Modulation of $K^+$ Channels by Intracellular ATP in Human Neocortical Neurons

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Jiang, Chun and Gabriel G. Haddad. Modulation of $K^+$ channels by intracellular ATP in human neocortical neurons. J. Neurophysiol. 77: 93–102, 1997. ATP-modulated $K^+$ channels play an important role in regulating membrane excitability during metabolic stress. To characterize such $K^+$ channels from the human brain, single channel currents were studied in excised inside-out patches from freshly dissociated human neocortical neurons. Three currents that were sensitive to physiological concentrations of ATP and selectively permeable to $K^+$ were identified. One of these currents had a unitary conductance of $\sim 47$ pS and showed a strong inward rectification with symmetric $K^+$ concentrations across the membrane. This $K^+$ current was inhibited by ATP in a concentration-dependent manner with an $IC_{50}$ (half-inhibition of channel activity) of $\sim 130 \mu M$. Channel activity also was suppressed by ADP, non-hydrolyzable ATP analogue AMP-PNP, and sulfonilurea receptor/channel blocker glibenclamide. The second $K^+$ current had a unitary conductance of $\sim 200$ pS and showed a weak inward rectification. Similarly, this current was inhibited by ATP ($IC_{50} = 350 \mu M$), AMP-PNP, and glibenclamide. Unlike the small-conductance ATP-inhibitable $K^+$ channel (S-$K_{ATP}$), activation of this large-conductance $K^+$ channel (L-$K_{ATP}$) required the presence of micromolar concentration of $Ca^{2+}$ in the internal solution, but charybdotoxin did not inhibit this channel. The third $K^+$ current was also $Ca^{2+}$ dependent and had a large conductance (280 pS). It was inhibited by external charybdotoxin, iberiotoxin, and tetraethylammonium. In contrast to the other two $K_{ATP}$ channels, ATP enhanced channel open-state probability and unitary conductance, and glibenclamide at concentration of $10–20 \mu M$ had no inhibitory effect on this current. $K_{ATP}$ channels that have single-channel and pharmacological properties similar to these three human ATP-modulated $K^+$ channels also were observed in experiments on rat neocortical neurons. These results therefore indicate that $K_{ATP}$ channels are expressed in human neocortical neurons, and two distinct $K_{ATP}$ channels (S-$K_{ATP}$ and L-$K_{ATP}$) exist in the human and rat neurons. The observation that ATP at different concentrations modulates different $K^+$ channels suggests that metabolic rate may be continuously sensed in neurons with resulting alterations in neuronal membrane excitability.

INTRODUCTION

Adenosine triphosphate (ATP) is an important metabolite and an inter- and intracellular messenger (Ashcroft 1988; Quayle and Standen 1994; Standen 1992; Surprenant et al. 1995; Takeno and Noma 1993). Among a number of target molecules that are modulated by intracellular ATP are ion channels whose activity determines the overall plasma membrane excitability and cellular responses to external stimuli (Hille 1992). The modulation of ion channel activity by ATP in excitable cells appears to have major physiological significance, because it enables a direct coupling of the change in intermediary metabolism to membrane excitability. During certain metabolic stress such as hypoxia and hypoglycemia, this ATP-mediated modulation of membrane excitability becomes specially important in cells that depend strongly on an exogenous energy supply such as central neurons. A decrease in membrane excitability resulting from the activation of ATP-inhibited $K^+$ ($K_{ATP}$) channels can slow down energy consumption and thus may influence cell survival during energy deprivation (Haddad and Jiang 1993).

Because these $K_{ATP}$ channels seem to play a role in modulating cellular membrane excitability during a decrease in intracellular ATP level, detailed studies of these channels may have implications on several neurological diseases or conditions. However, such studies have rarely been carried out in central neurons (Ashford et al. 1988, 1990a; Jiang et al. 1994; Ohno-Shosaku and Yamamoto 1992), in contrast with a rich documentation in a number of peripheral tissues (Ashcroft et al. 1984, 1990a; Cook and Hales 1984; Noma 1983; Spruce et al. 1987; Standen et al. 1989; Wang and Giebisch 1991). In fact, we do not even know if these $K_{ATP}$ channels are present in human central nervous system (CNS). To determine whether ATP-modulated $K^+$ channels are present in human neurons and study their properties, we performed patch-clamp experiments and took advantage of a rather rare opportunity to obtain human neocortical tissue from neurosurgery. In addition to human neurons, we studied rat neocortical neurons to see if ion channels with similar properties are present in both species. Our results have not only demonstrated that $K_{ATP}$ channels are present in human neocortical neurons, but also have shown that physiological concentrations of ATP modulates coexisting $K^+$ channels differently in both human and rat CNS.

