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FUNCTIONAL EXPRESSION OF LARGE-CONDUCTANCE Ca^{2+}-ACTIVATED POTASSIUM CHANNELS IN LATERAL GLOBUS PALLIDUS NEURONS

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Abstract—The presence of large-conductance Ca^{2+}-activated potassium (BK) channels, which are considered to play an important role in the excitability of neurons, in the highly-excitable lateral globus pallidus (LGP) neurons has yet to be confirmed. In this study, we confirmed the functional expression of BK channels in mouse LGP neurons and investigated the characteristics of their single-channel currents using inside-out patch-clamp recordings. These BK channels had a conductance of 276 pS, were activated by the elevation of both the transmembrane potential and intracellular calcium concentration ($[Ca^{2+}]_i$), and were completely blocked by the BK channel-specific blocker paxilline (100 nM). In addition, the channel currents were sensitive to high-energy phosphate compounds and low internal pH. The cellular function of these BK channels was then investigated by nystatin-perforated whole-cell recording. Paxilline (100 nM) had no effect on the frequency and half-width of the action potential (AP) in LGP neurons under control conditions, but significantly attenuated the hyperpolarization that was caused by carbonyl cyanide m-chlorophenylhydrazone (CCCP), an inhibitor of ATP synthesis. In addition, the pancreatic β-cell type ATP-sensitive potassium channel (K_{ATP} channel) blocker tolbutamide (0.25 mM) also attenuated the hyperpolarization, in a manner similar to paxilline. The voltage-dependent potassium channel blocker tetraethylammonium (TEA, 2 mM) significantly decreased the frequency and increased the half-width of the AP in LGP neurons under control conditions, and attenuated CCCP-induced hyperpolarization to an extent close to that of paxilline. The results presented here suggest that functional BK channels are present in LGP neurons, and may behave as partners of K_{ATP} channels in the regulation of neuronal activity under metabolic stress conditions.

Key words: BK channel, lateral globus pallidus, functional expression, action potential, CCCP, hyperpolarization.

The presence or absence of large-conductance Ca^{2+}-activated potassium (BK) channels in the lateral globus pallidus (LGP) is an important question for understanding the regulation role of the LGP in the output of the basal ganglia circuitry, the center which is involved in motor control and some higher functions of the nervous system.

The function of the LGP is known to be sensitive to metabolic stress. LGP infarction has been found in rats exposed to carbon monoxide (CO) poisoning and middle cerebral artery occlusion (Piantadosi et al., 1997; Dihne et al., 2002), while lesions in the globus pallidus and systemic motor dysfunction have been found simultaneously in some patients suffering hypoxic encephalopathy such as carbon monoxide intoxication (Prockop and Chichkova, 2007). Such dysfunctions may be explained by the alteration of neuronal activation in metabolic stress. The majority of neurons in the LGP, projective nuclei of the basal ganglia, are highly excitable GABAergic neurons (Baranauskas et al., 1999; Kita, 2007). More than 70% of LGP neurons exhibit a continuous firing rate of 2–40 Hz (Kita and Kitai, 1991; Bengtson and Osborne, 2000). Although the involvement of ion channels in the regulation of neuronal activity under different metabolic conditions has been investigated in various brain areas that are vulnerable to metabolic stress, it has not been investigated in LGP neurons so far.

BK channels are considered to be involved in the regulation of neuronal activity under physiological and metabolic stress conditions. They are reported to be involved in modulating the frequency or half-width of action potentials (AP) in neostriatal neurons, neostriatal cholinergic interneurons, cerebellar purkinje cells, and CA1 hippocampal pyramidal cells (Pineda et al., 1992; Bennett et al., 2000; Sausbier et al., 2004; Gu et al., 2007). It is reported that the open probability of BK channels decreased during extracellular O_2 deprivation in mice neocortical neurons (Liu et al., 1999). Charybdotoxin, a blocker of intermediate-conductance potassium (IK) and BK channels, attenuates the hyperpolarization induced by metabolic stress in midbrain dopaminergic neurons and striatal large aspiny interneurons (Pisani et al., 1999; Marinelli et al., 2000). Treatment with BK channel blockers aggravates ischemic cell death in hippocampal and cerebrocortical regions (Runden-Pran et al., 2002; Katsuki et al., 2005), while treatment with a BK channel opener reduces the ischemic infarct volume in the cortex (Gribkoff et al., 2001). It thus seems likely that BK channels are important in the excitability of neurons of the LGP and their response to metabolic stress.

BK channels are activated by an increase in membrane potential and intracellular calcium concentration ($[Ca^{2+}]_i$). Those in brain regions such as the ventromedial

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP, action potential; BK, channel, large-conductance Ca^{2+}-activated potassium channel; $[Ca^{2+}]_i$, intracellular calcium concentration; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; LGP, lateral globus pallidus; K_{ATP} channel, ATP-sensitive potassium channel comprised by inwardly rectifying K^+ channel and sulfonylurea receptor; P_{open}, open probability; TEA, tetraethylammonium.

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hypothalamic nucleus, motor cortex, suprachiasmatic nucleus and substantia nigra compacta are also known to be modulated by decreases in intracellular pH or ATP (Treherne and Ashford, 1991; Lee et al., 1995; Hall et al., 1997; Su et al., 2010), such as occur in ischemic injury (Lipton, 1999). The characteristics of BK channels may vary according to location within the brain. Here, we studied the functional expression of the BK channel in LGP neurons using patch-clamp electrophysiology recording techniques. We found that functional BK channels are present in LGP neurons, and may be involved in the regulation of neuronal activity under metabolic stress conditions.

