurones were prepared by a method similar to that described previously (Ashford, Boden & Treherne, 1990). In brief, the motor area of the cerebral cortex was isolated from 300–400 μm coronal slices (Sprague–Dawley rats, 100–300 g). The isolated cortical sections were incubated for 10 min in Pronase E (1·5 mg ml^{-1}) in ACSF and dispersed by trituration.

Electrophysiological recording and data analysis

Single channel currents were recorded from cell-attached and excised membrane patches using standard patch-clamp recording procedures (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Recording pipettes, when filled with electrolyte, had resistances of 8–15 MΩ. Current recordings were obtained using a patch-clamp amplifier (EPC-7, List Electronic, Germany) and recorded onto digital audio tape. The tape was later replayed through an 8-pole Bessel filter (−3 dB at 1 kHz) and digitized at 5 kHz with a 12-bit analog-to-digital converter (Data Translation Inc., Marlborough, MA, USA). The data were analysed on a 386-based microcomputer (Elonex plc, London, UK) using PAT 6.2 software (patch-clamp analysis program 6.2, J. Dempster, University of Strathclyde, UK). Current amplitudes and open-state probability (P_o) were determined as described previously (Dempster, 1988). In multichannel patches, channel activity was determined by integration of the current signal over 40–120 s at a constant voltage and the result expressed as the function $N_f P_o$ where N_f is the number of functional channels and P_o the open-state probability. The mean open-state probability for individual channels was obtained from the value of $N_f P_o$ once the number of functional channels in the patch was established. In order to determine whether a particular agent produced a significant change in channel activity, data were subjected to one-way analysis of variance (ANOVA) using the computer program 'Modstat' (Shareware, Modern Microcomputers, Mechanicsville, VA, USA) as implemented on the Elonex 386 microcomputer.

Data for the variation of P_o with membrane potential (V) were fitted to the following equation:

$$P_o = P_{max}(1 + e^{-A(V - V_{0.5})})^{-1}, \quad (1)$$

where P_{max} is the maximum open-state probability, $V_{0.5}$ the membrane potential at which P_{open} is half of P_{max} and A is a constant related to the slope of the curve. The best fit values for P_{max}, $V_{0.5}$ and A were obtained from non-linear regression using Kaleidagraph 3.0 (Abelbeck Software, Reading, PA, USA) as implemented on the Macintosh LC III microcomputer.

To assess if the channels were acting independently of each other in the presence of various modulating agents, the percentage time that each channel was open in a given patch was compared with that predicted by the binomial distribution:

$$P(r) = 100 \frac{n!}{r!(n-r)!} p^r (1-p)^{n-r}, \quad (2)$$

where $P(r)$ is the percentage time that 0, 1, 2...n channels are open, n is the total number of channels in the membrane patch, r is the number of channels open and p is the probability that any given channel is open.

In order to assess the pH sensitivity of BK_{Ca} channel activity, the data obtained in the presence of varying conditions of intracellular pH were fitted by non-linear regression to the following equation:

$$P_o = 1/[1 + (pK/pH)^n], \quad (3)$$

where P_o is the channel activity at the test pH, pK is the logarithm of the apparent dissociation constant and n is the Hill coefficient.

Single channel records used for illustrative purposes were replayed into a chart recorder (Gould 2200) which had a nominal frequency response of 140 Hz. All recordings illustrated in the text, except

where stated otherwise, were recorded from inside-out patches at a holding potential of +20 mV in the presence of 140 mM KCl and 1 μM Ca^{2+}; i.e. the pipette contained solution B while the bath contained solution C. The potential across the membrane 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 means ± S.E.M. When presented graphically, the S.E.M. is represented by the associated error bars where larger than the symbols.

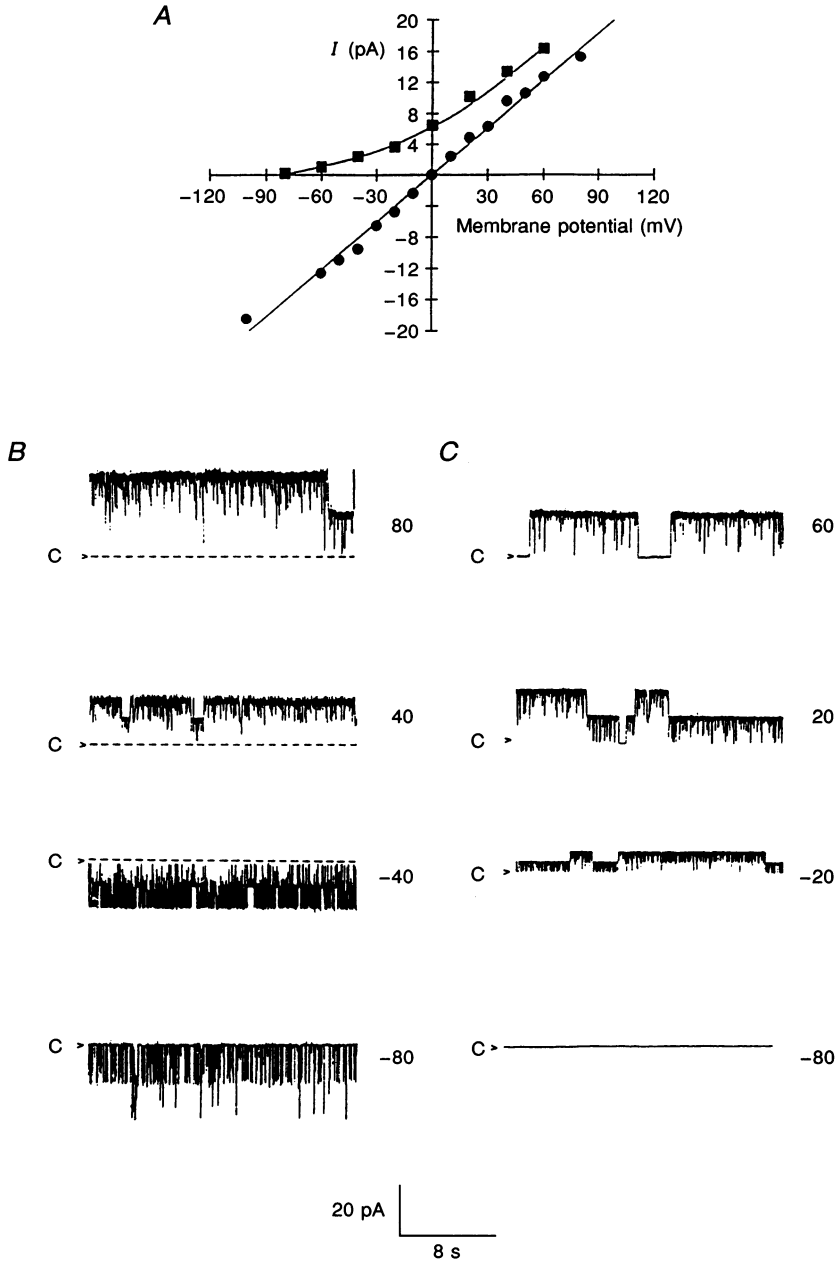

Figure 1. Single channel current–voltage relationship of the BK_{Ca} channel

A, ■, currents recorded in a quasi-physiological cation gradient, i.e. 135 mM NaCl and 5 mM KCl in the pipette (solution A) and 140 mM KCl in the bath ($n = 7$). The line drawn through the points represents the Goldman–Hodgkin–Katz constant current equation for a channel of 245 pS under this ionic gradient. ●, currents recorded with symmetrical 140 mM KCl bathing both surfaces of the membrane. The line drawn through these points is the line of best fit by linear regression and gives a conductance of 245·0 ± 10·1 pS ($n = 8$). *B*, single BK_{Ca} channel currents recorded from an inside-out patch recorded at various holding potentials in the presence of symmetrical 140 mM KCl. *C*, single BK_{Ca} channel currents recorded from an inside-out patch recorded at various holding potentials in the presence of a quasi-physiological ionic gradient. The holding potential of the patch (in millivolts) is indicated to the right of each recording in *B* and *C*. C, closed state.

RESULTS

Effect of Ca^{2+} concentration and membrane potential upon BK_{Ca} channel activity recorded from rat motor cortical neurones

Isolated inside-out patch recordings ($n = 241$) made from acutely dissociated neurones from the motor cortex of male Sprague–Dawley rats contained 5·8 ± 0·6 ($n = 192$) BK_{Ca} channels per patch. Under conditions of symmetrical 140 mM KCl, the BK_{Ca} channel displayed a linear current–voltage relationship (Fig. 1) with a mean single channel conductance (over the range ±80 mV) of 245·0 ± 10·1 pS ($n = 8$). 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 exhibited pronounced outward rectification at positive potentials and the current–voltage relationship was close to that predicted by the Goldman–Hodgkin–Katz constant current equation for this K^+ gradient (Fig. 1). The extrapolated reversal potential under these recording conditions was approximately −80 mV ($n = 7$), close to the calculated value of −84 mV for a K^+ selective conductance.