METHODS

Methods for dissociation of human neocortical neurons were similar to those previously described (Cummins et al. 1993; Jiang and Haddad 1992). Briefly, a small portion of human cortical tissue was obtained from patients with epilepsy who had suffered from recurring seizures identified by long-term electroencephalograph or corticoencephalograph. The tissue was obtained from the anterior portion of the temporal lobe (mostly from the middle temporal gyrus). The temporal tissue obtained for these studies was removed solely to gain access to the hippocampus and was judged to have normal excitability from intracranial chronic depth and subdural electrode recordings and/or intraoperative cortical surface electrocorticography. Previous studies from this laboratory and others have demonstrated that nerve cells in such excised human neocorti-
cral tissue are electrophysiologically and histologically normal (Cummins et al. 1993; Jiang and Haddad 1992). Neocortical tissue was removed by neurosurgeons (Drs. Dennis D. Spencer and Charles Duncan) and a portion (0.5–1.0 cm³) of the human brain tissue was placed in ice-cold artificial cerebrospinal fluid solution (0–1°C) containing (in mM) 124.0 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2.0 MgSO₄, 2.0 CaCl₂, 26.0 NaHCO₃, and 10.0 D-glucose; the solution was oxygenated with 95% O₂-5% CO₂ (pH 7.4) and rapidly transferred to the laboratory where the tissue was then sectioned into 300-μm slices. The sections were incubated for 1 h with oxygenated N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) buffer containing (in mM) 140 NaCl, 2.5 KCl, 1 MgCl₂, 1 CaCl₂, 25 D-glucose, 10 HEPES, and trypsin (0.2–0.3%, Sigma type X1) at 35°C (pH 7.40). Sections then were washed in oxygenated HEPES-buffer and maintained for ≥6 h.

Neurons also were harvested from the temporal cortex of Sprague-Dawley rats (10–20 days). The rats were killed with inhalation of saturated methoxyflurane and decapitated. The brain was removed rapidly, chilled in 0–1°C Ringer solution and prepared as a tissue block. The tissue block from the temporal cortex was removed by neurosurgeons (Drs. Dennis D. Spencer and Charles Duncan) and a portion (0.2±0.3%, Sigma type XI) at 35°C (pH 7.40) was sectioned transversely into 300-μm slices. The sections were maintained in a low Na+/Ca²⁺ buffer. Using a dissecting microscope, the gray matter (presumably including layers III, IV, and V) was cut free from the rest of the slice. The tissue of interest then was dissociated by gentle trituration with fire-polished Pasteur pipettes. Cells were plated in 35-mm Petri dishes and observed with Hoffman modulation optics. Recordings were obtained only from cells that had a pyramidal shape of soma with a single thick proximal dendrite (presumably pyramidal neurons) and did not show visible evidence of injury. Flat or swollen cells or cells with a grainy membrane appearance were not studied as previously described (Cummins et al. 1993; Jiang and Haddad 1994a,b; Kay and Wong 1986).

Patch-clamp experiments were performed at room temperature (~24°C). Fire-polished patch pipettes (2–4 MΩ) were made from 1.2 mm borosilicate capillary glass using a Sutter P-84 puller (Sutter Instrument, Novato, CA). Whole cell currents were studied in the voltage-clamp mode, and single channel currents were recorded from inside-out patches (Hamill et al. 1981) using an Axo-Patch 200A amplifier (Axon Instruments, Foster City, CA). To study whole cell currents, perforated patches were performed as described previously (Jiang et al. 1994). The tip of the recording pipette was filled with a solution containing (in mM) 150 KCl, 0.2 MgCl₂, 10 D-glucose, and 10 HEPES (pH 7.4), and the remainder of the pipette was filled with the same solution plus 100 μg/ml nystatin (Sigma, stock solution 25 mg/ml in dimethylsulfoxide). After formation of a gigohm seal, perforation was monitored by measuring series resistance with an Axo-Patch 200A amplifier. Patches were accepted when the series resistance was <50 MΩ. Current records were low-pass filtered (Bessel, 4-pole filter, ~3 dB at 2 kHz), digitized (10 kHz, 12-bit resolution) with pClamp 5.5.1 software (Axon Instruments), and stored on computer disk for later analysis (Jiang and Haddad 1994a,b; Jiang et al. 1994).