EXPERIMENTAL PROCEDURES

Cell preparation

P15-20 C57BL/6 mice, in which the functional response of GABAergic receptors is similar to that of adult mice (Ben-Ari et al., 2007), were obtained from the Animal Experiment Center of Peking University Medical Department (PR China). After decapitation, brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) for 1 min. Coronal slices (thickness: 300–350 μM) containing the LGP were sectioned in ACSF with a vibroslicer (ZERO 1, Dosaka, Japan). The ACSF contained (in mM): 145 NaCl, 4 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 HEPES, pH 7.4 (NaOH). The solution was saturated with 100% O_2. All experiments were carried out at room temperature (22–25 °C). The slices were conditioned by pre-incubating in ACSF for 30 min. Here, we used enzymatic rather than mechanical methods to isolate neurons from rodent brain tissue, since these methods have been used extensively for animals older than 2 weeks (Koyama et al., 1999; Liu et al., 1999; Yamada et al., 2001; Shimada et al., 2007; Vicente et al., 2010; Su et al., 2010). Trypsin was used here at a lower concentration than that in the previous studies on BK channels (Meis and Pape, 1997; Liu et al., 1999; Spreadbury et al., 2004) to avoid any impact of enzymes on the open properties of BK channels (Armstrong and Roberts, 2001; Spreadbury et al., 2004).

Brian slices were exposed to trypsin (0.1 mg/ml, in ACSF) for 10–15 min at 30 °C. The LGP were then punched out from both sides of the slices (Suppl. Fig. 1A–C), and tissue chunks were dissociated by gentle pipetting with fire-polished glass pipettes in poly-lysine-coated dishes (Falcon) under a microscope (CK30, Olympus, Japan) (Yamada et al., 2001). The dissociated cells were perfused with ACSF at a flow rate of 2–2.5 ml/min and were used for a maximum of 8 h. Cells were photographed under a phase contrast microscope (IX-70, Olympus, Japan) equipped with a digital camera (1412M, DVC, USA) and image acquisition software (IPLab 3.6, Scanalytics, USA). All efforts were made to minimize the number of animals used and their suffering, and all experiments were approved by the Institutional Animal Administration Committee of the Institute of Biophysics, Chinese Academy of Sciences.

Patch-clamp recording

Single channel currents were recorded using the inside-out patch-clamp configuration. Fire-polished pipettes used here were made from borosilicate glass capillary (P-97 puller, Sutter, USA; 2002-A polisher, Yibo, China) and had a resistance of 4–9 MΩ when filled with pipette solution containing (in mM): 140 KCl and 10 HEPES, pH 7.2 (tris(hydroxymethyl)aminomethane, Tris). The bath solution contained (in mM): 140 KCl, 0.2 $MgCl_2$, 10 HEPES and the indicated amounts of $CaCl_2$, at pH 7.2 (Tris). Beakers and tubes (Azlon, PP, USA; Tagon R3603, Japan; Hibiki polyethylene tube, Japan) with good chemical resistance were used throughout the calcium-dependent measurements, and 1 mM EGTA was used to clean free Ca^{2+} in the perfusion tubes before each experiment. Solution pH values of 7.2–5.0 were obtained by adjusting with HCl (final concentration ~5 mM) and pH 8.0 was obtained by adjusting with Tris (added at a concentration of ~11 mM); the pH of the resulting solutions was steady during the experimental period (fluctuations of less than 0.1 U within 8 h).

Cellular roles of BK channels were investigated under control and chemical metabolic stress conditions. Isolated single cells were used to facilitate precise regulation of the extracellular environment. Nystatin-perforated whole-cell recording, which permits only small monovalent ions to pass through the clamped patch, was used in this experiment to avoid leakage of large molecules and ensure that steady intracellular conditions were maintained during the recording period (Akaike and Harata, 1994). The transmembrane current was clamped at zero. Pipettes used in nystatin-perforated whole-cell recording had a resistance of 3–7 MΩ when filled with solution containing (in mM): 140 KCl, 10 HEPES, 50 μg/ml nystatin, at pH 7.2 (Tris). The bath solution was ACSF under control conditions, and the perfusion solution was changed to ACSF containing 200 nM carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), an inhibitor of ATP synthesis, for the metabolic stress experiment. Serial resistance and membrane capacitance were compensated as high as possible by the amplifier without producing an oscillation in the potential signal.

In all of the above experiments, reagents were directed to recorded patches or cells via a tube that was located at a distance of 3–5 mm. Paxilline, trypsin, nystatin, Tris, ATP, GTP, CCCP, tobutamide and tetraethylammonium (TEA) were purchased from Sigma, USA. ADP and AMP were purchased from Amresco (USA). Electrophysiological signals were amplified with an Axonpatch 200B patch-clamp amplifier (Axon Instruments, USA), digitized with Digidata 1322A (Axon Instruments, USA). Data were acquired with pCLAMP software (version 8.0, Axon Instruments). The Axonpatch 200B amplifier was designed for single channel recording, and is also used for investigating changes in action potential by current-clamp recording (Zhang et al., 2003; Ohbuchi et al., 2010). A low-pass filter of 2 kHz and a matched sampling frequency of 4 kHz were used.