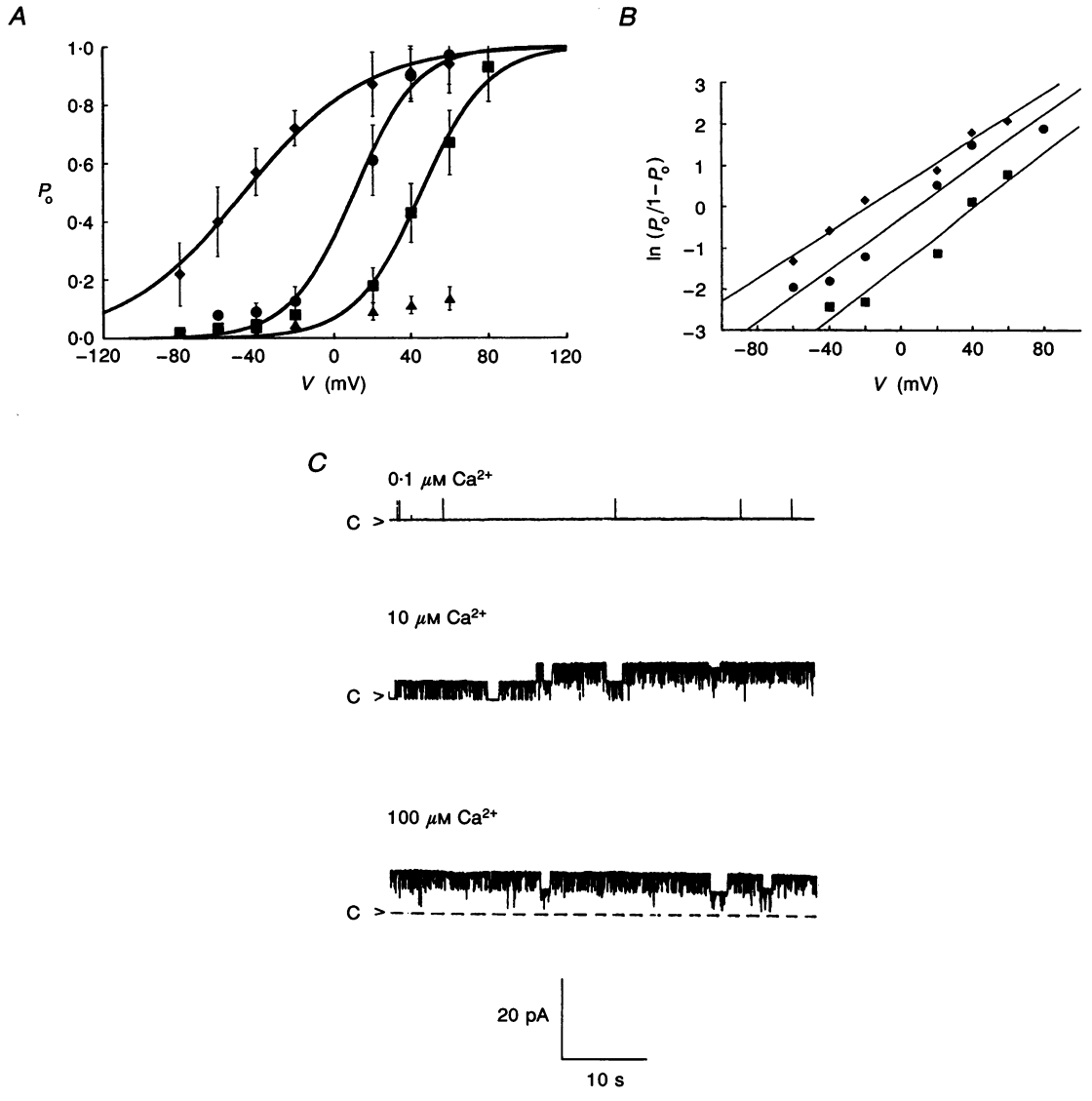

Figure 2. The effect of Ca^{2+} and voltage on BK_{Ca} channel activity

A, effect of membrane potential upon channel P_o as a function of intracellular Ca^{2+}. ◆, 100 μM Ca^{2+}; ●, 10 μM Ca^{2+}; ■, 1 μM Ca^{2+}; and ▲, 0·1 μM Ca^{2+}. Each point is the mean of 4–8 determinations. The fitted lines are the lines of best fit to a Boltzman distribution (eqn (1)). *B*, linearization of the data presented in *A*. *C*, single channel recordings of BK_{Ca} channel activity in the presence of varying concentrations of intracellular Ca^{2+}. The values of P_o were as follows: 0·1 μM Ca^{2+}, 0·08; 10 μM Ca^{2+}, 0·65; 100 μM Ca^{2+}, 0·89.

In addition to a high unitary conductance and K^+ selectivity, the activity of the channel was dependent on both membrane voltage and the concentration of Ca^{2+} in contact with the intracellular surface of the channel. The open-state probability of the channel increased with membrane depolarization and/or increasing concentrations of Ca^{2+} to the intracellular surface of the membrane patch (Fig. 2). On the basis of these findings, the channel was identified as a large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel (Barrett, Magleby & Pallotta, 1982).

In agreement with previous reports, the channel observed in the present study exhibited some variability in Ca^{2+} sensitivity (Moczydlowski & Latorre, 1983). However, in general, at 0·1 μM Ca^{2+} there was little channel activity observed at a membrane potential of +20 mV, and the introduction of 10 μM or 100 μM Ca^{2+} was consistently associated with a dramatic rise in channel activity which was maintained throughout the period of exposure to Ca^{2+} (Fig. 2*C*). As noted for other channels of this type, variation of the Ca^{2+} concentration bathing the extracellular surface did not affect channel activity.

Data for the variation in channel open probability (P_o) with membrane potential (V) were fitted to the Boltzman equation (eqn (1), see Methods) for a two-state model which allowed estimates to be obtained for $V_{0.5}$ (the membrane potential at which P_o is half the maximum fitted value of P_o) at different Ca^{2+} concentrations (Fig. 2*A*). The mean value for $V_{0.5}$ moved to more hyperpolarized potentials as the free cytoplasmic Ca^{2+} concentration was increased: 45·2 ± 1·9 mV at 1 μM Ca^{2+} ($n = 7$), 10·3 ± 2·7 mV at 10 μM Ca^{2+} ($n = 6$) and −46·3 ± 2·0 at 100 μM Ca^{2+} ($n = 5$). This resulted in a 10·3 mV change in $V_{0.5}$ per e-fold increase in intracellular Ca^{2+} concentration. From the slope of $\ln(P_o/(1 - P_o))$ *versus* membrane potential, the channel underwent an e-fold change in P_o per 31·2 ± 1·6 mV (1 μM Ca^{2+}), 33·4 ± 1·8 mV (10 μM Ca^{2+}) or 28·2 ± 2·2 mV (100 μM Ca^{2+}) change in membrane holding potential (Fig. 2*B*).

Changes in channel activity associated with the variation in intracellular Ca^{2+} concentration were well fitted to the binomial expression (eqn (2), see Methods) suggesting that these channels act independently of each other in the presence of varying levels of intracellular Ca^{2+} alone.

The effect of pH upon BK_{Ca} channel activity

The concentration of protons present at the intracellular surface has previously been shown to be an important

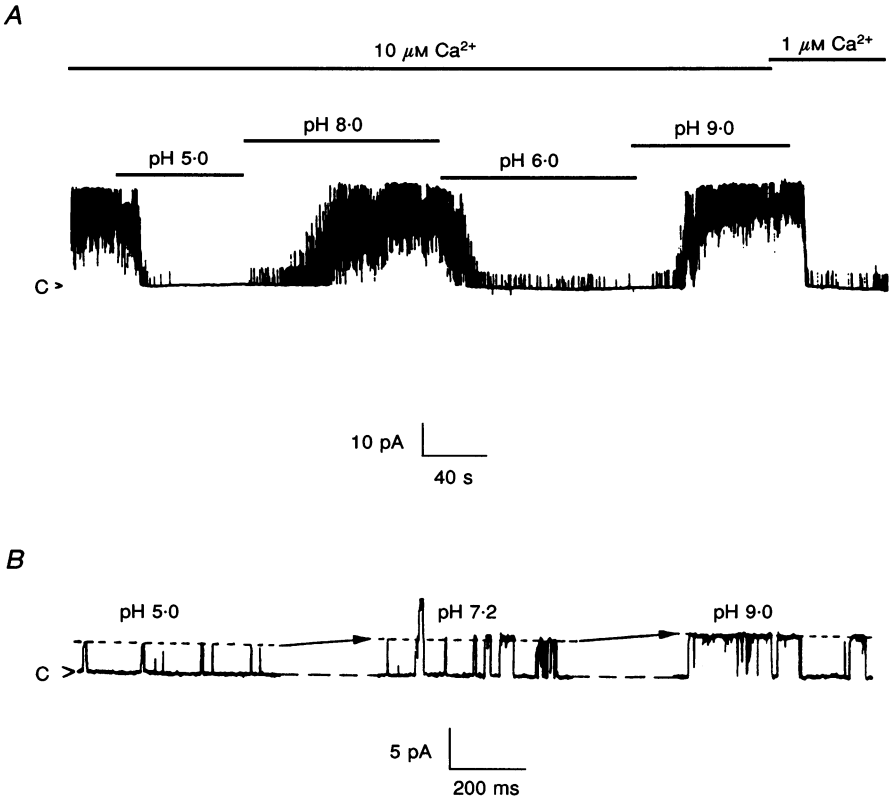

Figure 3. The effect of intracellular pH upon BK_{Ca} channel activity

A, representative record of channel activity at the indicated pH in the presence of 10 μM Ca^{2+}. The values of $N_f P_o$ were as follows: pH 7·2, 3·86; pH 5·0, 0·0; pH 8·0, 3·92; pH 6·0, 0·27; pH 9·0, 4·81. *B*, the effect of intracellular pH upon BK_{Ca} channel current amplitude.

modulator of BK_{Ca} channel activity in peripheral tissues (Cook, Ikeuchi & Fujimoto, 1984). Similar findings were observed in the present investigation. As illustrated in Fig. 3, acidification of the intracellular compartment led to a dramatic inhibition of BK_{Ca} channel activity in the presence of 10 μM Ca^{2+}. At pH 6·0 and 5·0, channel P_o at +20 mV was reduced from 0·67 ± 0·08 (at pH 7·2, $n = 5$) to 0·03 ± 0·01 ($n = 5$) and 0·01 ± 0·002 ($n = 4$), respectively. Conversely, when the concentration of protons bathing the intracellular solution was reduced, there was an increase in channel P_o from 0·64 ± 0·06 ($n = 6$, pH 7·2) to 0·82 ± 0·12 ($n = 4$) at pH 8·0 and to 0·85 ± 0·05 at pH 9·0 ($n = 4$). In addition to changes in channel P_o, lowering the intracellular pH reduced the single channel current amplitude, whilst alkalization caused a slight increase in amplitude (see Fig. 3*B*). At pH 5·0, the BK_{Ca} channel exhibited a single channel current amplitude of 3·88 ± 0·06 pA ($n = 4$) at +20 mV compared with 5·28 ± 0·05 pA ($n = 4$) at pH 9·0.

