

Functional expression of a large-conductance Ca^{2+} -activated K^{+} channel in mouse substantia nigra pars compacta dopaminergic neurons

Wenting Su^{a,b}, Xueqin Song^{a,b}, Juan Juan Ji^{a,*}

^a State Key Laboratory of Brain and Cognitive Sciences, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Road, Chaoyang District, Beijing, 100101, China

^b Graduate University of Chinese Academy of Sciences, 19A Yuquan Road, Shijingshan District, Beijing, 100049, China

ARTICLE INFO

Article history:

Received 8 October 2009

Received in revised form

17 December 2009

Accepted 22 December 2009

Keywords:

Substantia nigra pars compacta

BK channel

Patch-clamp

Single-channel properties

Functional expression

ABSTRACT

The existence of large-conductance Ca^{2+} -activated K^{+} (BK) channels in substantia nigra pars compacta (SNc) has been a matter of debate. Using the patch-clamp technique in the inside-out configuration, we have recorded BK channel currents in SNc dopaminergic neurons. The channel has a conductance of 301 pS with a slight inward rectification and is both voltage- and calcium-dependent. Paxilline, a specific BK channel blocker, can completely block the channel, while tetraethylammonium (TEA), a nonspecific blocker of voltage-gated potassium channels, reduces its conductance and a high concentration of TEA (30 mM) inhibits its activity. ATP and GTP reduce the channel activity, while ADP is less potent, and AMP has no effect. The channel is also sensitive to changes in intracellular pH. Our results indicate that functional BK channels are expressed in SNc and suggest the possibility that the BK channel may be involved in the response of SNc dopaminergic neurons to metabolic stress.

© 2009 Elsevier Ireland Ltd. All rights reserved.

Large-conductance Ca^{2+} -activated K^{+} (BK) channels have been shown to play important roles in the modulation of neuron excitability and transmitter release [18,21]. It has been unclear whether BK channels are expressed in the mammalian substantia nigra pars compacta (SNc), a brain region which contains neurons with high excitability and plays key roles in movement control. A putative BK channel gene (*hSlo*) was cloned from a human substantia nigra cDNA library by hybridization screening and showed high homology with the mouse *Slo* gene [4], while a later report on *Slo* immunoreactivity and *in situ* hybridization studies suggested that there are no BK channels in SNc dopaminergic neurons, since neither *Slo* protein nor *Slo* mRNA was detected in rat SNc [15]. However, faint staining in the SNc was detected when *Slo* immunohistological analysis was performed on mouse brain tissue [23]. In addition, a charybdotoxin-sensitive whole-cell current (charybdotoxin is a blocker of calcium-dependent potassium channels including BK channels) and a hypoxia-inhibited potassium channel (~230 pS) were recorded from SNc neurons during a hypoxic/hypoglycemic stimulus [8,12,19]. Further proof of the existence of BK channels in SNc neurons is thus still necessary.

The purpose of this study was to obtain direct electrophysiological evidence of the functional expression and describe single-channel properties of BK channels from freshly isolated mouse SNc dopaminergic neurons.

Experiments were approved by the local animal care committee. C57BL/6 mice (15–20 d, both sexes) were obtained from the Animal Experiment Center of Peking University. After decapitation, whole brains were quickly removed and placed in ice-chilled oxygenated artificial cerebral spinal fluid (ACSF) which contained (in mM): 145 NaCl, 4 KCl, 1 MgCl_2 , 2 CaCl_2 , 10 glucose and 10 HEPES (pH 7.4, adjusted with NaOH). Coronal slices (300 μm thick) containing SNc were obtained with a vibratome (Dosaka, ZERO 1, Japan). The slices were transferred to oxygenated ACSF and incubated for 30 min at room temperature (22–24 °C). Following 10–15 min pronase enzyme treatment at 31 °C, lateral SNc were cut free from the rest of the slice (see Supplementary Materials Fig. S1). Single cells were then mechanically dissociated with glass pipettes. The isolated cells were allowed to settle for 30 min on poly-L-lysine-coated culture dishes, and were then continuously superfused with ACSF at room temperature throughout the entire experimental process and the ACSF solution was bubbled with 100% O_2 .