Data analysis

Data were analyzed with Clampfit software (version 10.0, Axon Instruments). In single-channel analysis, the data were filtered at 1000 Hz. In whole-cell recordings, we focused on the alteration of frequency and half-width of the AP rather than other parameters in order to avoid distortion of the fast signal in current-clamp mode caused by the amplifier and the recording configuration affecting the interpretation of our results. The nystatin-perforated configuration causes a large access resistance (~100 MΩ) (Akaike and Harata, 1994) that affects spike duration and AP amplitude. Severe filtering is required to achieve an acceptable signal–noise ratio, and AP shape is also altered. Statistical analysis was carried out with Origin 7.5 (Microcal). Data are presented as the mean±standard error (n=number of patches or cells). The significance of differences between means was tested with the Student's *t*-test, and differences were considered significant at $P<0.05$.

RESULTS

Most of the isolated LGP neurons were oval or triangular in shape, with a longitudinal diameter of 13–30 μm (Suppl. Fig. 1D). The quality of acutely isolated LGP neurons was tested by nystatin-perforated whole-cell recordings in 60 cells with membrane potentials that were more negative than −40 mV and had a spontaneous firing frequency higher than 1 Hz. In these neurons, the membrane potential, AP frequency, half-width were −56.3±0.9 mV, 9.6±

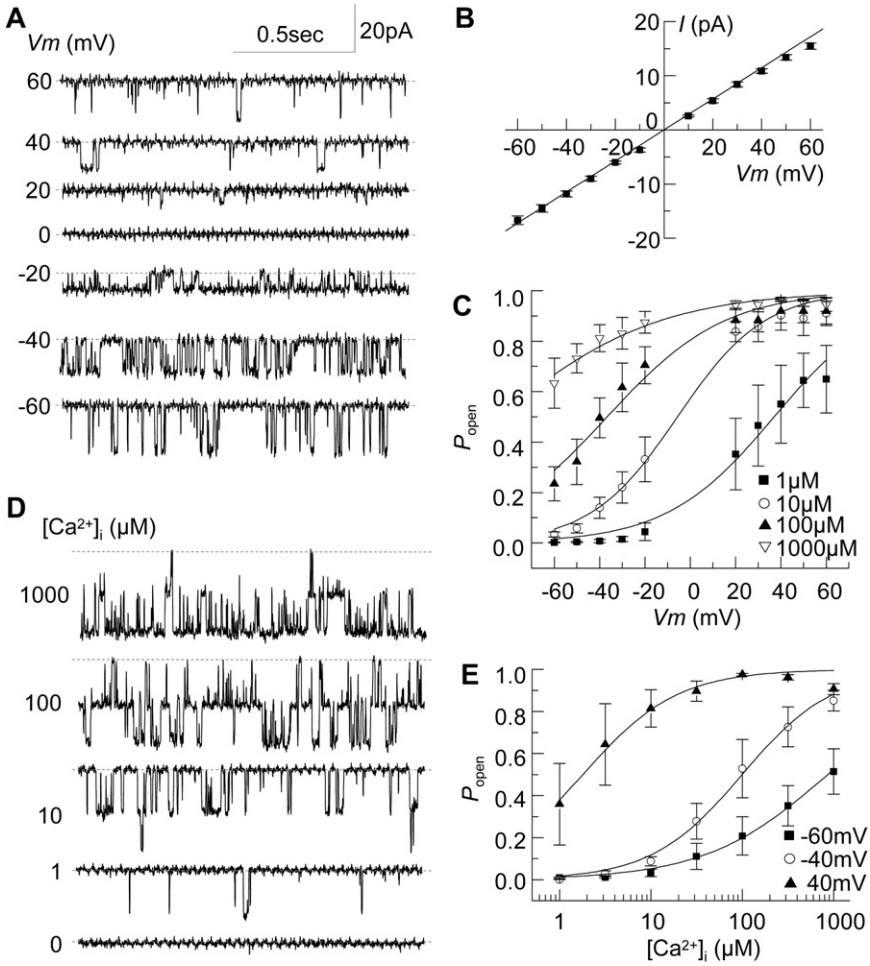

Fig. 1. Characteristics of BK channels in LGP neurons. (A) Representative recordings of single-channel currents at transmembrane potentials from −60 to 60 mV at a $[Ca^{2+}]_i$ of 100 μM (the dashed line represents the closed level). (B) Current–voltage relationship. Data from −60 to 0 mV were fitted linearly. (C) The relationship between P_{open} and transmembrane potential ($[Ca^{2+}]_i$: 1 μM, n=5; 10 μM, n=5; 100 μM, n=7; 1000 μM, n=6). The $P_{open}-V_m$ curve was obtained by fitting the Boltzmann equation $[y=A_2+\frac{A_1-A_2}{1+e^{(x-x_0)/dx}}]$ to the data. (D) Representative recordings of $[Ca^{2+}]_i$ -dependent single-channel currents at a transmembrane potential of −60 mV. (E) The relationship between P_{open} and $[Ca^{2+}]_i$ (transmembrane potential: −60 mV, n=6; −40 mV, n=4; 40 mV, n=4). The $P_{open}-[Ca^{2+}]_i$ curve was obtained by fitting the Hill equation $[y=V_{max}\frac{x^n}{k^n+x^n}]$ to the data.