For single channel studies, identical solutions were applied to the bath and recording pipettes, and these contained (in mM) 150 KCl, 1 ethylene glycol-bis(β-aminoethly ether)-N,N,N',N'-tetracetic acid (EGTA), 0.2 ADP (Mg⁴⁺ salt), 10 MgCl₂, and 10 HEPES (pH 7.4, free Ca²⁺ < 10 nM). In some experiments, CaCl₂ was added to the internal solution to achieve a free Ca²⁺ concentration of ~1 μM at pH 7.4. A perfusion system was used to administer agents in parallel to patches at a rate of ~1 ml/min. This perfusion system allows a fast exchange of perfusion solutions in ~200 ms. Single-channel data were analyzed by further filtering (0–1,000 Hz) with a Gaussian filter. Events <200 μs were ignored. The unitary conductance was calculated by measuring current amplitudes in the I/V plot using a slope voltage command. The open-state probability (P_open) was calculated as described previously (Davies et al. 1992; Jiang and Haddad 1994a,b; Jiang et al. 1994; Quayle et al. 1988; Sigworth and Sine 1987; Standen et al. 1989): 1) P_open values were calculated from stretches of data (obtained using the Fetchex software) having a total duration of 18–36 s. The time, t, spent at current levels corresponding to j = 0, 1, 2, ..., N channels open was first measured. The P_open was then expressed as a function of t_j

\[ P_{\text{open}} = \sum_{j=1}^{N} t_j/TN \]

where N is the number of channels active in the patch and T is the duration of recordings. 2) For segmented data (obtained using the Clampex software), stimulating artifact and leak currents were first removed by subtracting these currents from each traces of records, and 50–60 records then were averaged. P_open values were calculated as P_open = I/mi, where I is the amplitude of averaged currents, n the number of channels active in the patch, and i the amplitude of single channel current.

Chemicals were all purchased from Sigma Chemicals (St. Louis, MO), except charybotoxin and iberiotoxin, which were obtained from RBI (Natick, MA) and levorcanalam (BRL 38227) which was a gift of SmithKline Beecham Pharmaceuticals.

Data are presented as means ± SD (n = number of patches) and differences in means were tested with the Student’s t-test and the χ² test and accepted as significant if P ≤ 0.05.

RESULTS

Glibenclamide- and levorcanalam-sensitive K⁺ currents in neocortical neurons

Whole cell currents were studied under voltage-clamp mode with the membrane potential held at ~60 mV. Symmetric K⁺ concentrations (150 mM) were used in both bath and pipette solutions. Na⁺ and Ca²⁺ currents were abolished using low Na⁺ (5 mM) solution plus 1 mM Co²⁺ in the bath. Figure 1 shows an example of the recording of whole
cell currents in a perforated patch from a human neocortical neuron. BRL 38227 (levcromakalim, a K$_{ATP}$ channel activator) at 5 μM markedly increased the whole cell inward current in this cell. The increase in inward current was inhibited by subsequent application of glibenclamide (20 μM), a sulfonylurea receptor blocker, to the bath solution (Fig. 1). These glibenclamide- and BRL38227-sensitive currents are not Na$^+$ currents, because the inward Na$^+$ currents were inhibited strongly in our Na$^+$-free or low Na$^+$ solutions (Jiang et al. 1994) and because these currents were activated with hyperpolarization rather than depolarization.

To characterize these levcromakalim and glibenclamide sensitive currents, single channel currents were studied in excised inside-out patches. Recording from giant inside-out patches (using patch pipettes with 6–8 μm tip openings and a resistance of 0.5–1 MΩ) with symmetric K$^+$ concentrations, we found that ATP (1 mM) significantly inhibited K$^+$ currents and AMP-PNP (0.5–1 mM), a nonhydrolyzable ATP analogue, had a similar effect. Interestingly, we found that currents that were inhibited by ATP seemed to have different conductances, indicating that more than one type of K$^+$ channels that are inhibited by ATP existed in these human neurons.

**Small conductance ATP-inhibitable K$^+$ channel**

Single channel activity was studied with equal K$^+$ concentrations (150 mM) and Na$^+$-free (or with 5 mM Na$^+$ in 2 patches) pipette and bath solutions. Using these solutions, a small-conductance current was seen. This channel was observed in nine patches from ~80 human neocortical neurons studied (1 patch per cell). Figure 2 shows an example of the effect of ATP on this small-conductance current in a human neocortical neuron. This channel had a high frequency of openings and closures with a baseline $P_{open} = 0.299$. Channel activity was inhibited strongly and rapidly when the patch was exposed to 1 mM of ATP (K$^+$ salt), and $P_{open}$ was reduced to 0.026. The inhibition by ATP was removed rapidly after washout ($P_{open} = 0.263$). Figure 3 shows an example of K$^+$ selective permeability of this channel in a human neocortical neuron. With equal concentrations of K$^+$ (150 mM) applied to both internal and external solutions, reversal potential of the current was 0 mV with a clear inward rectification (Fig. 3A). Single channel conductance, which was almost linear from −10 to −90 mV, averaged 47 ± 3 pS ($n = 9$). When the KCl concentration on the internal surface was reduced to 54 mM (96 mM KCl was replaced by the same amount of NaCl), the unitary conductance decreased to ~40 pS, and the reversal potential was shifted to 23.4 ± 1.0 mV, $n = 3$ (Fig. 3B), which is close to the calculated Nernst potential for K$^+$ (25.6 mV). Thus this demonstrates that the channel is K$^+$ selective.

