Cholinergic EPSCs and their potentiation by bradykinin in single paratracheal ganglion neurons attached with presynaptic boutons

Jian-Rong Zhou,1 Tetsuya Shirasaki,2 Fumio Soeda,2 and Kazuo Takahama2

1Laboratory of Presymptomatic Medical Pharmacology, Faculty of Pharmaceutical Sciences, Sojo University, Kumamoto, Japan; and 2Department of Environmental and Molecular Health Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

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Zhou JR, Shirasaki T, Soeda F, Takahama K. Cholinergic EPSCs and their potentiation by bradykinin in single paratracheal ganglion neurons attached with presynaptic boutons. J Neurophysiol 112: 933–941, 2014. First published May 28, 2014; doi:10.1152/jn.00055.2014.—We have found that bradykinin (BK) potentiates the nicotine-induced currents in airway paratracheal/parabronchial ganglia (PTG) neurons. In this study, we investigated if BK affects the cholinergic synaptic transmission in rat PTG neurons attached with synaptic buttons. Excitatory postsynaptic currents (EPSCs) were recorded in acutely dissociated PTG neurons attached with presynaptic boutons. EPSC frequency was increased in the high-K+ external solution without affecting their amplitude. Activation and deactivation kinetics also did not change in the high-K+ solution. Cd2+ inhibited the EPSC frequency at 10−7 M and also amplitude at higher concentrations without changing the kinetics. Mecamylamine inhibited both the amplitude and frequency of EPSCs and reduced the activation and deactivation kinetics. 10−8 M BK potentiated the EPSC amplitude to 1.37 ± 0.19 times of preappraisal control. In addition, its frequency was increased to 2.04 ± 0.41 times. BK did not affect the activation and deactivation kinetics. The effects of BK were mimicked by [Hyp1]-BK, a B2 kinin receptor agonist, whereas HOE 140, a B2 kinin receptor antagonist, abolished the effects of BK. In conclusion, BK potentiates the cholinergic synaptic transmission via B2 kinin receptors in the PTG. Since predominant control of airway function is thought to be exerted by cholinergic nerves arising from the PTG, the present findings might underline at least partly the inflammatory pathological conditions of the lower airway.

PREDOMINANT CONTROL OF AIRWAY function is thought to be exerted by cholinergic nerves arising from the paratracheal/parabronchial ganglia (PTG) that are situated on the serosal surface of the dorsal tracheal wall. PTG neurons seem not to be simple parasympathetic relay neurons. Firstly, the collateral branches of tachykinin-containing C fibers project to the PTG neurons (Kummer et al. 1992) and their excitability can be modulated by a peripheral reflex via sensory C fibers (Canning et al. 2002). Secondly, inflammatory mediators may directly modulate the function of PTG neurons because proinflammatory substances and antigen exposure affect their function (Kajekar and Myers 2000; Kajekar et al. 2003; Ito et al. 2000; Myers and Undem 1993; Myers and Undem 1995). If this is the case, it seems likely that alteration of the function of PTG neurons by inflammatory mediators underlies the induction and/or maintenance of pathological conditions accompanied by airway inflammation. Thus the PTG are thought to be not only integrative sites for the neuromodulation of normal airway function but also important sites for pathogenesis in airway inflammation associated with airway diseases.