0.8 Hz and 2.9±0.1 ms, respectively. These firing characteristics are in accordance with those recorded for LGP neurons *in vivo* and in brain slices (Kita and Kitai, 1991; Chan et al., 2004), indicating that the isolated neurons here were well-preserved and suitable for electrophysiological studies.

Single BK channel currents were recorded in LGP neurons

Single BK channel currents were recorded on 707 of 2000 patches excised from isolated LGP neurons. Among the 707 patches, 217 patches were used for investigation of single channel characteristics, and the average number of current levels per patch was 2.3±0.1. No notable rundown of channel activity was observed during the 30 min recording period (n=6).

Basic characteristics of BK currents were first investigated under a symmetrical $[K^+]_o/[K^+]_i$ of 140 mM. The current-voltage relationship was assessed at a transmembrane potential range of −60–60 mV; in order to obtain a moderate P_{open} even at −60 mV, $[Ca^{2+}]_i$ was set at 100 μM. The I–V curve was linear at −60∼0 mV and 0∼60 mV with a mean conductance of 276±12 pS and 263±9 pS, respectively (n=7); the reversal potential was 0 mV (Fig. 1A, B). The relationship between the channel open probability (P_{open}) and the voltage was examined at $[Ca^{2+}]_i$ of 1, 10, 100 and 1000 μM (Fig. 1C). Channel activation was dependent on transmembrane potential at all the $[Ca^{2+}]_i$ tested, and the $V_{1/2}$ values obtained from fitting the Boltzmann equation to P_{open}-voltage data decreased as $[Ca^{2+}]_i$ increased. The P_{open}-$[Ca^{2+}]_i$ relationship was investigated at $[Ca^{2+}]_i$ from 0 to 1000 μM and at transmembrane potentials of −60, −40 and 40 mV. Channel activation was dependent on $[Ca^{2+}]_i$ for all the transmembrane potentials tested and the EC_{50} (half-maximal effective concentration, k) values obtained from fitting the Hill equation to P_{open}-$[Ca^{2+}]_i$ data decreased as transmembrane potential depo-

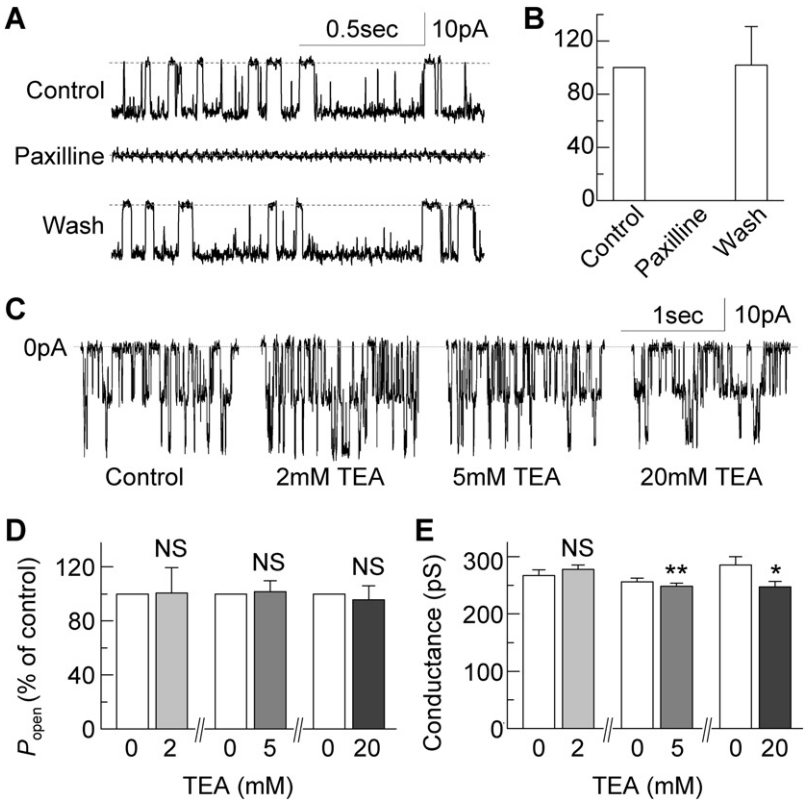

Fig. 2. Effects of paxilline and TEA on BK channels in LGP neurons. (A) Representative recordings of single-channel currents on internal application of paxilline. (B) Graphs showing the effects of paxilline on the P_{open} of the BK channel. (C) Representative recordings of single-channel currents at different concentrations of internal TEA (2 mM, $n=5$; 5 mM, $n=5$; 20 mM, $n=3$). (D, E) Graphs showing the effects of different concentrations of internal TEA on P_{open} and the conductance of the BK channel. Currents were recorded at a transmembrane potential of −60 mV and $[Ca^{2+}]_i$ of 100 μM. *, ** $P<0.05$, 0.01; NS (no significant difference between two samples): $P>0.05$.

larized (Fig. 1E). It should be noted that the channel was totally closed in Ca^{2+}-free solution, as was the case in the solution containing 1 mM EGTA, and that it was clearly open at 1 μM $[Ca^{2+}]_i$, and had a P_{open} that was significantly increased at 3 μM $[Ca^{2+}]_i$ ($P<0.05$, $n=14$); thus the deviation of $[Ca^{2+}]_i$ here was less than 1 μM.