The mean value for $V_{0.5}$ occurred at more hyperpolarized potentials as intracellular pH was increased: 10·3 ± 2·8 at pH 7·2; −22·9 ± 2·8 at pH 8; −26·5 ± 2·5 at pH 9·0 (Fig. 4*A*). The data obtained at each holding potential were also fitted to a modified version of the Hill equation (eqn (3), Fig. 4*B*). At +20 mV, the best fit values to this equation were obtained with $pK = 6·67 \pm 0·23$ and $n = 1·28 \pm 0·14$ and the corresponding values for +40 mV and +60 mV were $pK = 6·51 \pm 0·16$ and $n = 1·48 \pm 0·21$ and $pK = 6·29 \pm 0·22$ and $n = 1·25 \pm 0·13$, respectively.

The effect of ATP upon BK_{Ca} channel activity

Recent investigations have shown that BK_{Ca} channels isolated from rat brain can be modulated by phosphorylation (Reinhart *et al.* 1991). Furthermore, it has been demonstrated that at least one type of neuronal BK_{Ca} channel can be activated by the intracellular application of ATP (Chung, Reinhart, Martin, Brautigan & Levitan, 1991). We therefore examined the effect of this nucleotide

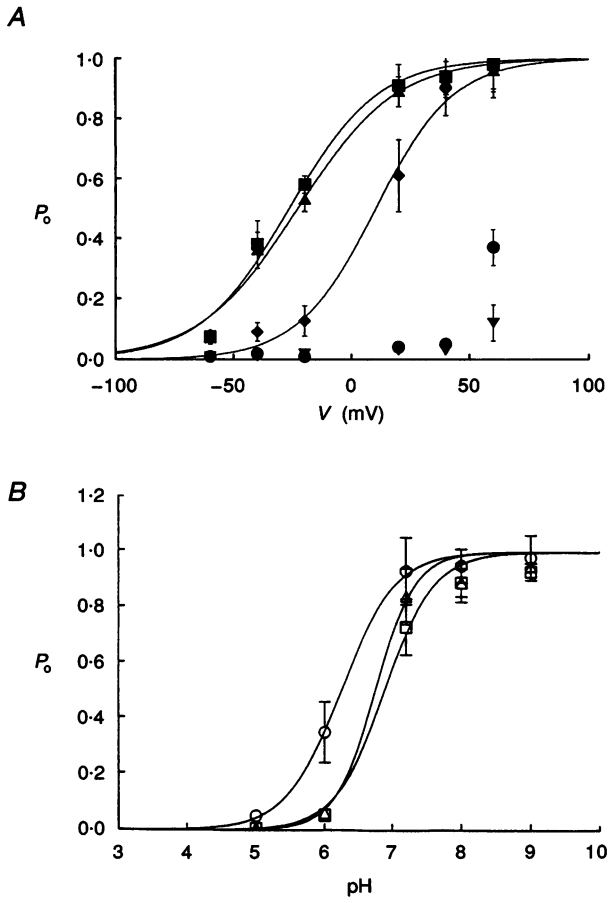

Figure 4. Effect of membrane potential on BK_{Ca} channel activity at different levels of intracellular pH

A, the relationship between the membrane potential and channel P_o in the presence of 10 μM free intracellular Ca^{2+} and varying intracellular pH. ■, pH 9·0; ▲, pH 8·0; ◆, pH 7·2; ●, pH 6·0; and ▼, pH 5·0. Each point is the mean of 3–5 determinations. The fitted lines are the lines of best fit to a Boltzman distribution. *B*, the relationship between intracellular pH and channel P_o at various membrane potentials. ○, +60 mV; △, +40 mV; and □, +20 mV. Data were taken from Fig. 4*A* and were fitted to eqn (3) using non-linear regression.

upon the activity of the BK_{Ca} channel observed in the present study. As depicted in Fig. 5*A*, application of 5 mM ATP to the cytoplasmic surface of an excised inside-out patch led to a large increase in channel activity. This concentration of ATP activated BK_{Ca} channel activity in 95% of the patches in which it was tested ($n = 42$). In the presence of 1 μM Ca^{2+}, application of 5 mM ATP increased the BK_{Ca} channel open probability from $0{\cdot}13 \pm 0{\cdot}03$ to $0{\cdot}64 \pm 0{\cdot}17$ ($n = 40$, see Table 1) at a membrane potential of +20 mV. Furthermore, 5 mM ATP also stimulated BK_{Ca} channel activity when applied in the presence of lower concentrations of intracellular Ca^{2+}. This is illustrated in Fig. 5*B* in the form of a Hill plot relating open probability to intracellular Ca^{2+} concentration. In the absence of ATP, increasing the Ca^{2+} concentration over the range 10 nM to 100 μM at a membrane potential of +20 mV was associated with a Hill coefficient ranging between 1·2 and 1·5. However, in the presence of 5 mM ATP the slope of the activation curve was reduced and was characterized by a Hill coefficient between 0·6 and 0·8.

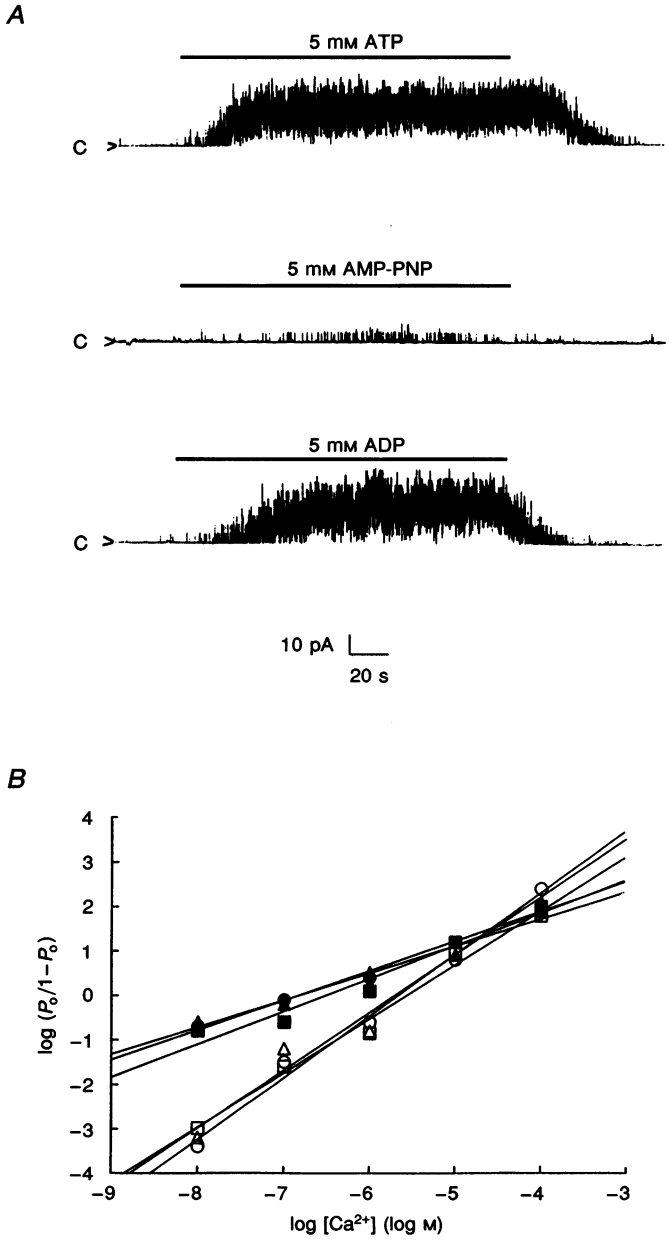

Figure 5. The effect of ATP upon BK_{Ca} channel activity

A, single channel recording illustrating the reversible activation of BK_{Ca} channel activity by ATP and ADP and the lack of effect of AMP-PNP. *B*, Hill plot illustrating the relationship between intracellular Ca^{2+} concentration and channel open probability in the presence (closed symbols) and absence (open symbols) of 5 mM ATP at the intracellular surface. Three representative experiments are shown for each.

Table 1. Effect of ATP concentration on BK_{Ca} channel open-state probability

[ATP] (mM)	Activation	n/total
0·1	0·07 ± 0·03 to 0·08 ± 0·04	0/6
0·5	0·11 ± 0·04 to 0·09 ± 0·03	0/6
1·0	0·12 ± 0·05 to 0·38 ± 0·08*	12/37
2·0	0·09 ± 0·05 to 0·57 ± 0·13*	24/24
5·0	0·13 ± 0·03 to 0·64 ± 0·17*	40/42
10·0	0·11 ± 0·03 to 0·84 ± 0·07*	5/5

The values in the right hand column indicate the number of times the effect was observed out of the total number of times the effect was tested for. * Significant ($P < 0·05$) increase in channel activity with respect to control.

Although the application of ATP increased BK_{Ca} channel activity over a wide range of intracellular Ca^{2+} concentrations (0·01–10 μM), the channel retained its voltage sensitivity. In the presence of 5 mM ATP and 1 μM Ca^{2+}, the channel underwent an e-fold change in P_o per 29·4 ± 0·9 mV change in membrane potential whilst in the presence of 1 μM Ca^{2+} alone, there was an e-fold change in P_o per 31·2 ± 1·6 mV change in membrane potential.

The degree of BK_{Ca} channel activation produced by ATP was, to some extent, concentration dependent (see Table 1 and Fig. 6*A*). Although concentrations of ATP above 2 mM were seen to increase BK_{Ca} channel activity consistently, ATP concentrations below 2 mM had more variable effects upon channel activity. For example, 1 mM ATP increased BK_{Ca} channel activity in only twelve out of thirty-seven experiments.

Using binomial analysis (Fig. 6*B*), good agreement between observed and predicted values of P_o were found when ATP was applied to the channel's intracellular surface at concentrations between 1 and 10 mM ($n = 18$). This finding suggests that ATP exerts its effects upon BK_{Ca} channel activity by increasing the P_o of each channel in the patch to a similar extent.