ATP inhibited this channel in a concentration-dependent manner. Channel activity started to respond to ATP concentrations on the cytosolic side at 30 μM and was abolished almost totally by 1.0 mM. The concentration of ATP that produced half-inhibition of channel activity (IC$_{50}$) was 130 μM. Channel activity also was inhibited significantly by glibenclamide (10–20 μM, $n = 3$), a sulfonylurea receptor/channel blocker (Fig. 3C). Other nucleotides such as ADP (1 mM, $n = 2$) and nonhydrolyzable ATP analogue, AMP-PNP (0.5–1.0 mM, $n = 4$), also inhibited this channel when applied to the internal surface. Similar to K$_{ATP}$ channels described in other tissues, this ATP-inhibited K$^+$ channel in human neocortical neurons showed an ADT-dependent rundown. Channel activity disappeared within 1–2 min after patch excision from the cell unless Mg-ADP was present in the internal solution. Activation of this K$^+$ channel is independent of free Ca$^{2+}$ in the cytosol because this current was observed using a Ca$^{2+}$-free internal medium (free Ca$^{2+} < 10$ nM, $n = 9$) and an increase in free Ca$^{2+}$ to micromolar concentration in this medium did not increase channel activity ($n = 2$). In addition, this K$^+$ channel was inhibited by internal Cs$^+$ (1 mM, $n = 2$).

An inward rectifying K$^+$ channel that had a similar conductance (46 ± 4 pS, $n = 7$) as the human K$^+$ channel described above also was found in rat neocortical neurons. This K$^+$ channel was inhibited by glibenclamide ($n = 3$), AMP-PNP ($n = 5$; Fig. 4) and ATP in a concentration-dependent manner (Fig. 5). Although BRL38227 did not...
FIG. 3. Characterization of small-conductance K<sub>ATP</sub> channel from a human neocortical neuron. A: single channel current was recorded in an inside-out patch. Patch was held at 0 mV, and a ramp potential was given from −100 to 100 mV. When internal and external solutions contained equal concentrations of K<sup>+</sup> (150 mM), channel showed an inward rectification with a reversal potential at 0 mV. In negative membrane potential (−10 to −90 mV), conductance is almost linear and it is 48 pS. B: when internal membrane surface was exposed to a solution containing 54 mM KCl and 96 mM NaCl, reversal potential was shifted to −23 mV (∆) and unitary conductance was reduced to 40 pS. Note that initial baseline currents are produced by inward K<sup>+</sup> gradient. C: inhibition of channel activity by ATP and glibenclamide. Same single channel currents as in A and B were studied when membrane potential was held at 0 mV and stepped to −80 mV. Single channel activity is shown in trace 2–6 in each panel. Closing levels are indicated by broken lines. Top traces in each panel show “macroscopic” currents by averaging 60 individual records. Two active channels (-----) are seen during baseline (Control). Both of these currents are inhibited when ATP (1 mM) was applied to internal solution (2nd panel labeled ATP). After washout of ATP, channel activity rapidly returned to baseline level. Channel activity is also markedly and reversibly inhibited by glibenclamide (10 μM).

affect baseline channel activity in excised patches, it enhanced <i>P</i><sub>open</sub> when these channels had been inhibited by ATP (0.011 ± 0.007 vs. 0.155 ± 0.028, <i>n</i> = 3, <i>P</i> < 0.05). These characteristics indicate that this inward rectifying K<sup>+</sup> channel found in both human and rat neocortical neurons belongs to the K<sub>ATP</sub> channel family.