Since we wanted to determine the function of the BK channel under metabolic stress-induced hyperpolarization, subsequent single-channel current recordings were carried out at a transmembrane potential of −60 mV and $[Ca^{2+}]_i$ of 100 μM.

The channel was completely blocked to zero open rate by the internal application of 100 nM paxilline, a specific blocker of BK channels ($n=4/4$) (Fig. 2A, B). The high conductance, dependence on transmembrane potential and $[Ca^{2+}]_i$, and sensitivity to paxilline described above are typical characteristics of BK channels.

For adding an aspect to compare LGP neuron BK channels with those reported previously, TEA, a non-specific blocker of voltage-dependent potassium channels, was used on the internal side of patches. TEA had no effect on P_{open} even at high concentrations (20 mM), but it did reduce single-channel conductance at concentrations of more than 5 mM ($n=3$–5) (Fig. 2C–E). This sensitivity to TEA was consistent with previous reports (Yoshida et al., 1991; Wang and Mathers, 1993; Jiang and Haddad, 1997).

BK channels are inhibited by internal high-energy phosphate compounds

The effect of high-energy phosphate compounds on BK channel currents was investigated at a transmembrane potential of −60 mV and $[Ca^{2+}]_i$ of 100 μM under a symmetrical $[K^+]_o/[K^+]_i$ of 140 mM. The internal application of ATP resulted in a dose-dependent decrease in the channel open probability (Fig. 3A). The Hill coefficient (n) and IC_{50} (half-maximal inhibitory concentration; k) were obtained from fitting the Hill equation to P_{open}-ATP data (Fig. 3B) and were −1.3 and 0.3 mM, respectively ($n=5$). 1 mM GTP inhibited P_{open} to 12.3%±2.7% of the control ($P_{open}=0.54\pm0.11$) ($n=5$, $P<0.001$), comparable to the effect caused by 1 mM ATP ($P>0.05$) ($n=7$) (Fig. 3C, D). Moreover, the effect of 2 mM ADP was less than that of 1 mM ATP ($n=5$, $P<0.005$), and no significant effect was observed for AMP even at a concentration of 3 mM ($n=4$) (Fig. 3C, D). In addition, 1 mM ATP+3 mM ADP and 3 mM ATP+1 mM ADP inhibited P_{open} to 16.4%±5.4% and 1.4%±0.5% of the control ($P_{open}=0.31\pm0.09$ and 0.32 ± 0.09), respectively ($n=4$, $P<0.001$) (Fig. 3E, F). The effects of the high-energy phosphate compounds presented above were immediate and reversible ($n=4$–7).