Bradykinin (BK) is a potent inflammatory peptide that has been implicated as a potential mediator of human airway diseases. In the lower airway, BK induces bronchoconstriction, mucus secretion, microvascular leakage, and cough (Barnes 1992). The mechanisms underlying BK-induced actions in the airway are considered to be largely indirect and to occur primarily through airway nerve activation (Ellis and Fozard 2002). BK stimulates afferent C fibers (Carr et al. 2003) and causes cholinergic reflex via brainstem neurons. We previously reported that BK activates B2 kinin receptors and induces the action potential generation via the inhibition of the M-type K+ current (M) in acutely dissociated PTG neurons (Mochidome et al. 2001). We also found that BK potentiated nicotine-induced currents via the B2 kinin receptor, pertussis toxin (PTX)-sensitive G protein, and phospholipase C pathway in dissociated PTG neurons (Zhou et al. 2006). However, nicotinic acetylcholine receptors are present not only in the synapse but also in the extrasynaptic sites in autonomic ganglia (Wilson Horch et al. 1996). Therefore, it is still unknown if BK potentiates cholinergic synaptic transmission or not. In this study, we tried to dissociate single neurons attached with presynaptic buttons from rat PTG and record excitatory postsynaptic currents (EPSCs) in their neurons. Then, we clarified their pharmacological properties and investigated the modulatory effect of BK on the EPSCs to elucidate the roles of BK on the cholinergic synaptic transmission under airway inflammation.

METHODS

Ethical approval. All experiments were conducted in strict accordance with the Guidelines of the Japanese Pharmacological Society and Kumamoto University for the Care and Use of Laboratory Animals. These protocols were submitted to and approved by an independent review committee.

Dissociation of paratracheal ganglia neurons attached with presynaptic boutons. PTG were at first taken out with the trachea from 12- to 18-day-old male/female Wistar rats (Kyudo, Fukuoka, Japan) that were under pentobarbital anesthesia (50 mg/kg ip), and then the PTG were detached from the trachea under stereomicroscope. The data presented in this study are derived from 20 rat litters, with each litter comprising ~12 pups. The PTG detached were then subjected to a mild enzyme digestion, that is, the ganglia were treated with 0.3% collagenase and 0.3% trypsin dissolved in the normal external solution (see below for composition) for 20 min at 34°C. Thereafter, the PTG

Address for reprint requests and other correspondence: J.-R. Zhou, Laboratory of Presymptomatic Medical Pharmacology, Faculty of Pharmaceutical Sciences, Sojo Univ., 4-22-1 Ikeda, Nishi-ku, Kumamoto 860-0082, Japan (e-mail: zhourj@ph.sojo-u.ac.jp).
were mechanically and gently dissociated to get single PTG neurons attached with synaptic boutons with fine pipettes in the normal external solution. Considering the remaining adhering tissue, such as adhering buttons or segments of PTG neurons, these isolated neurons would be difficult to achieve GQ seals with patch pipettes. Then, dissociated neurons were incubated in the normal external solution for 15–30 h at 20°C. Thus the enzyme effect might be minimized, and the alive neurons would be better for the patch-clamp recording.

Electrophysiological recordings. Electrical measurements were performed by using the nystatin-perforated patch-clamp recording technique (Horn and Marty 1988; Akaike and Harata 1994). The resistance of electrode for the patch-clamp recording was 5–8 MΩ. Synaptic currents were recorded with an Axopatch-200B patch-clamp amplifier (Molecular Devices, Union City, CA) and were acquired with Clampex 9 data acquisition software (Molecular Devices) after digitization with a Digidata 1200B (Molecular Devices). The signals were filtered at 1 kHz using a facility of the amplifier and sampled at 5 kHz. All experiments were performed at room temperature (21–24°C). Recordings were conducted in the neurons that had resting membrane potentials of −55 mV or less.

Data analysis was performed with Clampfit 9 (Molecular Devices), Origin 7.5 (Microcal, Northampton, MA), and/or Excel 2003 (Microsoft, Redmond, WA). The results are given as means ± SE. The cumulative probability distributions on interevent interval and peak amplitude of spontaneous EPSCs were analyzed within individual cells using the Kolmogorov-Smirnov test. Statistical significance across the neuron groups was determined by the use of paired or unpaired t-test. *P < 0.05 was considered significant.