**Large conductance ATP-inhibitable K<sup>+</sup> channel**

Under the same experimental conditions, we observed a large-conductance current in 5 patches out of ~70 patches studied in human neocortical neurons. This channel had a very low baseline channel activity with the same internal and external solutions described above. When free Ca<sup>2+</sup> in the internal solution was increased to micromolar level, however, channel activity markedly increased. As in the previously described small conductance current, we found that ATP, glibenclamide and AMP-PNP also inhibited this current (<i>n</i> = 5). Figure 6 shows an example of this current from a human neocortical neuron, in which single channel currents were recorded using symmetric K<sup>+</sup> concentrations on either sides of the patch membrane (free Ca<sup>2+</sup> ≈ 1 μM). Two channels with fast flickering activity were seen during baseline in this patch. The slope conductance of this current was 180 pS (Fig. 6A), and <i>P</i><sub>open</sub> 0.211 (Fig. 6B). Channel activity was inhibited markedly when the cytosolic side of
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FIG. 4. Small-conductance K<sub>ATP</sub> channel in a rat neocortical neuron. Single channel current was studied in same condition as Fig. 7C. An active channel is seen in this inside-out patch. Channel activity is reversibly inhibited by ATP (1.0 mM) and AMP-PNP (1 mM).

the patch membrane was exposed to 1.0 mM of ATP ($P_{\text{open}} = 0.015$). The inhibitory effect of ATP was reversible and channel activity rapidly returned to baseline level after washout ($P_{\text{open}} = 0.223$).

With symmetric K⁺ across the membrane, this channel had a slope conductance of 145–230 pS (196 ± 27 pS, $n = 5$) with a weak inward rectification (Fig. 7A). When the internal K⁺ concentration was reduced to 54 mM, the reversal potential was shifted to near 24 mV (calculated reversal potential is 25.6 mV), indicating that this channel is highly K⁺-selective (Fig. 7B).

It is worth noting that besides a relatively wide range of conductances, two patterns of channel activity were seen. The 180-pS K<sub>ATP</sub> channel in Fig. 6 had a high frequency of channel openings and closures, whereas the 230 pS one inwashout ($P_{\text{open}} = 0.223$). With symmetric K⁺ across the membrane, this channel Fig. 7 showed a relatively low flickering activity. Because they had a much larger single channel conductance (4±5 pS, $n = 5$) with a weak inward rectification (Fig. 7A), we pooled them together as a single group.

As for the small-conductance K<sub>ATP</sub> channel, this large-conductance K⁺ channel was inhibited by ATP in a concentration-dependent manner with an IC<sub>50</sub> of 350 μM (Fig. 8A). This K⁺ channel also was inhibited by AMP-PNP (0.5–1.0 mM, $n = 4$) and by glibenclamide (10 μM, $n = 2$; Fig. 8B). Also, channel activity ceased within 3–4 min after excision in the absence of MgADP in the internal solution. In addition, this channel was sensitive to internal Cs⁺ (1 mM, $n = 2$) and modestly inhibited by internal tetraethylammonium (TEA, 20 mM; Fig. 8B). Unlike the small-conductance K<sub>ATP</sub> channel, however, activation of this large-conductance K⁺ channel required the presence of micromolar concentration of Ca<sup>2+</sup> in the bath (cytosolic) solution. Although this channel was activated by Ca<sup>2+</sup> and had a large conductance, this channel still was seen when charybdotoxin (50 or 100 nM) was present in the pipette (extracellular) solution ($n = 2$).

In rat neocortical neurons, we also observed a K⁺ channel that was inhibited by ATP, glibenclamide and AMP-PNP. As for its human neocortical counterpart, this K⁺ channel in rat had a conductance of 188 ± 33 (n = 5) and showed a weak inward rectification. Therefore, these data indicate that this large conductance K⁺ channel also belongs to the K<sub>ATP</sub> channel family. To differentiate this channel in human and rat neocortical neurons from the other much smaller K<sub>ATP</sub> channel, we term the large-conductance channel as L-K<sub>ATP</sub> channel and the small-conductance one as S-K<sub>ATP</sub> channel.

FIG. 5. Concentration-dependent inhibition of ATP. Data were obtained from an inside-out patch of a rat neocortical neuron. Single channel currents were recorded ($V_m = −100$ mV) with equal concentrations of K⁺ in both internal and external solutions. $P_{\text{open}}$ is normalized to baseline level ($P_{\text{open}}/P_{\text{open,CTL}}$). Data are fitted using the Hill equation: $y = 1/(1 + ([\text{ATP}] / K)^h)$, where $y = P_{\text{open}}/P_{\text{open,CTL}}$; [ATP] = internal ATP concentration; $K = IC_{50}$ (half-blocking concentration, which is 140 μM), and $h$ (Hill coefficient) = 1.5. ATP at 30 μM has evident inhibitory effect on channel activity and $P_{\text{open}}$ reaches almost 0 when ATP concentration is 1.0 mM.
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This channel had a slope conductance of 277 ± 24 pS (n = 8), was voltage activated and did not show inward rectification (Fig. 9, A, C, and D). This channel was almost completely blocked by charybdotoxin (50 nM), iberiotoxin (100 nM) or external TEA (2 mM) but was not sensitive to internal TEA (40 mM (n = 4)). These characteristics indicated that this current belonged to the BK (or Maxi K) type of K⁺ channels.