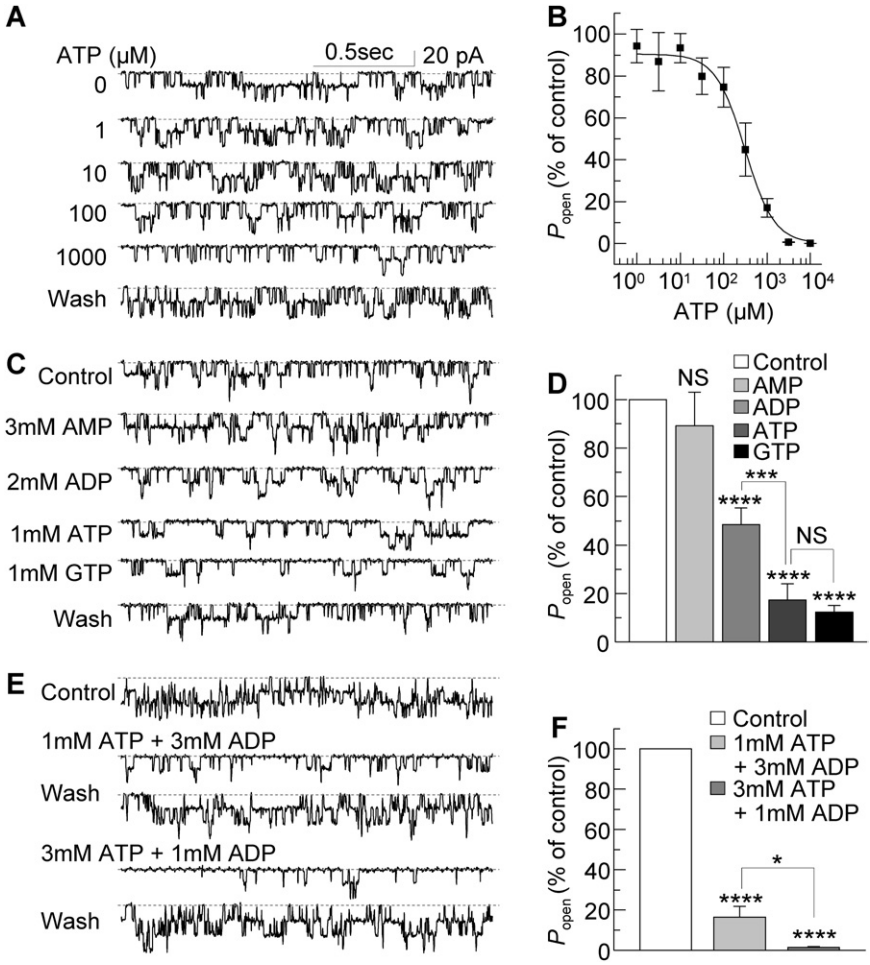

Fig. 3. Sensitivity of BK channels in LGP neurons to high-energy phosphate compounds. (A) Representative recordings with different concentrations of internal ATP. (B) The relationship between P_{open} and concentrations of internal ATP. The curve of the ATP effect on P_{open} was obtained by fitting the Hill equation [$y=V_{max}\frac{x^n}{k^n+x^n}$] to the data. (C, D) Representative recordings of single-channel currents when AMP, ADP, ATP, or GTP was applied internally and their corresponding statistical graphs. (E, F) Representative recordings and statistical graphs of the effect on BK channels of internal application of different mixtures of ATP and ADP. Recordings were made at a transmembrane potential of −60 mV and a $[Ca^{2+}]_i$ of 100 μM. *, ***, **** $P<0.05$, 0.005, 0.001; NS: $P>0.05$.

BK channels are inhibited by low internal pH

The effect of pH on the activity of BK channels in LGP neurons was investigated at internal pHs of 5.0, 6.0, 7.2 and 8.0 at a $[Ca^{2+}]_i$ of 100 μM and a transmembrane potential of −60 mV under a symmetrical $[K^+]_o/[K^+]_i$ of 140 mM (Fig. 4). At pH 7.2 (control), P_{open} and conductance were 0.34±0.18 and 277±12 pS, respectively ($n=4$). At pH 5.0 and pH 6.0, P_{open} decreased significantly to 5.4%±0.9% ($P<0.001$), and 84.4%±14.5% ($P>0.05$), respectively, and conductance decreased significantly to 225±13 pS ($P<0.005$), and 249±8 pS ($P<0.005$), respectively ($n=4$). At pH 8.0, the corresponding values increased to 144.5%±41.6% ($P>0.05$) and 285±11 pS ($P>0.05$), respectively ($n=4$). The effect of pH was reversible in three patches.

BK channels may be involved in the response to metabolic stress

Here, only neurons with resting membrane potentials that were more negative than −40 mV and had a spontaneous firing frequency greater than 1 Hz were used for investigating the roles of BK channels at the cellular level.

In control conditions, the external application of paxilline (100 nM), a BK channel-specific blocker, had no significant effect on the frequency and half-width of the AP ($n=7$, $P>0.05$) (Fig. 5A, C, D). External application of TEA (2 mM), a voltage-dependent potassium channel blocker, caused a decrease in frequency of 20.5%±4.2% and an increase in the half-width of 29.4%±3.1% ($n=26$, $P<0.001$) (Fig. 5A, C, D).

During external CCCP (200 nM)-mimicked metabolic stress, the membrane potential of LGP neurons demonstrated a hyperpolarization of 10.7±0.7 mV within 4.2±0.8 min ($n=27/29$, $P<0.001$) (Fig. 5B, E). The external application of paxilline (100 nM) attenuated the hyperpolarization by 5.7±1.3 mV ($n=29$, $P<0.001$). In addition, the effect of external TEA (2 mM) ($n=32$) and tolbutamide, a specific blocker for the inwardly rectifying K^+ channel and sulfonylurea receptor comprised ATP-sensitive potassium channel (K_{ATP} chan-

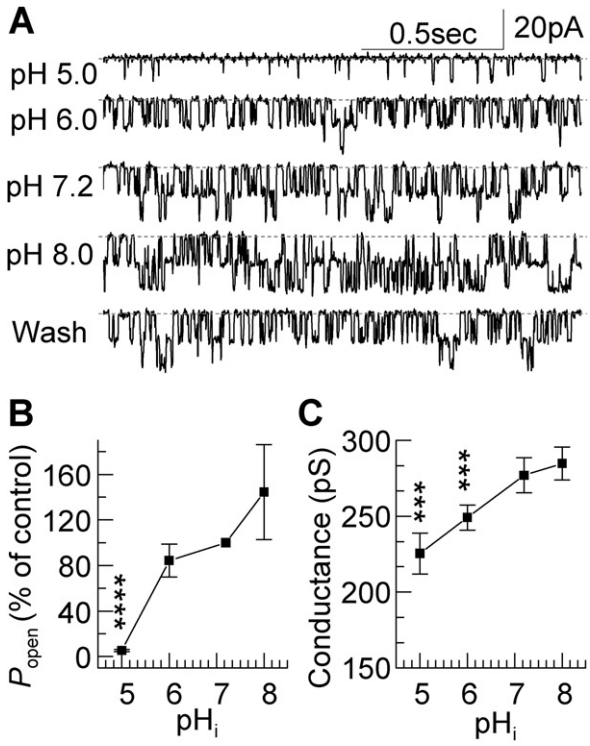

Fig. 4. Sensitivity of BK channels in LGP neurons to internal pH. (A) Representative recordings of BK currents at different internal pHs. P_{open} increased significantly as internal pH increased. (B, C) The effect of internal pH (pH_i) on the P_{open} and conductance of BK channels at pH values ranging from 5.0 to 8.0. Recordings were made at a transmembrane potential of −60 mV and a $[Ca^{2+}]_i$ of 100 μM. ***, **** $P<0.005$, 0.001.

nel) (0.25 mM) ($n=32$) was almost equal to the effect of external paxilline ($P>0.05$) (Fig. 5B, E, F).