The inside-out configuration of patch-clamp recording was used to record single-channel currents from BK channels. Data were acquired using an Axopatch 200B patch-clamp amplifier (Axons Instruments, USA) and pClamp 9.0 software (Axon Instruments, USA). Borosilicate capillary glass pipettes (Sutter Instruments, USA) were pulled using a micropipette puller (model P-97, Sutter Instruments, USA), fire-polished using a polisher (2002-A, Yibo, China) and filled with a solution of (in mM) 140 KCl and 10 HEPES (pH 7.2, adjusted with Tris) to obtain a resistance of 3–10 M Ω . The bath solution containing (in mM): 0.1 CaCl_2 , 0.2 MgCl_2 , 140 KCl and 10 HEPES (pH 7.2, adjusted with Tris), and the drug solution (test drugs were

* Corresponding author. Tel.: +86 10 64888534; fax: +86 10 64888534.

E-mail address: jjj@ibp.ac.cn (J.J. Ji).

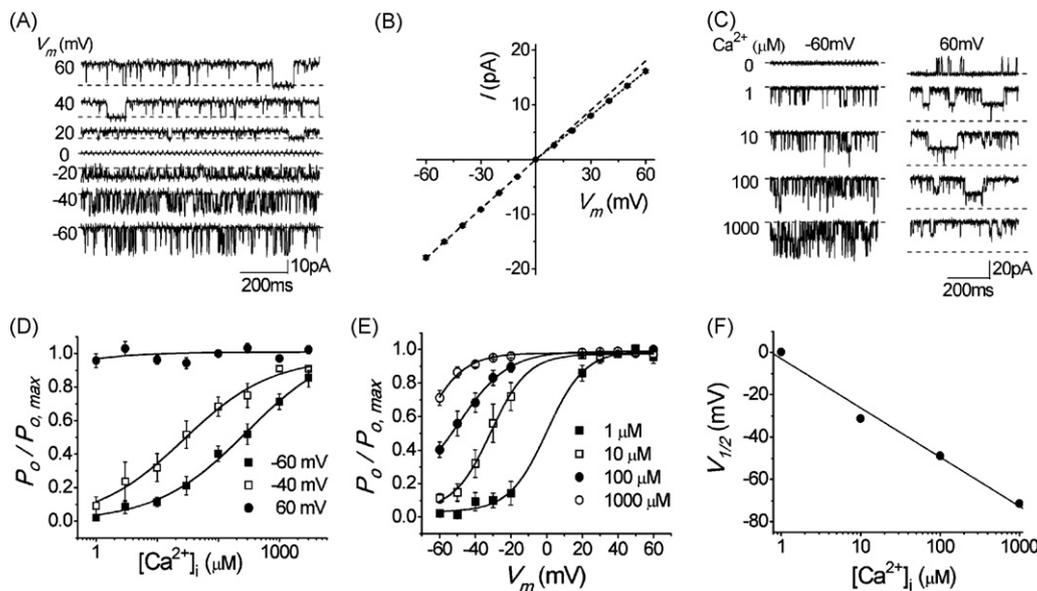


Fig. 1. Basic characteristics of the BK channel in SNc dopaminergic neurons. (A) Single-channel currents recorded from an inside-out patch at various membrane potentials in symmetrical 140 mM K^+ . $[Ca^{2+}]_i$ was 100 μM . Dashed lines represent the closed channel state. (B) I - V relationship obtained in symmetrical K^+ . No activity was visible at 0 mV. Dashed and dotted lines correspond to experimental data from -60 to 0 mV and from 0 to 60 mV, respectively, fitted using linear regression. (C) Single-channel currents recorded from a patch at indicated $[Ca^{2+}]_i$ and -60 mV (left) or 60 mV (right). (D) Relationship between normalized P_o ($P_o/P_{o,max}$) and $[Ca^{2+}]_i$ at -60 , -40 and 60 mV. The data are fitted by the Hill equation. (E) The activation curves for various $[Ca^{2+}]_i$ are best fitted by the Boltzmann equation. (F) Half-activation potential ($V_{1/2}$) for various $[Ca^{2+}]_i$ obtained from the data in (E).

dissolved in bath solution), were administered separately using a silicon tube with a tip diameter of 0.3 mm at a distance of about 3 mm from the patch being recorded. Bath solution was adjusted with Tris or HCl to the various pH values used in pH-sensitivity experiments. Data obtained when channels remained open in the Ca^{2+} -free solution at -60 mV were discarded. Single-channel currents were filtered at 2 kHz with a low-pass four-pole Bessel filter and digitized at 4 kHz using a 12-bit A/D converter (Digidata 1322A, Axon Instruments, USA). Paxilline, TEA, ATP, ADP, AMP, GTP, Tris and HEPES were purchased from Sigma (St. Louis, MO, USA).