Unlike its effect on S-K,ATP and L-K,ATP channels, ATP enhanced channel activity of the BK channel in human neocortical neurons when applied to the cytosolic side in the presence of Mg²⁺. P(open) was increased from 0.096 ± 0.011 to 0.172 ± 0.014 (n = 5, P < 0.05; Fig. 9B). Also, ATP enhanced the unitary conductance of this BK channel. The slope conductance increased from 267 ± 6 to 295 ± 7 (n = 5, P < 0.01; Fig. 9, C and D). AMP-PNP at concentrations of 0.5–1.0 mM did not consistently affect this channel, although we saw a slight increase in channel activity in three out of eight patches. Glibenclamide at a concentration of 10–20 μM had no effect on this BK channel (n = 4).

**FIG. 6.** Inhibition of large-conductance current by ATP in a human neocortical neuron. Single channel currents were studied using solutions containing symmetric K⁺ (150 mM) with a free-Ca²⁺ concentration of 1 μM. A: patch was held at 0 mV and a ramp potential was given from −75 to 75 mV. Under this condition, 2 active channels were seen, and they showed an inward rectification. In negative membrane potential (0 to −50 mV), linear slope conductance is 180 pS. B: when membrane potential was held at −30 mV, same channels as in A had a P(open) of 0.211 during baseline. Application of ATP (1 mM) to cytosolic membrane caused a marked inhibition of both of these currents (P(open) = 0.015). Channel activity recovers after washout of ATP (P(open) = 0.223).

**Large conductance Ca²⁺-activated K⁺ channel**

We also observed a very large conductance outward current in inside-out patches from human neocortical neurons, which was different from both channels described above. This current had very low channel activity when the internal solution contained no Ca²⁺ (free Ca²⁺ < 10 nM). However, as for the L-K,ATP channel, when Ca²⁺ concentration in the internal solution rose to micromolar concentrations, channel activity markedly increased. This channel was observed in eight patches from 15 human neocortical neurons (1 patch/cell). This channel had a slope conductance of 277 ± 24 pS (n = 8), was voltage activated and did not show inward rectification (Fig. 9, A, C, and D). This channel was almost completely blocked by charybdotoxin (50 nM), iberiotoxin (100 nM) or external TEA (2 mM) but was not sensitive to internal TEA (40 mM (n = 4)). These characteristics indicated that this current belonged to the BK (or Maxi K) type of K⁺ channels.
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Our current studies have shown for the first time that at least two distinct K⁺ATP channels exist in the CNS of humans and rats. Evidence for the existence of more than one subtype of K⁺ATP channels also can be found from a large number of previous studies indicating that K⁺ATP channels are different in their biophysical and pharmacological properties, such as conductance (ranging from 7 to 250 pS) (Ashcroft et al. 1984; Ashford et al. 1988, 1990a; Bonev and Nelson 1993; Cook and Hales 1984; Jiang et al. 1994; Kajioaka et al. 1991; Kirsch et al. 1990; Lorenz et al. 1992; Noma 1983; Ohno-Shosaku and Yamamoto 1992; Sellers et al. 1992; Spruce et al. 1987; Standen et al. 1989; Tung and Kurachi 1991; Wakatuki et al. 1992; Wang and Giebisch 1991), rectification (both inward and outward rectification) (Ashford et al. 1988; Ashford et al. 1988, 1990a; Bonev and Nelson 1993; Cook and Hales 1984; Kakei and Noma 1984; Noma 1983; Standen et al. 1989; Trube and Hescheler 1984), sensitivity to ATP (IC₅₀ level spans from 50 µM to up to mM) (Ashcroft et al. 1984; Ashford et al. 1988, 1990a; Bonev and Nelson 1993; Cook and Hales 1984; Kakei et al. 1985; Noma 1983; Noma and Shibasaki 1985; Schmid-Antomarchi et al. 1987; Spruce et al. 1987; Standen et al. 1989; Wang and Giebisch 1991) and receptor affinity to sulfonylureas (Gopalakrishnan et al. 1991; Treherne and Ashford 1991; Xia and Haddad, 1991; Zini et al. 1991).

This BK channel, which had a large conductance and was blockable by charybdotoxin, also was seen in rat neocortical neurons (n = 4). Its channel activity (both P_open and conductance) was enhanced by intracellular ATP as in the human neuronal BK channel.