Mg^{2+} is required for the activation of the BK_{Ca} channel by ATP

To investigate the mechanism of ATP activation of the BK_{Ca} channel, the effects of the non-hydrolysable ATP analogue AMP-PNP were examined (Fig. 5). This nucleotide was tested in a total of four patches at the same concentration as that of ATP, which had previously been shown to stimulate channel activity (1–5 mM). In contrast to ATP, AMP-PNP was unable to alter BK_{Ca} channel activity. This finding suggests that ATP hydrolysis may be involved in the process by which ATP stimulates BK_{Ca} channel activity. Many biological processes which utilize ATP as a high energy phosphate donor require the divalent cation Mg^{2+} as a cofactor (Wacker, 1969). In order to determine if this was true in the present situation, the effect of this cation was examined upon the channel activation produced by ATP. When Mg^{2+} was removed from the intracellular solution, ATP was no longer able to activate the channels present (Fig. 7*A*).

The stimulation of BK_{Ca} channel activity by 1 mM ATP was also reduced by increasing the concentration of intracellular Mg^{2+} from 1 to 2 mM. This is illustrated in Fig. 7*B* which shows activation of BK_{Ca} channel activity by 1 mM ATP ($N_f P_o$ increased from 0·36 to 1·65). However, when the intracellular concentration of Mg^{2+} was increased to 2 mM, there was a reduction in channel $N_f P_o$ to 0·51. Similar results were obtained in three further experiments where 1 mM ATP was applied to stimulate channel activity ($N_f P_o$ reduced from 1·48 ± 0·57 ($n = 3$) in the presence of 1 mM ATP and 1 mM Mg^{2+} to 0·67 ± 0·22 ($n = 3$) in the presence of 1 mM ATP and 2 mM Mg^{2+}). In contrast, increasing the concentration of Mg^{2+} in the presence of 10 μM Ca^{2+} induced no significant change in channel activity ($n = 3$, data not shown) in the absence of ATP.

These results suggest that the effects produced by ATP upon the BK_{Ca} channel require Mg^{2+} as a cofactor but higher concentrations of this cation serve to inhibit this activation. Attempts to investigate the relationship between channel activity and free ATP^{4-} concentration were not made due to these dual actions of Mg^{2+}.

Since many ATP-mediated processes which require Mg^{2+} involve substrate phosphorylation, the effects of the chemical phosphatase 2,3-butanedione monoxime (BDM, 5 mM) were examined. In the absence of ATP, this compound was without effect upon channel activity in the presence of either 1 μM or 10 μM Ca^{2+}. However, 5 mM BDM was found to reduce the level of activation produced by 1 or 2 mM ATP by 42·3 ± 10·4% ($n = 4$; Fig. 8*A*), providing evidence that the channel or an associated protein may be phosphorylated in the process of BK_{Ca} channel activation. This hypothesis was further supported by the finding that ATP-induced BK_{Ca} channel activation was reduced by the concomitant application of alkaline

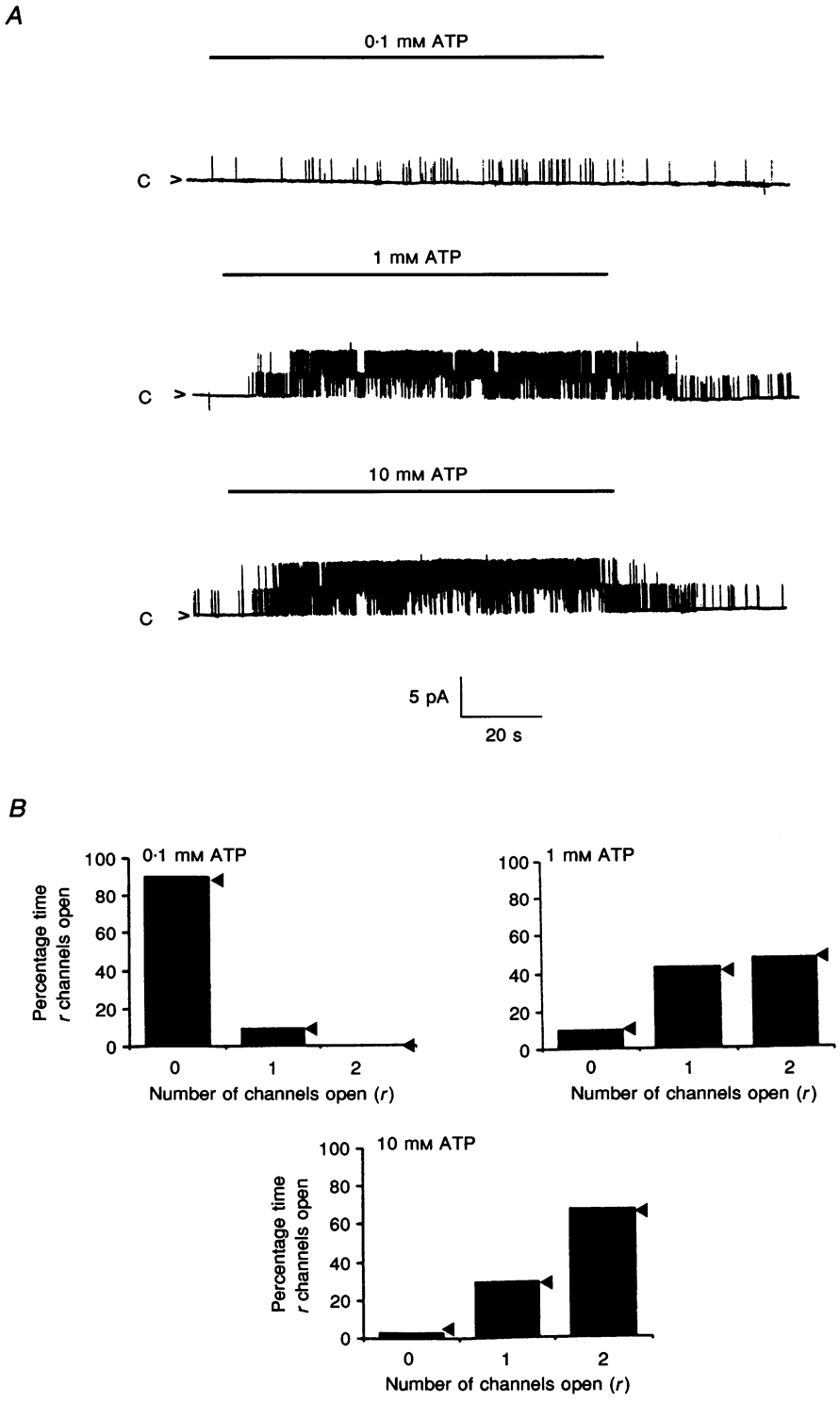

Figure 6. Effect of increasing concentration of ATP on channel activity

A, representative records of BK_{Ca} channel activity at the indicated concentration of ATP. The values for $N_f P_o$ were as follows: control, 0·16; 0·1 mM ATP, 0·20; 1 mM ATP, 1·38; 10 mM ATP, 1·64. *B*, plots of percentage time during which 0, 1 or 2 BK_{Ca} channels were simultaneously open in the excised membrane patch recording presented in Fig. 4*A*. Bars, observed values; arrowheads, predicted values.

phosphatase. In four patches tested, 100 U ml^{-1} alkaline phosphatase reduced the level of BK_{Ca} channel activation produced by 2 mM ATP by 63·2 ± 9·4% (Fig. 8*B*).

The results presented above suggest that ATP may affect BK_{Ca} channel activity via its ability to act as a phosphate donor. Such a process is likely to require the presence of a kinase or ATPase in order to facilitate the transfer of the phosphate group to the recipient molecule. To test for the presence of kinase activity, the effects of the non-specific kinase inhibitors staurosporine and H-7 were examined upon the ATP-mediated activation of the BK_{Ca} channel. Both agents inhibited the activation of channel activity by 2 mM ATP. For example, 50 μM H-7 reduced channel activity by 59·4 ± 5·2% ($n = 3$, data not shown) in the presence of 2 mM, while 10 μM staurosporine reduced channel activity stimulated by 2 mM ATP by 67·3 ± 6·2% ($n = 3$, data not shown). The effects of these agents were poorly reversible and appeared to reduce patch stability. Application of the peptide inhibitor of PKA, protein kinase inhibitor (PKI; Walsh & Glass, 1991), also inhibited BK_{Ca} channel activity in the presence of ATP. BK_{Ca} channel activity was reversibly inhibited by 5 μg ml^{-1} of this compound, reducing the activation produced by 1 or 2 mM ATP by 88·6 ± 8·6% ($n = 4$; Fig. 8*C*). In contrast, PKI (5 μg ml^{-1}) failed to affect BK_{Ca} channel activity stimulated by 10 μM Ca^{2+} alone ($n = 3$, not shown).