All single-channel data were further filtered (200–2000 Hz) with a Gaussian filter before being analyzed using Clampfit 9.0 (Axons Instruments, USA) and Origin 8.0 (OriginLab, Northampton, MA, USA). Open-state probability (P_o) values were calculated with the following equation: $P_o = NP_o/N$, where NP_o is the total open-state probability determined by Clampfit 9.0, and N is the number of channels activated in the patch. Normalized values of P_o were obtained by $P_o/P_{o,max}$ in experiments to determine the voltage- and Ca^{2+} -dependence of channel activity, and by $P_o/P_{o,control}$ in other experiments, where $P_{o,max}$ was the maximum P_o , and $P_{o,control}$ was P_o under control conditions (-60 mV, $[Ca^{2+}]_i$ 100 μM and pH 7.2).

Experimental results are presented as means \pm SE, and n is the number of patches. Statistical significance was calculated with the Student's t test and a p value of <0.05 was considered to be significant.

The density of dopaminergic neurons in lateral SNc is higher than that in other nuclei which are located dorsally and ventrally to lateral SNc [6] and it was shown here in the tyrosine hydroxylase (TH) immunostaining result (see Supplementary Materials Fig. S1D). Thus, we concluded that the dopaminergic neurons we obtained were mainly from the SNc (see Supplementary Materials Fig. S1). In addition, 88% (299/341) of the cells whose body length exceeded 15 μm were TH-positive (see Supplementary Materials Fig. S2) and these neurons were used for further investigations of BK channels from SNc dopaminergic neurons.

When equal concentrations of K^+ (140 mM) were applied to the pipette and bath solutions, large-conductance potassium channel currents were observed in 31.1% (399/1283) of all the successfully

sealed patches and the average number of channels per patch was 2.2 (861/399).

The BK channels of SNc neurons exhibited a slight inward rectification in its current-voltage (I - V) relationship (Fig. 1A and B). The slope conductance was 301 ± 6 pS from -60 to 0 mV and 269 ± 5 pS from 0 to 60 mV ($n = 17$ –21; Fig. 1B); P_o was 0.36 ± 0.05 at -60 mV ($n = 30$) and 0.88 ± 0.06 at 60 mV ($n = 14$), maintaining $[Ca^{2+}]_i$ at 100 μM .

The Ca^{2+} -dependence of the BK channel was examined at various membrane potentials. When V_m was held at -60 mV, increasing the $[Ca^{2+}]_i$ markedly increased channel activity (Fig. 1C). It is worth noting that in the Ca^{2+} -free bath solution the channel was completely inactivated when V_m was held at -60 mV, while at 60 mV the channel opened occasionally (Fig. 1C). The relationship between normalized P_o ($P_o/P_{o,max}$) and $[Ca^{2+}]_i$ was best fitted by the Hill equation. The half-activation concentration and the Hill coefficient were 293.0 μM and 0.6 at -60 mV, 27.1 μM and 0.6 at -40 mV and 0.002 μM and 0.5 at 60 mV ($n = 3$ –30; Fig. 1D).

The voltage-dependence of the BK channel at various $[Ca^{2+}]_i$ is shown in Fig. 1E, in which normalized P_o ($P_o/P_{o,max}$) is plotted as a function of BK voltage at different $[Ca^{2+}]_i$. The curve was fitted with the Boltzmann equation and the values of $V_{1/2}$ were 0.3, -31.2 , -48.8 and -71.4 mV at 1, 10, 100 and 1000 μM of $[Ca^{2+}]_i$, respectively ($n = 4$ –30; Fig. 1F).

Paxilline, a specific BK channel blocker, can be applied both intra- and extra-cellularly [14,22] and is used to confirm BK channels when using the inside-out configuration [2]. Paxilline showed a marked inhibitory effect on the channel as expected (Fig. 2A). 1 and 10 nM paxilline inhibited channel activity by $19 \pm 6\%$ ($n = 5$; $p < 0.005$) and $28 \pm 11\%$ ($n = 3$; $p < 0.05$), respectively, and 1 μM paxilline completely blocked channel activity ($n = 3$, $p < 0.001$, Fig. 2C). Our results thus confirm that the channels we recorded here were BK channels.