Besides these three K⁺ channels, we have observed several other types of K⁺ channels whose unitary conductances and other single channel properties were different from the three ATP-modulated K⁺ channels. Because the modulation of these channels by intracellular ATP was not seen, we did not perform detailed studies on these channels.

DISCUSSION

K⁺ATP channels are present in a number of tissues and cell types, including pancreatic β-cells, cardiac myocytes, skeletal muscles, smooth muscles, and rodent central neurons (Ashcroft at al. 1984; Ashford et al. 1988, 1990a; Cook and Hales 1984; Noma 1983; Ohno-Shosaku and Yamamoto 1992; Spruce et al. 1987; Standen et al. 1989; Wang and Giebisch 1991). The S-K⁺ATP channel found in the present study appears to be a novel neuronal type of K⁺ATP channel. Its biophysical and pharmacological properties are different from those of K⁺ATP channels that have previously been found in central neurons. Although Ashford et al. (1988) have observed a K⁺ATP channel in cultured rat cortical neurons that has a conductance of 50 pS, the current shows a clear outward rectification and a low sensitivity to ATP. Also in cultured rat cortical neurons, Ohno-Shosaku and Yamamoto (1992) have identified another K⁺ATP channel, whose rectification and ATP-sensitivity are comparable with the S-K⁺ATP channel. Inhibited this channel. Internal tetraethylammonium at a concentration of 20 mM suppressed slightly this channel.

In Fig. 7, P_open (normalized to baseline P_open) is plotted as function of ATP concentration in internal solutions. Half-blocking concentration (IC₅₀ level spans from 50 µM to up to mM) (Ashcroft et al. 1984; Ashford et al. 1988, 1990a; Bonev and Nelson 1993; Cook and Hales 1984; Kakei et al. 1985; Noma 1983; Noma and Shibasaki 1985; Schmid-Antomarchi et al. 1987; Spruce et al. 1987; Standen et al. 1989; Wang and Giebisch 1991) and receptor affinity to sulfonylureas (Gopalakrishnan et al. 1991; Treherne and Ashford 1991; Xia and Haddad, 1991; Zini et al. 1991).
A: voltage-dependent activation of BK channel in 2 rat neocortical neurons. Currents were recorded with symmetric concentrations of K⁺ applied to both sides of inside-out patches (free Ca²⁺ = 1 μM). Channel activity (P_open, normalized to maximum level) started to increase near a membrane potential (V_m) of 20 mV. Full activation was reached when V_m was ≥80 mV. Relation of P_open to V_m can be described with Boltzmann expression: y = 1/(1 + exp(-K_v/V_m/k)), where y = normalized P_open, V_m = membrane potential, K_v = 52 mV (V_m at 50% of P_open), and k = 9 mV. Note that data at 0 mV point are missing, because these potentials are very close to reversal potentials.

B: enhancement of channel activity of 300-pS outward current by ATP in a human neocortical neuron. Symmetric concentrations of K⁺ were applied to both sides of inside-out patch, and membrane potential was held at -40 mV. One active channel was seen during baseline with P_open = 0.077. When patch was exposed to an internal solution containing 1 mM of ATP, 2nd channel that had a similar conductance was activated and P_open increased to 0.171. Channel activity decreased after washout of ATP, and P_open returned to baseline level within 1 ± 2 min. Note that bottom traces in each pair of traces showed expanded segments of top traces located between 2 arrows.

C: augmentation of unitary conductance of BK currents by ATP. Single channel currents were recorded from a rat neocortical neuron in an inside-out patch. Using symmetric K⁺ concentrations and a ramp command potential (0 to 50 mV), 2 active channels were observed. Both of these channels had same conductance of 270 pS.

D: when patch was exposed to an internal solution containing 1 mM ATP, unitary conductance increased by ~10% to 300 pS.

that have previously been described in rat cortical, hypothalamic and substantia nigra neurons (Jiang et al. 1994; Sellers et al. 1992; Treherne and Ashford 1991). These K_ATP channels have a unitary conductance of 220–250 pS and are Ca²⁺-activated. K_ATP channels with a large conductance and Ca²⁺ sensitivity also have been described in other tissues. For instance, previous studies have shown that the activity of K_ATP channels in skeletal and smooth muscles is stimulated by an increase in free Ca²⁺ concentrations in the cytosol (Kajioka et al. 1990; Krippeit-Drews and Uönnendonker 1992; Silberberg and van Breemen 1990). In spite of the fact that the L-K_ATP channel is Ca²⁺-dependent and has a large conductance, our data do not support the idea that it belongs to the classical BK type of Ca²⁺-activated K⁺ channels, because it has a number of biophysical and pharmacological properties different from those of BK channels (see below). It may be worth mentioning that the L-K_ATP channel found in the present study has a range of conductances. Two examples shown in Figs. 6 and 7 are 180 and 230 pS, respectively. The 180-pS K_ATP channel shows a high frequency of channel open and closed activity, whereas the 230 pS one has a relatively low flickering activity. Because we do not have other evidence suggesting that each of them belongs to a distinct channel family, we tentatively pooled them together as a single group.