In the absence of kinase inhibitors, the effects of ATP were found to be rapidly reversible following washout (e.g. Figure 5*A*). This rapid loss of channel activity suggests that BK_{Ca} channels may also be subject to regulation by closely associated phosphatase activity. To test this hypothesis, the effect of the phosphatase inhibitor okadaic acid (Cohen, Holmes & Tsukitani, 1990) was examined upon the stimulation of BK_{Ca} channel activity by 2 mM ATP. In the three patches tested, application of 2 mM ATP alone reversibly increased BK_{Ca} channel N_fP_o from 0·18 ± 0·08 to 1·34 ± 0·39. The subsequent reapplication of 2 mM ATP in the presence of 10 μM okadaic acid, did not alter either the degree of BK_{Ca} channel activation (N_fP_o increased from 0·16 ± 0·07 to 1·38 ± 0·35 ($n = 3$)) or the rate at which the

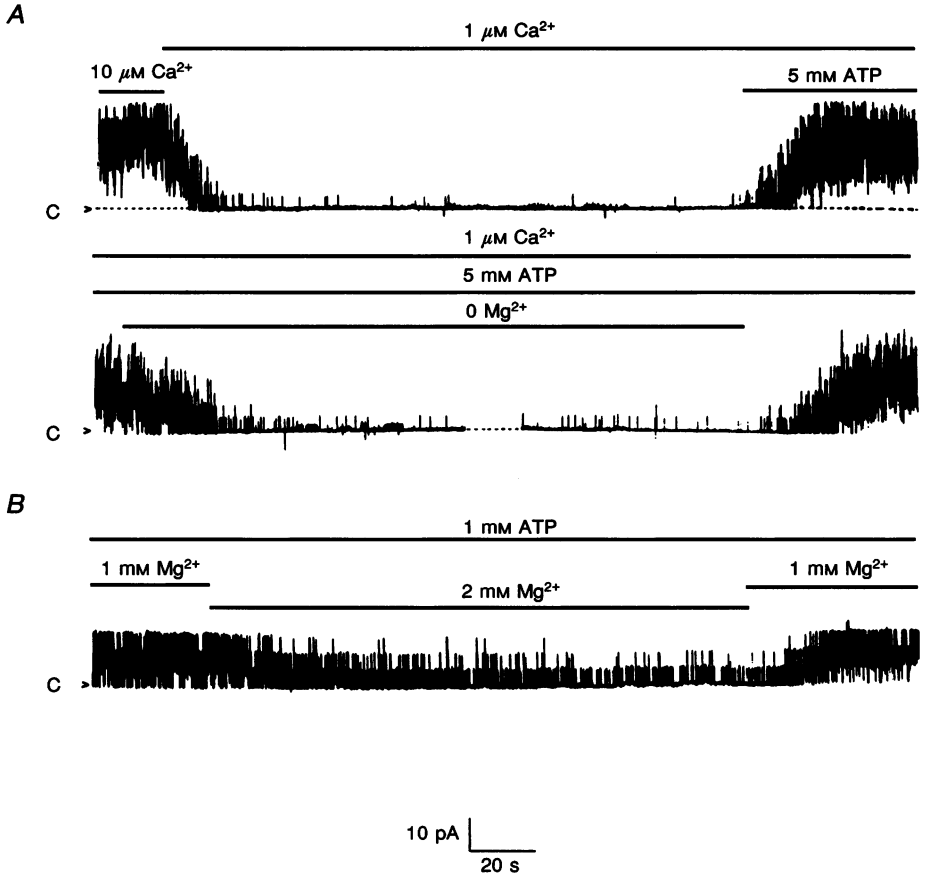

Figure 7. Mg^{2+} ions have a dual effect upon BK_{Ca} channel activity stimulated by intracellular ATP

A, continuous recording of BK_{Ca} channel currents demonstrating that removal of intracellular Mg^{2+} inhibits the ATP-mediated activation. Note that approximately 40 s of experimental data have been omitted from the figure (dashed line). *B*, continuous recording of BK_{Ca} channel currents showing that elevation of intracellular Mg^{2+} inhibits the ATP-mediated activation of BK_{Ca} channel activity.

effects of ATP were reversed (Fig. 9*A*). This suggests that an okadaic acid-sensitive phosphatase is not responsible for reversal of the ATP-mediated stimulation of BK_{Ca} channel activity.

Okadaic acid was also tested in four separate patches shown to be insensitive to 1 mM ATP (N_fP_o unchanged at $0{\cdot}08 \pm 0{\cdot}02$ ($n = 4$) in the presence and absence of 1 mM ATP). In two of these patches, the subsequent reapplication of 1 mM ATP in the presence of 10 μM okadaic acid increased BK_{Ca} channel N_fP_o from 0·12 to 0·96 in one patch and from a P_o of 0·04 to 0·49 in another patch containing a single BK_{Ca} channel (Fig. 9*B*).

In a small number of experiments the sensitivity of the BK_{Ca} channel to 1 or 2 mM ATP declined with time following patch excision ($n = 5$ out of 40 experiments). This is illustrated in Fig. 10 where BK_{Ca} channel sensitivity to 1 mM ATP was completely lost over a period of 30 min. However, subsequent application of the catalytic subunit of PKA to the patch in the presence of 1 mM ATP restimulated channel activity. Identical effects were observed in two further experiments. In control experiments, the application of PKA alone in the absence of ATP failed to affect BK_{Ca} channel activity ($n = 3$, not shown).

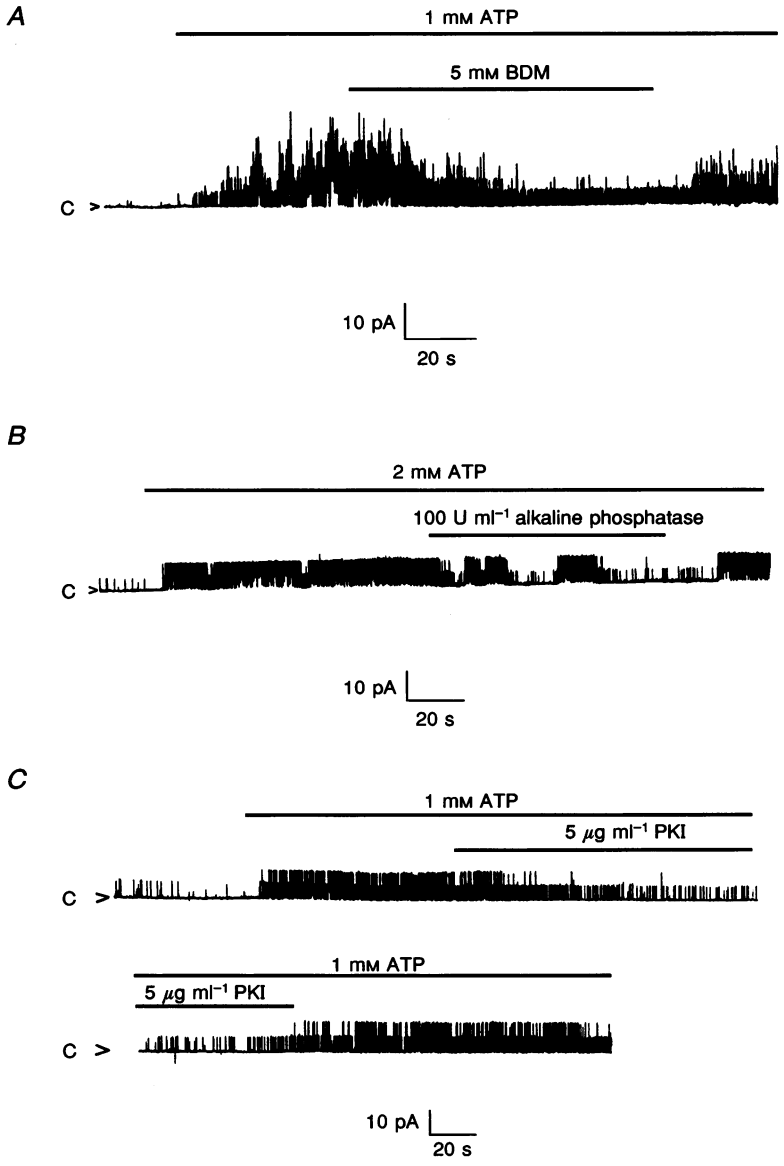

Figure 8. Phosphatases and the kinase inhibitor PKI inhibit ATP-mediated BK_{Ca} channel activity

Continuous recordings of BK_{Ca} channel currents show that 5 mM 2,3 butanedione monoxime (BDM; *A*), 100 U ml^{-1} alkaline phosphatase (*B*) and 5 μg ml^{-1} PKI, the peptide inhibitor of PKA (*C*) inhibit the ATP-mediated activation of BK_{Ca} channel activity. The membrane potential was +30 mV in *C*.

Phosphorylation has been reported to modulate the activity of Ca^{2+} channels (see Hille, 1992). Therefore, in order to exclude the possibility that the effects seen in the present study were due to the local influx of Ca^{2+} from the pipette solution, the activation produced by ATP was shown to be unaffected when Ca^{2+} was omitted from the pipette solution ($n = 4$) or when the Ca^{2+} channel blockers Cd^{2+} (100 μM) or La^{3+} (10 μM; see Hille, 1992), were added to the pipette solution to block any Ca^{2+} channels present in the patch ($n = 5$ and $n = 3$ for Cd^{2+} and La^{3+}, respectively).

These findings therefore suggest that the BK_{Ca} channels present upon the somata of neurones from the rat motor cortex can be modulated by phosphorylation. Similar findings have previously been reported by Chung *et al.* (1991), who have shown that ATP was able to stimulate directly the activity of a reconstituted charybdotoxin-insensitive BK_{Ca} (type II) channel from cortical synaptosomes. However, the channel in the present study was inhibited by charybdotoxin. When applied to the external surface of excised outside-out patches, 30 nM charybdotoxin reduced BK_{Ca} channel activity by $78{\cdot}3 \pm 4{\cdot}2\%$ ($n = 4$, data not shown). The effects of this agent were partially reversible following its removal from the bath solution.