A reversible and concentration-dependent decrease in single-channel current amplitude was observed during TEA bath application (Fig. 2B). 2, 10 and 30 mM TEA reduced the channel current amplitude by $4 \pm 1\%$ ($n = 9$; $p < 0.0001$), $10 \pm 1\%$ ($n = 8$; $p < 0.0001$) and $19 \pm 2\%$ ($n = 6$; $p < 0.0001$), respectively (Fig. 2D). TEA

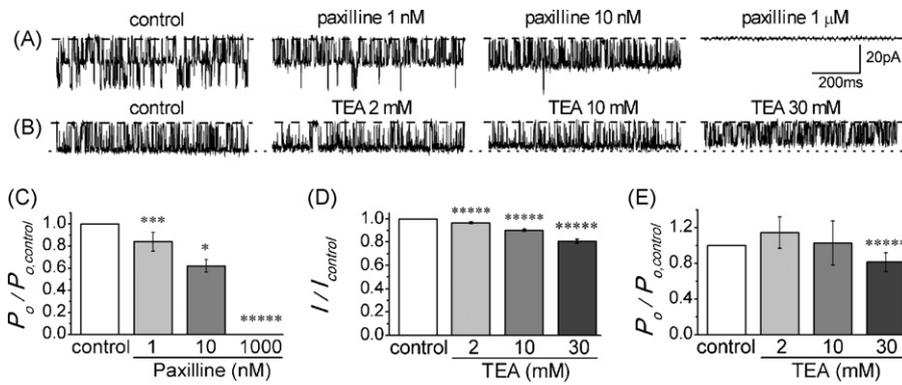


Fig. 2. Effects of blockers on activity and current amplitude of the BK channel. (A and C) Single-channel currents and histogram of normalized P_o ($P_o/P_{o,control}$) with or without different concentrations of paxilline. (B) Single-channel currents recorded from a patch in 0 mM (control), 2, 10 or 30 mM TEA. The dotted line indicates the channel open-state under control conditions. (D) Histogram of normalized $I/I_{control}$ and (E) normalized P_o ($P_o/P_{o,control}$) for different concentrations of TEA. V_m was -60 mV and $[Ca^{2+}]_i$ was $100 \mu M$. * Represents a significant difference compared to values obtained under control conditions at a level of $p < 0.05$; * $p < 0.05$; *** $p < 0.005$; ***** $p < 0.0001$.

at low concentrations (2 and 10 mM) had no effect on the activity of the channel but a decline of $18 \pm 10\%$ ($n = 6$; $p < 0.05$) in P_o was observed at a concentration of 30 mM TEA (Fig. 2E). The product of P_o and channel conductance also decreased by $33 \pm 9\%$ ($n = 6$; $p < 0.0001$) when 30 mM TEA was applied.

Pharmacology experiments were used to detect the ATP-sensitivity of the channel. Fig. 3A displays a typical single-channel recording showing that the channel was inhibited by ATP in

a concentration-dependent manner. 1 and 3 mM ATP inhibited channel activity by $78 \pm 4\%$ ($n = 24$; $p < 0.0001$) and $95 \pm 3\%$ ($n = 7$; $p < 0.0001$), respectively. The concentration of ATP required to half-inhibit channel activity was $305.1 \mu M$ and the Hill coefficient was 0.9 ($n = 7-24$; Fig. 3B).

We also examined the effects of GTP, ADP, AMP and a cocktail of 1 mM ATP and 2 mM ADP. As shown in Figs. 3C and D, 1 and 3 mM GTP inhibited channel activity by $53 \pm 12\%$ ($n = 6$; $p < 0.0005$)