Solution and chemicals. The ionic composition of the normal external solution was the following (in mM): 131.7 NaCl, 5 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 10 HEPES, and 11.5 glucose. The pH was adjusted to 7.4 with NaOH. For the 30-mM K⁺ external solution, 25 mM NaCl were replaced by equimolar KCl. The composition of the patch pipette solution was the following (in mM): 80 KCl, 70 K-gluconate, and 10 HEPES. The pH was adjusted to 7.2 with KOH. Nystatin was dissolved in pure methanol and added to the internal solution at a final concentration of 400 μg/ml just before use. A rapid application of the external solution was performed with the “Y-tube” technique (Murase et al. 1990) that allows the complete exchange within 30 ms.

BK, [Hyp³]-BK, and d-arginyl-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]-BK (HOE 140) were purchased from the Peptide Institute (Osaka, Japan). Collagenase, nystatin, and trypsin were purchased from Sigma (St. Louis, MO). All reagents except nystatin were dissolved in the normal external solution at the optimum concentrations.

Fig. 1. Spontaneous transient inward currents in dissociated paratrabecular/parabronchial ganglia (PTG) neurons. A: representative records showing spontaneous transient inward currents in the 5-, 20-, and 30-mM K⁺ external solutions at a holding potential of −60 mV. B: distribution of spontaneous transient inward current amplitude in the normal and high-K⁺ external solutions. C: representative cumulative probability plots for interevent interval and amplitude of transient currents. Plots were obtained from the records partly shown in A. Circle and triangle indicate the results recorded in the 5- and 30-mM K⁺ solutions, respectively. D: effect of increasing in external K⁺ concentration on amplitude and frequency of spontaneous currents. The amplitude and frequency of spontaneous currents at each concentration were normalized to the mean amplitude and frequency of spontaneous currents recorded during pretest in the 5-mM K⁺ solutions, respectively. Data are shown as means ± SE (n = 3 to 5). *P < 0.05 vs. 5 mM K⁺. E: analysis of activation and deactivation kinetics of transient inward currents. a: Averaged current traces in the 5- and 30-mM K⁺ solutions. Representative traces were obtained from the records partly shown in A. Activation and deactivation phases of averaged traces were well fitted with single exponential function, respectively (white and gray traces). b: Time constants of activation and deactivation kinetics of averaged traces in the 5- and 30-mM K⁺ solutions (n = 5).
RESULTS

Transient inward currents in dissociated single PTG neurons with synaptic boutons. In neurons mechanically dissociated after gentle enzyme treatment, spontaneous transient inward currents were recorded at a holding potential of −60 mV in normal external solution containing 5 mM K+ (Fig. 1A). The frequency of transient inward currents was increased when K+ concentration in the external solution was increased to 20 and 30 mM (Fig. 1A). The increase in frequency was statistically significant, whereas the peak amplitude were almost the same at each external K+ concentration (Fig. 1, C and D). In the 30-mM K+ external solution, the cumulative probability distribution of interevent interval, but not the peak amplitude, was shifted to the left (Fig. 1C). The difference was significant in Kolmogorov-Smirnov test (P < 0.0001). Transient currents recorded in 5- and 30-mM K+ external solutions were, respectively, averaged and their activation and deactivation kinetics were analyzed (Fig. 1E). When activation and deactivation phases of current traces were fitted with exponential curves, both activation and deactivation phases were well fitted with the following single exponential function in Eqs. 1 and 2, respectively.

\[
I = I_{\text{peak}} e^{-\tau_1} + C_a \quad (1)
\]

\[
I = I_{\text{peak}} (1 - e^{-\tau_2}) + C_b \quad (2)
\]

where \(I\) is the absolute current level, \(I_{\text{peak}}\) the peak current amplitude, \(\tau\) relative time, \(\tau_1\) time constant, and \(C_a\) and \(C_b\) constants related to the basal current level. From the curve fitting, the activation and deactivation time constants were given as 1.68 ± 0.19 and 15.94 ± 0.96 ms in the 5-mM K+ solution.