ADP can mimic ATP and stimulate BK_{Ca} channel activity

ADP also produced enhancement of BK_{Ca} channel activity (Fig. 5*A*). When applied at a concentration of 5 mM, ADP increased BK_{Ca} channel open probability from $0{\cdot}08 \pm 0{\cdot}02$ to $0{\cdot}48 \pm 0{\cdot}11$ ($n = 15$). Furthermore, in experiments where lower concentrations of ATP were capable of stimulating channel activity, similar concentrations of ADP were also seen to stimulate BK_{Ca} channel activity. As found for ATP, the effects of ADP upon BK_{Ca} channel activity were dependent upon the presence of intracellular Mg^{2+} ($n = 4$; Fig. 11*A*). Similarly, the activation produced by ADP was inhibited by co-application of BDM or PKI; 5 mM BDM inhibited the activation produced by 1 or 2 mM

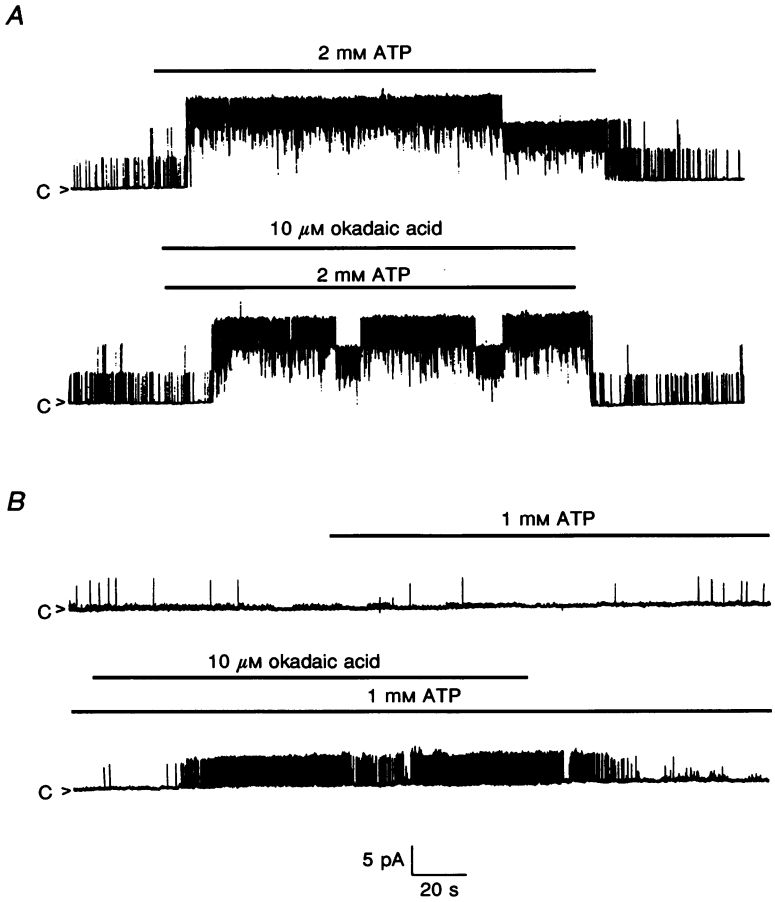

Figure 9. The action of okadaic acid upon the ATP-mediated modulation of BK_{Ca} channel activity

Continuous recordings of BK_{Ca} channel currents show that 10 μM okadaic acid does not affect the activation of BK_{Ca} channel activity produced by 2 mM ATP (*A*), and in 50% of patches where ATP was shown to be ineffective subsequent application of 10 μM okadaic acid induced BK_{Ca} channel sensitivity to 1 mM ATP (*B*).

ADP by 36 ± 7·2% ($n = 5$, not shown) whilst 5 μg ml^{-1} PKI reduced the activation induced by these concentrations of ADP by 43·2 ± 5·6% ($n = 3$; Fig. 11*B*).

The ability of ADP to activate this channel was also inhibited by the concomitant application of hexokinase and glucose in order to remove any ATP produced via the transphosphorylation of ADP by enzymes present within the patch. In the presence of 2 mM ADP, application of 20 U ml^{-1} hexokinase and 10 mM glucose led to a 71·2 ± 7·2% ($n = 4$) reduction in BK_{Ca} channel activity at +20 mV (Fig. 11*C*). In contrast, neither 20 U ml^{-1} hexokinase ($n = 2$) or 10 mM glucose alone ($n = 2$) were able to inhibit the effects of ADP upon channel activity. Furthermore, the combination of 20 U ml^{-1} hexokinase and 10 mM glucose was unable to inhibit channel activity stimulated by 10 μM Ca^{2+} ($n = 2$). On the basis of these findings, it is possible that ADP may stimulate BK_{Ca} channel activity via its conversion to ATP. The transformation of ADP to ATP is known to be catalysed physiologically by the enzyme adenylate kinase (Noda, 1973). To determine if this enzyme might be responsible for the ADP-mediated activation of BK_{Ca} channel activity, the specific adenylate kinase inhibitor Ap_5A (Lienhard & Secemski, 1973) was applied to the channel in the presence of 2 mM ADP. Ap_5A (10 μM) reduced BK_{Ca} channel activity by 41·2 ± 4·3% ($n = 5$; data not shown). In contrast, 100 μM Ap_5A had no effect upon BK_{Ca} channel activity stimulated by 10 μM Ca^{2+} ($n = 3$) or 2 mM ATP ($n = 3$).

Nucleotide specificity for activation of the BK_{Ca} channel

In addition to ATP and ADP, the ability of several other adenine nucleotides to stimulate BK_{Ca} activity was examined in order to determine the specificity of the enzymes associated with BK_{Ca} channel modulation. The adenine derivatives AMP (1–5 mM, $n = 3$), AMP-PNP (5 mM, $n = 4$) and cAMP (1–5 mM, $n = 5$) were all without effect upon channel activity (see Table 2). In contrast, the thiophosphate ATPγS stimulated BK_{Ca} channel activity but to variable degrees. In four patches tested, the effect of 5 mM ATPγS was much less potent than ATP (P_o increased from 0·07 ± 0·01 to 0·26 ± 0·07 ($n = 4$)). However, in three further patches ATPγS produced a large increase in channel activity (P_o increased from 0·08 ± 0·01 to 0·67 ± 0·12 ($n = 3$)). The activation of BK_{Ca} channel activity induced by this nucleotide was rapidly reversed following its removal regardless of the level of activation produced.

Other nucleotides tested without effect upon BK_{Ca} channel activity included GDP (1–5 mM, $n = 4$), GMP (1 mM, $n = 2$), GTPγS (0·1–1 mM, $n = 4$) and GDPβS (0·1–1 mM, $n = 3$). Furthermore, both GTPγS and GDPβS were unable to affect the ability of ATP to stimulate BK_{Ca}

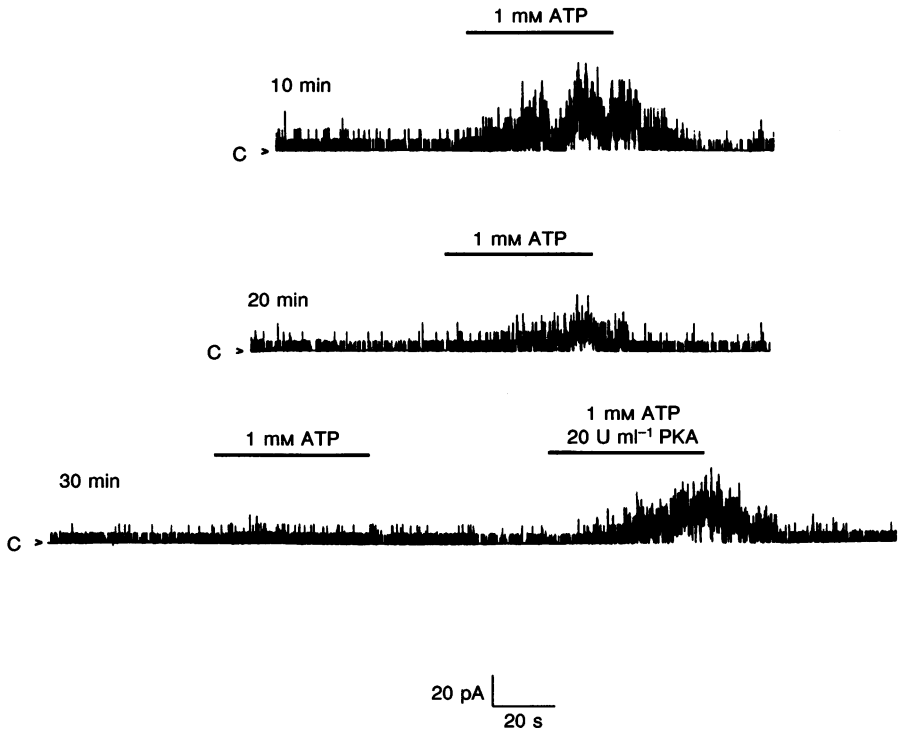

Figure 10. Loss of BK_{Ca} channel ATP sensitivity with time

Continuous recording of BK_{Ca} channel currents illustrating the time-dependent loss of ATP sensitivity following patch excision and the reversal of this effect by concomitant application of PKA and ATP.

channel activity ($n = 2$ for each, not shown). In contrast, GTP had variable effects upon BK_{Ca} channel activity. In five out of eight experiments, 5 mM GTP was unable to increase BK_{Ca} channel activity in patches where ATP was shown to be effective. In three further experiments, 5 mM GTP induced a large activation of BK_{Ca} channel activity (Table 2).

BK_{Ca} channel activity was consistently activated by CTP (2–5 mM) (Table 2; Fig. 12). This enhancement of channel activity was also Mg^{2+} dependent (channel activity reduced by $89{\cdot}5 \pm 3{\cdot}2\%$ ($n = 3$) upon removal of intracellular Mg^{2+}; Fig. 12*A*), and was inhibited by PKI (Fig. 12*B*) and BDM (channel activity reduced by $51{\cdot}3 \pm 4{\cdot}3\%$ ($n = 3$) and $58{\cdot}2 \pm 3{\cdot}4\%$ ($n = 3$) by 5 μg ml^{-1} PKI and 5 mM BDM, respectively).