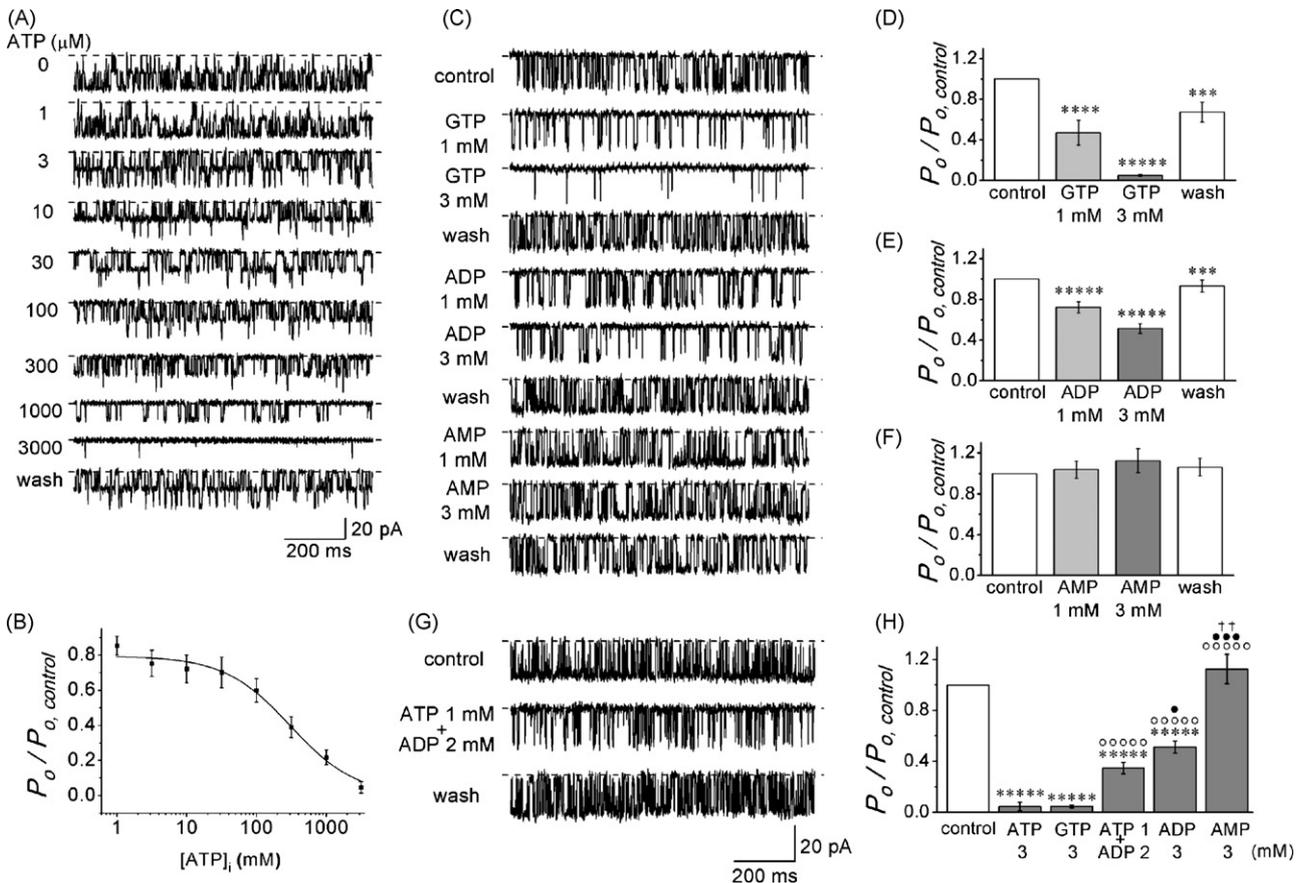


Fig. 3. Effects of intracellular phosphate compounds on BK channel activity. (A) Single BK channel currents obtained from a patch exposed to different concentrations of ATP at -60 mV. (B) Relationship between normalized P_o ($P_o/P_{o,control}$) and $[ATP]_i$, fitted by the Hill equation. (C) Single-channel currents recorded from a patch exposed to 1 or 3 mM of GTP, ADP and AMP. (D–F) Histograms of the effects on channel activity of GTP, ADP and AMP, respectively. (G) Single-channel currents obtained using a cocktail of 1 mM ATP and 2 mM ADP. (H) The effect of nucleotides (3 mM) on activity of the BK channel. V_m was -60 mV and $[Ca^{2+}]_i$ was $100 \mu M$. **, ○, ●, † denote the statistical significance of comparisons with control conditions, 3 mM ATP, the cocktail of 1 mM ATP and 2 mM ADP, and 3 mM ADP, respectively, calculated with the Student's t test. The number of these symbols (1–5) represents $p < 0.05$, 0.01, 0.005, 0.001 and 0.0001, respectively.