Since paxilline and tolbutamide can both reverse CCCP-induced hyperpolarization, BK channels in LGP neurons may be considered as partners of K_{ATP} channels in the neuronal response to metabolic stress. In contrast to paxilline under control conditions, TEA produced a decrease in the frequency and an increase in the half-width of the AP; however, both TEA and paxilline reversed CCCP-induced hyperpolarization to a similar extent. Thus BK channels in LGP neurons seem more suited to selective regulation under metabolic stress than other TEA-sensitive potassium channels.

DISCUSSION

Our results provide the first electrophysiological evidence of the functional expression of BK channels in LGP neurons. The combination of single-channel characteristics of the BK channels in LGP neurons detected here were unique since they were sensitive to both high-energy phosphate compounds and low pH. On the basis of single-channel characteristics and the effect of paxilline at the cellular level, we deduce that BK channels in LGP neurons are probably involved in the hyperpolarization of membrane potential that is induced by metabolic stress, and seem to be more easily regulated by specific blockers in metabolic stress conditions than in physiological conditions. Considering that the majority of neurons in the LGP are GABAergic neurons, the results presented here suggest the BK channels may be involved in regulating excitability of GABAergic neurons under metabolic stress conditions in the LGP.

Here, since the soma of LGP neurons was used for our investigations of BK channels, we can conclude that the BK channels detected are indeed located in LGP neurons. Many previous reports have indicated that immunoreactivity for Slo1, the BK channel α subunit, is concentrated in the LGP in rats (Knaus et al., 1996; Grunnet and Kaufmann, 2004; Sausbier et al., 2006). However, Slo1 mRNA *in situ* hybridization signals in rat LGP have been negative when detected using synthetic antisense DNA probes specific to the C-terminal domain of the mouse *slo1* sequence (Knaus et al., 1996), and only weakly positive when detected with *in vitro* transcribed antisense RNA probes specific to the N-terminal domain of the mouse *slo1* sequence (Chang et al., 1997). Although positive immunohistochemical staining of β4 subunits has been found in the LGP, corresponding mRNA expression has not yet been reported (Piwonska et al., 2008). Our results therefore provide the first direct evidence for the functional expression of BK channels in LGP neurons.

The single-channel characteristics of BK channels in LGP neurons are similar to those of BK channels containing β4 subunits. Previous studies with cloned subunits indicate that different BK channel β subunits (β1, 2, 3, 4) exhibit different effects on voltage-dependency under fixed $[Ca^{2+}]_i$. At $[Ca^{2+}]_i \leq 10$ μM, $V_{1/2}$ values varied greatly, regardless of whether chelators were used (McManus et al., 1995; Brenner et al., 2000; Lippiat et al., 2003). At higher $[Ca^{2+}]_i$ (>10 μM), $V_{1/2}$ values of BK channels in LGP neurons (−36.6 mV at 100 μM $[Ca^{2+}]_i$ and −84.8 mV at 1 mM $[Ca^{2+}]_i$) were almost the same as values previously reported for BK channels composed of α and β4 subunits (−38.9 mV at 100 μM $[Ca^{2+}]_i$ and −57.4 mV at 1 mM $[Ca^{2+}]_i$) (Wang et al., 2006), providing supporting evidence that BK channels in the LGP are composed of α and β4 subunits. Interestingly, the frequency and width of the AP in dentate gyrus cells, whose BK channels are composed of α and β4 subunits, was unaffected by paxilline, just as was the case in LGP neurons (Brenner et al., 2005).

BK channels in LGP neurons are sensitive to both high-energy phosphate compounds and low pH. High-energy phosphate compounds chelate free calcium in solution. In our experiments, when 1 mM ATP, or 2 mM ADP was added to a solution containing 100 μM $[Ca^{2+}]_i$, the $[Ca^{2+}]_i$ decreased to about 20 and 50 μM, respectively (as calculated using Maxchelator; www.stanford.edu/~cpatton/maxc.html). At $[Ca^{2+}]_i$ of 20 and 50 μM, P_{open} is 0.08 and 0.14, respectively, about 40% and 70% of that at a $[Ca^{2+}]_i$ of 100 μM (0.21), according to the P_{open}-$[Ca^{2+}]_i$ curve shown in Fig. 1E. However, during ATP and ADP treatments, the experimental values of P_{open} were about 20% and 50% of the control, respectively, lower than that predicted from the P_{open}-$[Ca^{2+}]_i$ curve. Thus, after sub-