Besides the K_ATP channels, our current studies have shown that a large conductance Ca²⁺-activated K⁺ channel present in human neocortical neurons is also modulated by ATP. This channel is similar to the BK channel previously described in a number of tissues, since it has a large conductance (~280 pS) and a high sensitivity to charybdotoxin, iberiotoxin, and external TEA. As with previous studies
(Chung et al. 1991), our results have shown that ATP enhances $P_{\text{open}}$ and the unitary conductance of this BK channel. This enhancement of $P_{\text{open}}$ by ATP appears to be mediated by a protein phosphorylation process since the effect can not be mimicked by the nonhydrolyzable ATP analogue AMP-PNP (see also Chung et al. 1991).

Although both BK channel and L-K$_{\text{ATP}}$ channel are Ca$^{2+}$-dependent and have a large conductance, there are clear differences between these two channels. For example, 1) ATP at micromolar concentration can suppress completely this channel, whereas the BK channels are stimulated by ATP. 2) The effect of ATP on the L-K$_{\text{ATP}}$ channel is not mediated by protein phosphorylation, but phosphorylation is required for the ATP effect on the BK channels (Chung et al. 1991). 3) The L-K$_{\text{ATP}}$ channel is sensitive to K$_{\text{ATP}}$ channel blockers and insensitive to charybdotoxin, whereas the BK channel is not sensitive to glibenclamide. It is also known that single channel conductance of K$_{\text{ATP}}$ currents varies among tissues and cell types, and K$_{\text{ATP}}$ channels with large conductances have been described previously in other tissues (Ashford et al. 1990a, b; Jiang et al. 1994; Krippett-Drewes and Uönnendonker 1992; Lorenz et al. 1992; Sellers et al. 1992).

The modulation of ion channel activity by intracellular metabolites such as ATP can have major impact on cellular function and response to metabolic stress (de Weille and Lazdunski 1990; Haddad and Jiang 1993; Rorsman et al. 1990; Standen 1992; Takeno and Noma 1993). In central neurons, K$_{\text{ATP}}$ channels are activated during hypoxia as we and others have previously shown (Grigg and Anderson 1989; Jiang and Haddad 1991; Jiang et al. 1994; Luhmann and Heinemann 1992; Mourre et al. 1989; Murphy and Greenfield 1992). Activation of these K$_{\text{ATP}}$ channels produces hyperpolarization or attenuation of the hypoxia-induced depolarization in neurons, with a resulting decrease in energy consumption and thus an increased likelihood of cell survival (Jiang and Haddad 1991; Mourre et al. 1991; Murphy and Greenfield 1992).

One important question that can be raised is why nerve cells are endowed with K$_{\text{ATP}}$ channels that have very different biophysical and pharmacological properties. Because S-K$_{\text{ATP}}$ can be activated around resting membrane potential, the modulation of this inward rectifier K$_{\text{ATP}}$ channel may be critical for constant adjustment of resting membrane potential (Quayle and Standen 1994; Standen 1992; Surprenant et al. 1995; Takeno and Noma 1993). The L-K$_{\text{ATP}}$ channel may be an important modulator of membrane potential when the cell is depolarized and when Ca$^{2+}$ increases such as during hypoxia or hypoglycemia. In addition, both S-K$_{\text{ATP}}$ and L-K$_{\text{ATP}}$ may play a role in determining membrane potential when intracellular ATP level drops. Because these channels have different sensitivities to ATP, it is possible that L-K$_{\text{ATP}}$ may be activated at an earlier time when ATP is declining. It is important to note that since the BK channel is activated with ATP, ATP can activate K$^+$ channels not only when it is abnormally low but also when it occurs at physiologic concentrations (>1 mM). Hence, it would seem that the different biophysical properties of K$^+$ channels described can be useful in various circumstances and levels of stress. Thus ATP (or other nucleotides) may be considered as sensors that can adjust neuronal membrane excitability.

In conclusion, our results have shown that three ATP-modulated K$^+$ channels exist in human and rat neocortical neurons. By modulating these different species of K$^+$ channels, ATP could serve as a regulator of cellular membrane excitability by changing K$^+$ channel activity in temporospatial-specific manner depending on ATP availability and demand in nerve cells.

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