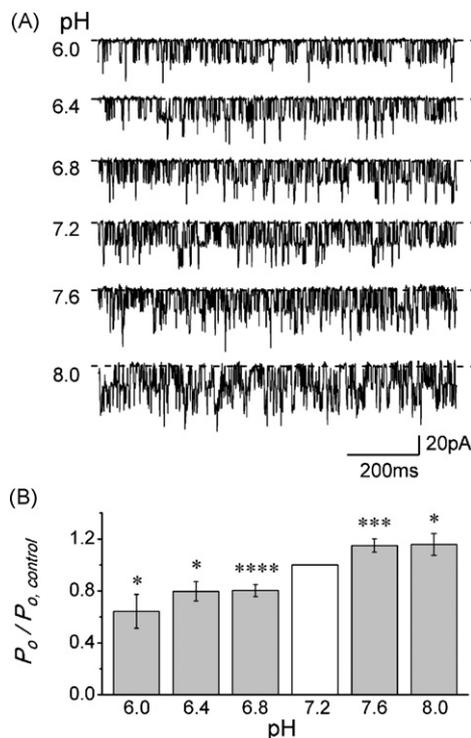


Fig. 4. Effects of pH on the activity of the BK channel. (A) Typical inside-out recordings showing BK channel activity at different $[pH]_i$. (B) Histogram of normalized P_o ($P_o/P_{o,control}$) at different $[pH]_i$. V_m was -60 mV and $[Ca^{2+}]_i$ was 100 μ M. * $p < 0.05$; *** $p < 0.005$; **** $p < 0.001$.

and $95 \pm 1\%$ ($n = 6$; $p < 0.0001$), respectively. ADP also inhibited BK channel activity but was less potent than ATP and GTP (Fig. 3C). 1 and 3 mM ADP reduced BK channel activity by $28 \pm 6\%$ ($n = 18$; $p < 0.0001$) and $49 \pm 5\%$ ($n = 5$; $p < 0.001$), respectively (Fig. 3E). In contrast, neither 1 nor 3 mM AMP showed any significant effects on the channel (Fig. 3C), although the mean P_o appeared to have a slight increase of $4 \pm 9\%$ ($n = 27$; $p > 0.05$) and $13 \pm 12\%$ ($n = 27$; $p > 0.05$), respectively (Fig. 3F). In addition, a cocktail of 1 mM ATP and 2 mM ADP resulted in a $59 \pm 8\%$ ($n = 6$; $p < 0.0001$) decrease in channel activity (Fig. 3G and H). Fig. 3H summarizes the effect of 3 mM adenosine or guanosine nucleotides. The inhibitory effect on channel activity decreased with reductions in high-energy phosphate and the effect of 3 mM GTP was similar to that of the same concentration of ATP. These results indicate that BK channel activity is depressed by high-energy phosphate compounds.

Single-channel currents recorded over a range of $[pH]_i$ are shown in Fig. 4A. Compared to activity at pH 7.2, BK channel activity was increased by $15 \pm 5\%$ ($n = 6$; $p < 0.005$) and $16 \pm 8\%$ ($n = 6$; $p < 0.05$) at pH 7.6 and 8.0, respectively. In contrast, BK channel activity was reduced by $20 \pm 5\%$ ($n = 10$; $p < 0.0005$), $21 \pm 8\%$ ($n = 4$; $p < 0.05$) and $36 \pm 13\%$ ($n = 4$; $p < 0.05$) at pH 6.8, 6.4 and 6.0, respectively (Fig. 4B).

In this study, we show here for the first time the direct single-channel electrophysiological evidence that BK channels are present in mouse SNc dopaminergic neurons. The channels possess typical BK channel characteristics: large-conductance, voltage- and Ca^{2+} -dependence and sensitivity to the specific blocker paxilline. In addition, these BK channels are inhibited by intracellular high-energy phosphate compounds including ATP, GTP and ADP, and were also sensitive to the pH of the intracellular solution.

The voltage- and Ca^{2+} -dependence of BK channels in SNc dopaminergic neurons are closest to those of BK channel complex composed of α and β_4 subunits, based on previous studies with cloned subunits [1,9,25]. Furthermore, the immunostaining

intensity of the β_4 subunit is strong in SNc [20]. Therefore, the BK channels recorded here were probably comprised of α and β_4 subunits.