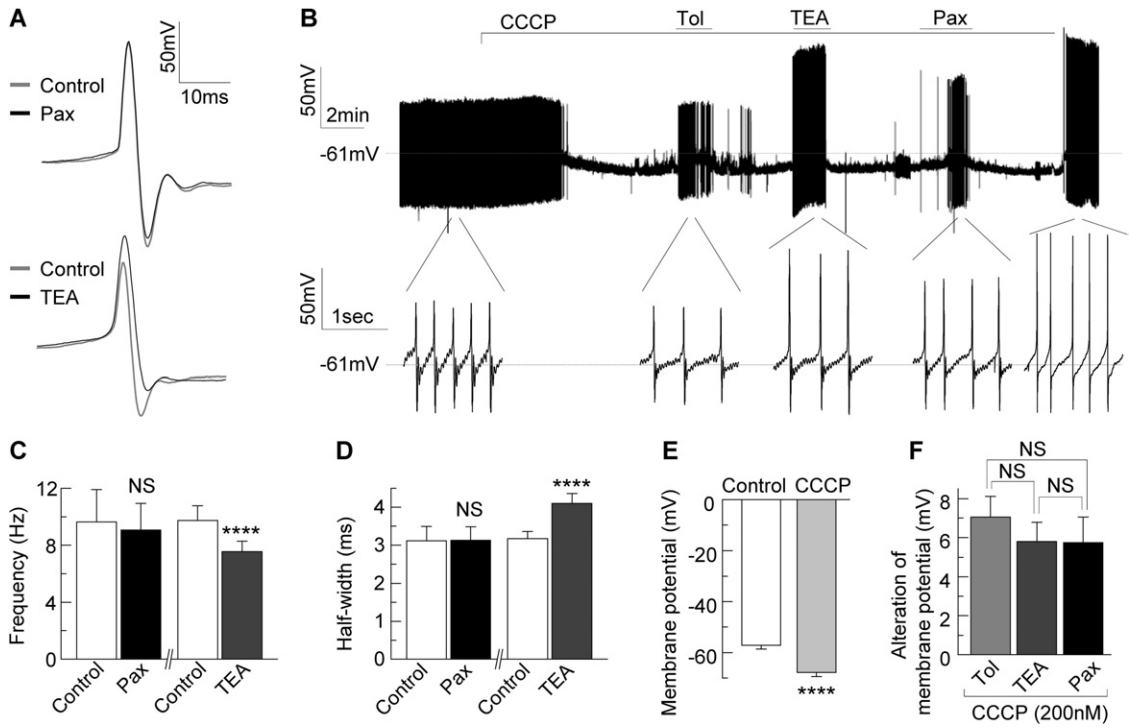

Fig. 5. Effect of BK channels on plasma membrane potential in LGP neurons. (A) Representative recordings showing the effect of paxilline and TEA on the AP of LGP neurons under control conditions. (B) Representative recordings showing the effects of tolbutamide (Tol), TEA and paxilline (Pax) on metabolic hyperpolarization caused by CCCP. In the cell indicated here, application of tolbutamide, TEA or paxilline could all reverse membrane hyperpolarization. (C, D) Effects of paxilline and TEA on the frequency and half-width of the AP. (E, F) Effects of CCCP and the extent of attenuation of CCCP-induced hyperpolarization by paxilline, TEA and tolbutamide. The amplitude of the AP was larger during washout than at the beginning of the treatment with CCCP, probably due to the decrease in access resistance because of the long time needed for CCCP to take effect. **** $P< 0.001$; NS: $P>0.05$.

tracting the chelation effect, the effect of high-energy phosphate compounds on the P_{open} of BK channels in LGP neurons was inhibitory. However, in previous reports, it has been suggested that, after subtracting the chelation effect, the effect of high-energy phosphate compounds on the P_{open} of BK channels in neurons from most brain regions was excitatory; only BK channels similar to those in LGP neurons are in neurons from the suprachiasmatic nucleus and ventromedial hypothalamic nucleus (Treherne and Ashford, 1991; Hall et al., 1997). The conductance and P_{open} of BK channels in LGP neurons was reduced by lowering the pH, similar to the situation in motor cortex neurons. However, the BK channel in motor cortex neurons is ATP-activated (Lee et al., 1995). It is worth mentioning that we recently found that a BK channel in substantia nigra pars compacta (SNc) neurons, which has a significantly larger conductance than the BK channel in LGP neurons, is also sensitive to both high-energy phosphate compounds and low pH (Su et al., 2010).

Paxilline significantly reverses CCCP-induced hyperpolarization in LGP neurons, suggesting that BK channels in LGP neurons may be involved in the response of membrane potential to metabolic stress. The inhibition of BK channels by high-energy phosphate compounds supports this hypothesis, since CCCP can result in a decrease of intracellular ATP. BK channels were not activated in the depolarization of the AP and the depolarization-induced $[Ca^{2+}]_i$ influx into LGP neurons but were activated in metabolic hyperpolarization, suggesting that ATP sensitivity may be the determining factor for the cellular function of the BK channel in LGP neurons, since the channel activity is quite consistent with the alteration of intracellular ATP. The effect of the K_{ATP} channel blocker tolbutamide on the hyperpolarization caused by CCCP also supports our hypothesis.

As discussed above, paxilline cannot easily alter neuronal excitability under control conditions but significantly affects metabolic stress-induced hyperpolarization of membrane potential in LGP neurons, thus suggesting that BK channels can be regulated selectively under metabolic stress without interfering with normal neuronal activity. We observed that paxilline aggravates the CCCP-induced decrease in mitochondrial redox potential in LGP in brain slices (Suppl. Fig. 2), indicating that BK channels in LGP neurons may be involved in protection against metabolic stress, and suggesting that selectively activated BK channels may be advantageous during metabolic stress. The LGP is vulnerable to metabolic stress, such as middle cerebral artery occlusion (Dihne et al., 2002) and CO intoxication (Prockop and Chichkova, 2007). Our results indicate that BK channels have a significant function in the LGP at both the cellular and tissue levels and may thus have a significant effect on the whole body under metabolic stress. Further research is required to

determine the precise role these newly discovered BK channels play in motor center dysfunction caused by ischemic stress.

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APPENDIX

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroscience.2010.06.026.

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