Recent studies have shown that NADH and NAD can modulate BK_{Ca} channel activity in arterial smooth muscle cells (Lee, Park, So & Earm, 1994). However, in the present study these compounds, together with NADPH and NADP were without effect upon BK_{Ca} channel activity when

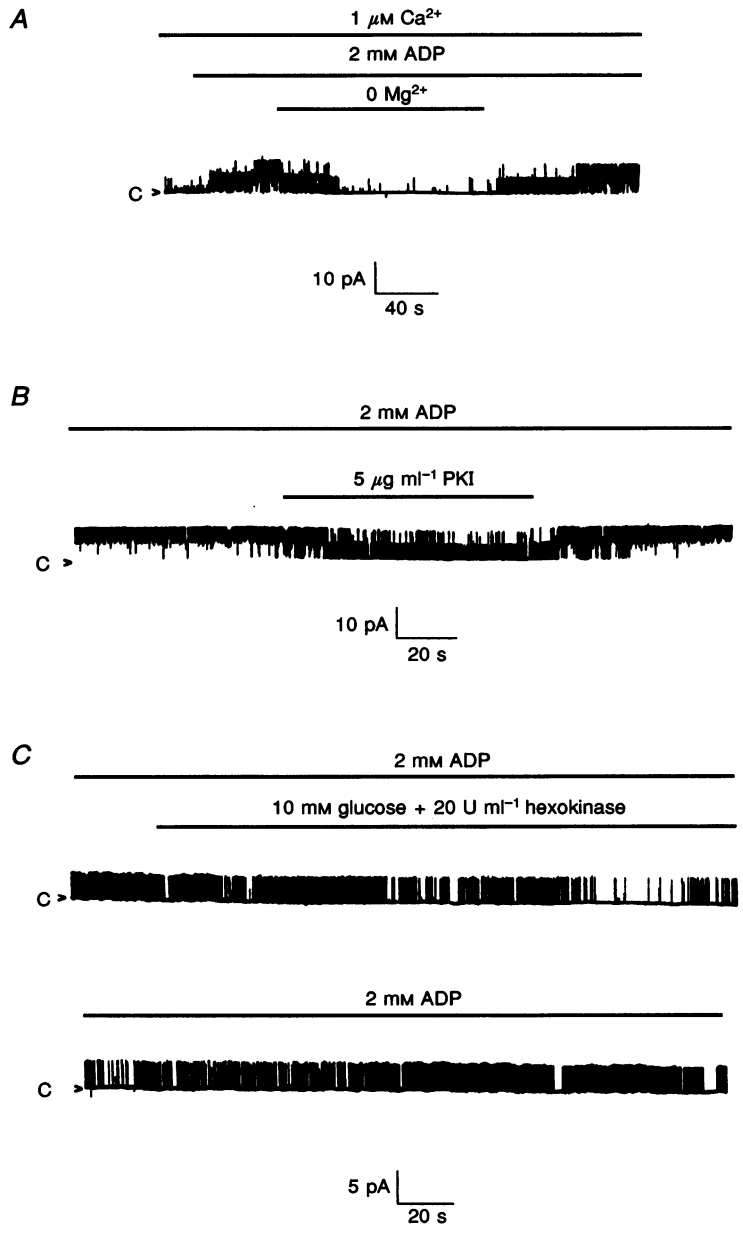

Figure 11. Effect of ADP on BK_{Ca} channel currents

Continuous recordings of BK_{Ca} channel currents illustrating that the actions of ADP are Mg^{2+} dependent (*A*), PKI dependent (*B*) and inhibited by the application of hexokinase in the presence of glucose (*C*).

Table 2. The effect of various nucleotides upon BK_{Ca} channel open-state probability

Nucleotide	Activation	*n*/total
5 mM ATP	0·13 ± 0·03 to 0·64 ± 0·17	40/42
	n.e.	2/42
5 mM ADP	0·08 ± 0·02 to 0·48 ± 0·11	15/15
5 mM AMP	n.e.	3/3
5 mM AMP-PNP	n.e.	4/4
5 mM ATPγS	0·07 ± 0·01 to 0·26 ± 0·07	4/7
	0·05 ± 0·01 to 0·67 ± 0·12	3/7
5 mM cAMP	n.e.	5/5
5 mM CTP	0·10 ± 0·02 to 0·52 ± 0·12	11/11
5 mM GTP	0·06 ± 0·02 to 0·59 ± 0·09	3/8
	n.e.	5/8
1 & 5 mM GDP	n.e.	3/3
1 mM GMP	n.e.	2/2
0·1 & 1 mM GTPγS	n.e.	4/4
0·1 & 1 mM GDPβS	n.e.	4/4
5 mM NAD	n.e.	4/4
5 mM NADH	n.e.	4/4
5 mM NADP	n.e.	4/4
5 mM NADPH	n.e.	4/4
5 mM UTP	0·00 to 0·01	1/5
	n.e.	4/5

The values in the right hand column indicate the number of times the effect was observed out of the total number of times the effect was tested for. No effect, n.e.

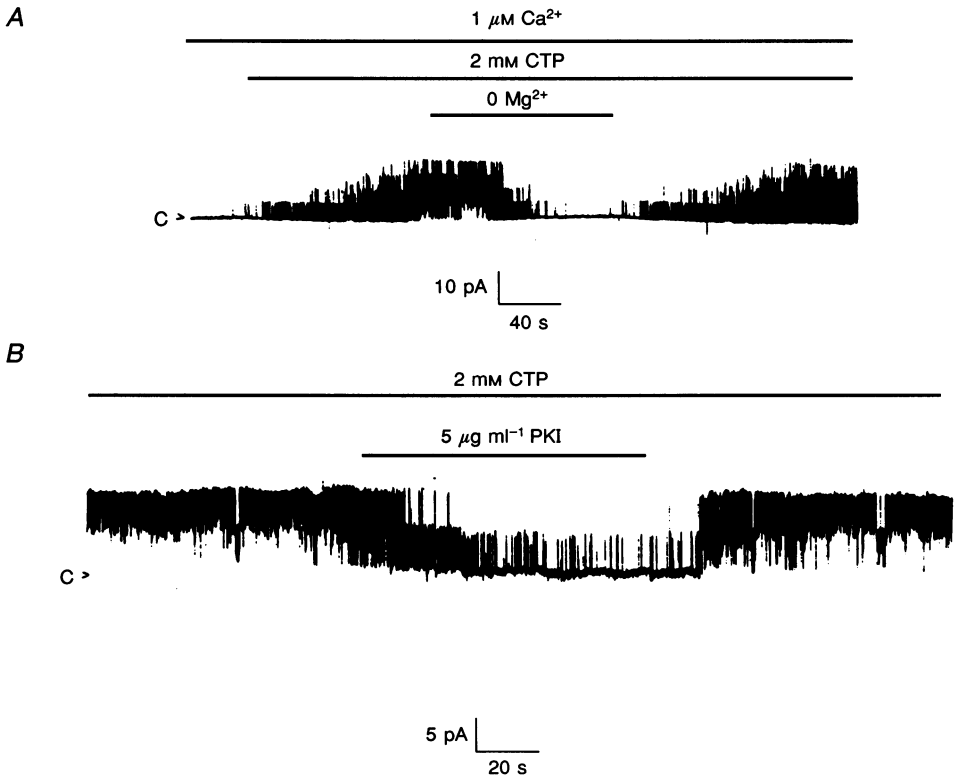

Figure 12. Effect of CTP on BK_{Ca} channel currents

Continuous recordings of BK_{Ca} channel currents illustrating that the actions of CTP are Mg^{2+} dependent (*A*), and PKI dependent (*B*).

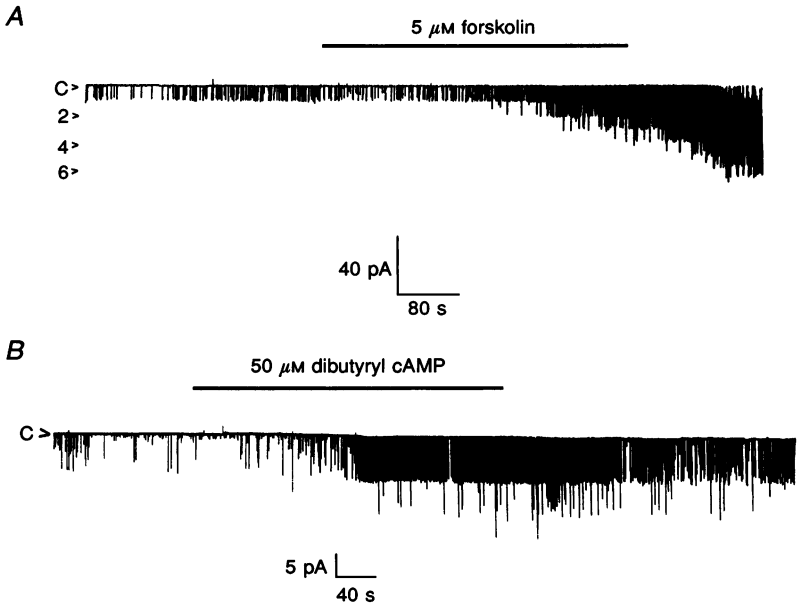

Figure 13. Effects of PKA activators on BK_{Ca} channel currents

Continuous cell-attached recordings of BK_{Ca} channel currents illustrating the effect of forskolin (*A*) and dibutyryl cAMP (*B*). Neurones were bathed in normal saline (solution A) whilst solution B (high KCl) was present in the pipette. The patch membrane potential was 0 mV in *A* and *B*.

applied to the intracellular surface of the channel at a concentration of 5 mM (Table 2).

Cell-attached recordings

The results presented above indicate that BK_{Ca} channel activity in neurones from the rat motor cortex can be modulated by phosphorylation in excised patches by an enzyme related to PKA. To examine if a similar system might regulate BK_{Ca} channel activity in the intact cell, the effects of PKA stimulants on cell-attached recordings of cortical neurones were investigated. Application of 5 μM forskolin, an adenylate cyclase activator (Seamon, Padgett & Daly, 1981), to the intact cell resulted in stimulation of BK_{Ca} channel activity. The activation was slow to develop and was sustained for long periods after the removal of forskolin from the bath solution (Fig. 13*A*). In eight out of eleven patches tested, 5 μM forskolin increased BK_{Ca} channel activity from a resting $N_f P_o$ of $0{\cdot}14 \pm 0{\cdot}04$ to $2{\cdot}94 \pm 0{\cdot}56$ ($n = 8$). Similarly, the membrane permeable cAMP analogue dibutyryl cAMP (50 μM) stimulated BK_{Ca} channel activity, increasing $N_f P_o$ from $0{\cdot}13 \pm 0{\cdot}08$ ($n = 3$) to $2{\cdot}43 \pm 0{\cdot}35$ ($n = 3$, Fig. 13*B*).