We compared properties of the BK channels in SNc dopaminergic neurons with those in other brain regions. Firstly, the conductance (301 pS) of BK channels in SNc was higher than the largest conductance that reported in other brain regions such as the cortex (277 pS) [13], hippocampus (266 pS) [11] and olfactory bulb (270 pS) [5], while it was similar to that in human meningioma cells (296 pS) [16]. Secondly, BK channels in SNc dopaminergic neurons showed a slight but evident inward rectification as has also been reported for BK channels of the olfactory bulb neurons [5]. Thirdly, the concentration of 2 mM TEA is enough to show inhibition on the channel current amplitude and 30 mM TEA inhibits the activity of BK channels in SNc. Thus, the sensitivity to TEA of BK channels in SNc is normal [13,26]. Fourthly, the mechanism of the inhibition of BK channels in SNc dopaminergic neurons by high-energy phosphate compounds may involve non-chelatory effects in addition to chelation of Ca^{2+} . We found that the normalized P_o values (relative to P_o of control) obtained during the application of the drug solutions of high-energy phosphate compounds were lower than the normalized P_o values at the concentrations of free Ca^{2+} remaining in the relevant test solutions (calculated using Maxchelator at <http://maxchelator.stanford.edu> with the data from Fig. 1D at -60 mV). Thus, excluding the chelation of Ca^{2+} , ATP inhibited BK channels in SNc, as in rat ventromedial hypothalamic and suprachiasmatic nucleus neurons [10,24], contrary to its effect in most brain tissues. Finally, BK channel activity in SNc dopaminergic neurons declined as intracellular pH value decreased (range 8.0–6.0), similar to results reported for other brain tissues, such as cortical [17] and hippocampus neurons [3]. Taken together, while each of the characteristics of SNc BK channels reported here has been described in other tissues, a channel with this combination of characteristics has not been reported before, suggesting that SNc BK channels may have a different assembly pattern of functional domains.

The fact that faint Slo protein immunoreactivity can be detected in mouse SNc [23] but that neither Slo protein nor Slo mRNA can be detected in rat SNc [15], may be attributed to differences in species or age of the experimental animals used. Another probable reason is the low expression of BK channels in SNc, which leads to problems in their detection at the molecular level. In addition, the possibility that the BK channel in SNc does not possess the protein domain that was used to raise specific antibodies in the immunohistochemical and molecular biology detections carried out in the reports discussed above cannot be excluded.

In conclusion, our results suggest that SNc BK channels can be regulated by several factors, such as Ca^{2+} , V_m , ATP and pH, and thus probably play important roles in modulating excitability of and dopamine release from SNc dopaminergic neurons in different metabolic phases, according to that happens in other tissues [7,21]. These results suggest that BK channels may be involved in cellular responses to metabolic stress in SNc dopaminergic neurons as well as the possibility that the BK channel is a target for modulating neurons during metabolic change.

Acknowledgements

We thank Lianwan Chen for TH immunostaining technical assistance and Dr. Joy Fleming and Dr. Shuyun Bai for critical reading of the manuscript. This work was supported by grants from the 100 Talents Program of the Chinese Academy of Sciences, the National Key Basic Research and Development Program ("973" projects 2005CB522804 and 2006CB504101), the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-217), and the National Natural Science Foundation of China (30370459).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2009.12.058.

References

- [1] R. Brenner, T.J. Jegla, A. Wickenden, Y. Liu, R.W. Aldrich, Cloning and functional characterization of novel large conductance calcium-activated potassium channel β subunits, hKCNMB3 and hKCNMB4, *J. Biol. Chem.* 275 (2000) 6453–6461.
- [2] H. Chapman, C. Piggot, P.W. Andrews, K.T. Wann, Characterisation of large-conductance calcium-activated potassium channels (BK_{Ca}) in human NT2-N cells, *Brain Res.* 1129 (2007) 15–25.
- [3] J. Church, K.A. Baxter, J.G. McLarnon, pH modulation of Ca²⁺ responses and a Ca²⁺-dependent K⁺ channel in cultured rat hippocampal neurones, *J. Physiol.* 511 (Pt 1) (1998) 119–132.
- [4] S.I. Dworetzky, J.T. Trojnacki, V.K. Gribkoff, Cloning and expression of a human large-conductance calcium-activated potassium channel, *Brain Res. Mol. Brain Res.* 27 (1994) 189–193.
- [5] T.M. Egan, D. Dagan, I.B. Levitan, Properties and modulation of a calcium-activated potassium channel in rat olfactory bulb neurons, *J. Neurophysiol.* 69 (1993) 1433–1442.
- [6] J.H. Fallon, S.E. Loughlin, *The Rat Nervous System*, second ed., Academic Press, Inc, California, 1995, p. 220, chapter 12: Substantia Nigra.
- [7] N. Furukawa, T. Takasusuki, T. Fukushima, Y. Hori, Presynaptic large-conductance calcium-activated potassium channels control synaptic transmission in the superficial dorsal horn of the mouse, *Neurosci. Lett.* 444 (2008) 79–82.
- [8] E. Guatteo, M. Federici, A. Siniscalchi, T. Knopfel, N.B. Mercuri, G. Bernardi, Whole cell patch-clamp recordings of rat midbrain dopaminergic neurons isolate a sulphonylurea- and ATP-sensitive component of potassium currents activated by hypoxia, *J. Neurophysiol.* 79 (1998) 1239–1245.
- [9] T.S. Ha, M.S. Heo, C.S. Park, Functional effects of auxiliary β 4-subunit on rat large-conductance Ca²⁺-activated K⁺ channel, *Biophys. J.* 86 (2004) 2871–2882.
- [10] A.C. Hall, R.M. Hoffmaster, E.L. Stern, M.E. Harrington, D. Bickar, Suprachiasmatic nucleus neurons are glucose sensitive, *J. Biol. Rhythms* 12 (1997) 388–400.
- [11] Y. Ikemoto, K. Ono, A. Yoshida, N. Akaike, Delayed activation of large-conductance Ca²⁺-activated K channels in hippocampal neurons of the rat, *Biophys. J.* 56 (1989) 207–212.
- [12] C. Jiang, G.G. Haddad, Oxygen deprivation inhibits a K⁺ channel independently of cytosolic factors in rat central neurons, *J. Physiol.* 481 (Pt 1) (1994) 15–26.
- [13] C. Jiang, G.G. Haddad, Modulation of K⁺ channels by intracellular ATP in human neocortical neurons, *J. Neurophysiol.* 77 (1997) 93–102.
- [14] H.G. Knaus, O.B. McManus, S.H. Lee, W.A. Schmalhofer, M. Garcia-Calvo, L.M. Helms, M. Sanchez, K. Giangiacomo, J.P. Reuben, A.B. Smith III, G.J. Kaczorowski, M.L. Garcia, Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels, *Biochemistry* 33 (1994) 5819–5828.
- [15] H.G. Knaus, C. Schwarzer, R.O. Koch, A. Eberhart, G.J. Kaczorowski, H. Glossmann, F. Wunder, O. Pongs, M.L. Garcia, G. Sperk, Distribution of high-conductance Ca²⁺-activated K⁺ channels in rat brain: targeting to axons and nerve terminals, *J. Neurosci.* 16 (1996) 955–963.
- [16] R. Kraft, K. Benndorf, S. Patt, Large conductance Ca²⁺-activated K⁺ channels in human meningioma cells, *J. Membr. Biol.* 175 (2000) 25–33.
- [17] K. Lee, I.C. Rowe, M.L. Ashford, Characterization of an ATP-modulated large conductance Ca²⁺-activated K⁺ channel present in rat cortical neurons, *J. Physiol.* 488 (Pt 2) (1995) 319–337.
- [18] B.Y. Ma, J.J. Ji, Structure and function of the BK_{Ca} channel, *Acta Biophys. Sinica* 23 (2007) 11–19.
- [19] S. Marinelli, G. Bernardi, P. Giacomini, N.B. Mercuri, Pharmacological identification of the K⁺ currents mediating the hypoglycemic hyperpolarization of rat midbrain dopaminergic neurones, *Neuropharmacology* 39 (2000) 1021–1028.
- [20] M. Piwonska, E. Wilczek, A. Szewczyk, G.M. Wilczynski, Differential distribution of Ca²⁺-activated potassium channel β 4 subunit in rat brain: immunolocalization in neuronal mitochondria, *Neuroscience* 153 (2008) 446–460.
- [21] L. Salkoff, A. Butler, G. Ferreira, C. Santi, A. Wei, High-conductance potassium channels of the SLO family, *Nat. Rev. Neurosci.* 7 (2006) 921–931.
- [22] M. Sanchez, O.B. McManus, Paxilline inhibition of the alpha-subunit of the high-conductance calcium-activated potassium channel, *Neuropharmacology* 35 (1996) 963–968.
- [23] U. Sausbier, M. Sausbier, C.A. Sailer, C. Arntz, H.G. Knaus, W. Neuhuber, P. Ruth, Ca²⁺-activated K⁺ channels of the BK-type in the mouse brain, *Histochem. Cell Biol.* 125 (2006) 725–741.
- [24] J.M. Treherne, M.L. Ashford, Calcium-activated potassium channels in rat dissociated ventromedial hypothalamic neurons, *J. Neuroendocrinol.* 3 (1991) 323–329.
- [25] B. Wang, B.S. Rothberg, R. Brenner, Mechanism of β 4 subunit modulation of BK channels, *J. Gen. Physiol.* 127 (2006) 449–465.
- [26] Y. Wang, D.A. Mathers, Ca²⁺-dependent K⁺ channels of high conductance in smooth muscle cells isolated from rat cerebral arteries, *J. Physiol.* 462 (1993) 529–545.