DISCUSSION

Characteristics of the cortical BK_{Ca} channel

The BK_{Ca} channel reported in this study has a single channel conductance of 245 pS in symmetrical 140 mM KCl, which compares well with unitary conductance values previously recorded for these channels (130–300 pS under similar ionic conditions; see Latorre *et al.* 1989). The voltage, Ca^{2+} and pH sensitivities of the channel are also similar to those reported for other BK_{Ca} channels. In addition, the channel under present investigation is modulated by ATP. The inability of a non-hydrolysable ATP analogue AMP-PNP and ATP in the absence of intracellular Mg^{2+} to mimic this effect suggests that ATP may be hydrolysed in the process of channel activation. This is supported by the observation that both synthetic and endogenous phosphatases reduce the effects of ATP upon BK_{Ca} channel activity. The ability of BDM, an oxime chemical phosphatase (Bergey, Reiser, Wiggins & Freeman, 1981) to inhibit BK_{Ca} channel activity stimulated by ATP but not by intracellular Ca^{2+} suggests that it does not inhibit the BK_{Ca} channel directly but interferes specifically with the ATP-mediated activation. Under the present experimental conditions, it is unlikely that BDM would attack the ATP molecule itself (Bergey *et al.* 1981) and thus it is probable that its effects arise from its ability to remove phosphate molecules from the channel or an associated molecule which, when phosphorylated, enhances BK_{Ca} channel activity.

The findings that the non-specific kinase inhibitors H-7 (Hidaki, Inagaki, Kawamoto & Sasaki, 1984) and staurosporine (Tamaoki, 1991) and the specific PKA inhibitor PKI (Walsh & Glass, 1991) inhibit the ATP-mediated activation of BK_{Ca} channel activity strongly

suggest that ATP increases BK_{Ca} channel activity via the activity of an associated kinase enzyme in some way related to PKA.

Mg^{2+} is an important cofactor in many biological phosphorylation processes (Wacker, 1969) and is often essential for ATP hydrolysis; this therefore accounts for its absolute requirement in the process of ATP activation. However, elevated concentrations of Mg^{2+} can inhibit PKA activity (Morton, 1992), which may explain the reduction of channel activity observed upon raising the Mg^{2+} concentration from 1 to 2 mM in the presence of ATP. This inhibitory effect was not due to chelation of ATP by Mg^{2+} since the ATP was added to the bath solution in the form of MgATP. It is also unlikely that the inhibitory effects of Mg^{2+} were due to direct channel inhibition since elevating the concentration of Mg^{2+} in the absence of ATP had no significant effect upon channel activity.

ATP *per se* also modulates the activity of other BK_{Ca} channels in rat brain. Chung *et al.* (1991) demonstrated that micromolar concentrations of ATP increase the activity of the type II, charybdotoxin-insensitive BK_{Ca} channel present in cortical synaptosomes. These effects of ATP were also dependent upon the presence of intracellular Mg^{2+} and required nucleotide hydrolysis. However, once activated by ATP, this channel remained active for the duration of the patch lifetime or until treated with the catalytic subunit of protein phosphatase I. This finding is at variance with the present study where the effects of ATP were rapidly reversed following its washout. The rapidity with which the channels lost their activity following the removal of ATP in the present study is perhaps indicative of closely associated phosphatase activity.

The effects of ATP are associated with a reduction in the Hill coefficient relating channel P_o to intracellular Ca^{2+} concentration from 1·4 to 0·7. It is therefore possible that ATP alters the number of Ca^{2+} binding sites needed to be occupied for channel activation. Alternatively, the nucleotide may modify the co-operativity between binding sites. In contrast, Reinhart *et al.* (1991) report that the PKA-mediated activation of type I BK_{Ca} channel from synaptosomal membranes was not associated with a change in Hill coefficient.

Nature of the associated kinase enzyme

PKA enzymes are typically composed of four subunits. In the absence of cAMP, two identical regulatory subunits are normally associated with two identical catalytic subunits and act to prevent catalytic activity (Morton, 1992). However, in the presence of cAMP, the cyclic nucleotide binds to the regulatory subunits and promotes the release of the active monomeric catalytic subunits which phosphorylate proteins using ATP as substrate. Classically, the regulatory subunits dictate the subcellular localization of PKA enzymes via their ability to associate with anchoring proteins which attach to membranes (Scott, 1993). In the present investigation, the effects produced by ATP were achieved in the absence of cAMP. This suggests that no regulatory subunits are associated with the kinase in the membrane patch or that they are in some way compromised so that they no longer regulate the enzyme's activity. It is doubtful that the effects seen could have arisen from the presence of free, soluble catalytic PKA subunits associated with the membrane patch since in most cases the channels retained ATP sensitivity for long periods after patch excision and following extensive patch perfusion. Therefore, the kinase responsible for the present effects could itself be strongly tethered to the intracellular surface of the excised patch. Recently, an isoform of the catalytic unit of PKA has been identified in bovine heart which bears a unique N-terminal region (Weimann, Kinzel & Pyerin, 1991). This Cβ2 isoform has a sequence of amino acids which may fold to form an amphipathic α-helix suitable for membrane anchorage. It is therefore possible that a PKA isoform similar to Cβ2 is associated with this BK_{Ca} channel. Alternatively, the BK_{Ca} channel protein itself may possess kinase-like activity. It has been shown that a dendrotoxin-sensitive K^+ channel purified from rat CNS has an endogenously associated kinase (Rehm *et al.* 1989).

Specificity of the associated kinase enzyme

ADP stimulated BK_{Ca} channel activity by a mechanism similar to that of ATP since both were Mg^{2+} dependent and inhibitable by PKI and BDM. However, the effects of ADP were also inhibited by the concomitant application of hexokinase and glucose. Under these conditions, hexokinase would consume any ATP present within the patch in order to phosphorylate glucose to glucose-6-phosphate (Crane & Sols, 1955). This finding is consistent with ADP stimulating BK_{Ca} channel activity via its conversion to ATP by transphosphorylating enzymes present within the excised patch. The ability of Ap_5A to inhibit the effects of ADP suggest that adenylate kinase, present in the rat cerebral cortex (Pradhan & Criss, 1976), may be involved in this process.

ATPγS also stimulated BK_{Ca} channel activity although its effectiveness was more variable. ATPγS possesses a terminal thiophosphate bond and has been reported to substitute for ATP in many processes which require phosphorylation (Yount, 1975). Surprisingly, the effect of ATPγS was reversible following its removal. This was unexpected as thiophosphorylated proteins are considered to be much more stable than their phosphorylated counterparts. The reasons for this phenomenon remain to be determined. ATPγS has been reported to have variable effects upon ATP-activated BK_{Ca} channels. In cortical synaptosomes ATPγS stimulated channel activity (Chung *et al.* 1991), whereas in pulmonary artery it had no effect (Robertson, Corry, Nye & Kozlowski, 1992). This

variability may simply reflect tissue specific differences in the nature of the kinase associated with BK_{Ca} channel activity.

CTP increased BK_{Ca} channel activity consistently an effect also Mg^{2+} dependent and sensitive to PKI and BDM. Thus, it is likely that the effects of CTP are mediated through the same pathway utilized by both ATP and ADP. The ability of CTP to mimic the effects of ATP is surprising, since it is not generally thought that PKA can utilize nucleotides other than ATP. Possible explanations are that the kinase responsible is not a classical form of PKA and has a different substrate specificity (perhaps supported by the apparent lack of regulatory domains) or, alternatively, CTP also acts as a substrate for transphosphorylating enzymes present within the patch.

Nature of the associated phosphatase enzyme

The inability of okadaic acid to affect the reversibility of the ATP-mediated stimulation of channel activity suggests that the associated phosphatase does not belong to the PP1 or PP2A class of protein phosphatases (Cohen & Cohen, 1989; Cohen *et al.* 1990). However, the protein phosphatases PP2B (Ca^{2+}–calmodulin dependent) and PP2C (Mg^{2+} dependent) are okadaic acid insensitive and so one of these enzymes may be involved in the rapid reversal of the ATP-stimulated increase in channel activity. Okadaic acid enhanced BK_{Ca} channel sensitivity to 1 mM ATP in two out of the four patches tested. This may be interpreted to suggest that more than one type of protein phosphatase is associated with BK_{Ca} channels in these neurones. In addition, the kinase may be more closely attached to the channel than the phosphatase, which could explain some of the variation in nucleotide sensitivity observed between patches.

Physiological role of the ATP-modulated BK_{Ca} channel in the rat motor cortex

The frequency with which the BK_{Ca} channel was observed in the present study indicates that rat motor cortex neurones express a high density of these channels. In central neurones BK_{Ca} channels have been implicated in the processes of spike repolarization and fast hyperpolarization following an action potential (Storm, 1993). The large conductance of the channels coupled with their Ca^{2+} and voltage sensitivity makes them extremely well suited for such a purpose.

The ability of ATP to stimulate directly BK_{Ca} channel activity in excised patches suggests the presence of an endogenous kinase enzyme devoid of regulatory subunits. The concentrations of intracellular ATP and Ca^{2+} present in central neurones have been estimated to be 3 mM and 160 nM, respectively (Duchen, Valdeolmillos, O'Neill & Eisner, 1990; Erecinska & Silver, 1994). In the presence of these levels of ATP and Ca^{2+} the BK_{Ca} channel would be expected to be highly active. However, as BK_{Ca} channels were found to have low activity in cell-attached patch recordings, it would appear likely that in the intact cell, channel activity is subject to regulation by other intracellular factors.

The activation of BK_{Ca} channels by agents which increase the concentration of intracellular cAMP raises the possibility that their activity is subject to regulation by neurotransmitters which modulate adenylate cyclase activity. Subtle changes in BK_{Ca} channel Ca^{2+} sensitivity induced by phosphorylation could affect the response of the channel to membrane depolarization and so alter the characteristics of the neuronal action potential. Furthermore, the increase in Ca^{2+} sensitivity produced by phosphorylation may be sufficient to cause activation of these channels at rest and thus neuronal hyperpolarization and inhibition of cell excitability. In addition to channel regulation by associated kinase enzymes, the channel also appears to be subject to regulation by an associated phosphatase enzyme, which could provide a second locus for neurotransmitters to alter neuronal excitability.

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Authors' present addresses

K. Lee: Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, 3831 Sam Jackson Park Road, Portland, OR 97201, USA.

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

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