**Fig. 2.** Effect of Ca²⁺ channel antagonist on spontaneous transient inward currents in the 30-mM K⁺ external solution. A: representative record showing the effect of Cd²⁺ on spontaneous transient inward currents. At first, the 30-mM K⁺ external solution was applied for 1 min as a precontrol. After a washout for 4 min with the 5-mM K⁺ solution, the neuron was pretreated with 10⁻⁶ M CdCl₂ for 20 s in the 5-mM K⁺ solution and then treated with it in the 30-mM K⁺ solution for 1 min. After control was recorded in the 30-mM K⁺ solution after a 5-min washout with the 5-mM K⁺ solution. B: concentration dependent inhibition by Cd²⁺ of amplitude and frequency of spontaneous transient inward current. The amplitude and frequency of spontaneous currents in the presence of 10⁻⁷, 10⁻⁶, or 10⁻⁴ M Cd²⁺ were normalized to the mean amplitude and frequency recorded in precontrol, respectively. Data are shown as means ± SE (n = 3). **P < 0.01 vs. individual control in the absence of Cd²⁺.

C: activation and deactivation kinetics of transient inward currents. a: Averaged current traces in the absence or presence of 10⁻⁶ M Cd²⁺. Representative traces were obtained from the records partly shown in A. Activation and deactivation phases were well fitted with single exponential function, respectively (white and gray traces). b: Time constants of activation and deactivation kinetics of averaged traces. Data are shown as means ± SE (n = 3).
solutions, respectively. In the 30-mM K⁺ solutions, these values were 1.57 ± 0.18 and 16.34 ± 1.76 ms, respectively, and were not significantly different from those in the 5-mM K⁺ solutions.

Cd²⁺, an inorganic Ca²⁺ channel blocker, concentration dependently reduced both the amplitude and frequency of the transient inward current in the 30-mM K⁺ external solution, although it only affected frequency at 10⁻⁷ M (Fig. 2, A and B). Activation and deactivation kinetics were not affected by Cd²⁺ (Fig. 2C). Activation and deactivation time constants were 1.14 ± 0.14 and 17.51 ± 1.01 ms in control, respectively, and 1.15 ± 0.12 and 18.13 ± 0.8 ms in the presence of 10⁻⁶ M Cd²⁺.

Mecamylamine, a nicotinic acetylcholine receptor antagonist, also inhibited the transient inward current in the 30-mM K⁺ external solution (Fig. 3). Mecamylamine at 10⁻⁵ M shifted the distribution of amplitude to the left (Fig. 3B) and the distribution of the event interval to the right (data not shown). The differences were significant in Kolmogorov-Smirnov test (P < 0.0001). Relative mean amplitude and frequency of transient inward currents were 0.68 ± 0.1 and 0.36 ± 0.1 times of control, respectively (Fig. 3C). In addition, mecamylamine significantly fastened both the activation and deactivation kinetics (Fig. 3D). Activation phase in the presence of mecamylamine was well fitted with the function in Eq. 1 described above. Activation time constants in the absence and presence of mecamylamine were 1.48 ± 0.08 and 0.63 ± 0.06 ms, respectively. On the other hand, the deactivation phase in the presence of mecamylamine was well fitted with following double exponential function.

\[ I = \sum_{i=1}^{3} I_i \cdot (1 - e^{-\tau_i}) + C \]  

where I is the absolute current level, Iᵢ peak current amplitude components, τᵢ time constants, and C constant. Deactivation time constant in the control was 16.89 ± 1.65 ms, and time constants in the presence of mecamylamine were 5.84 ± 0.53 and 15.58 ± 0.68 ms.

The results suggest that the transient inward current recorded is cholinergic fast EPSCs.

**Potentiation of EPSCs by BK.** Neurons were pretreated with 10⁻⁸ M BK for 20 s in the 5-mM K⁺ external solution and then BK 10⁻⁸ M was applied for 1 min in the 30-mM K⁺ external solution. In the 30-mM K⁺ external solution, BK increased the EPSC amplitude and its frequency to 1.37 ± 0.2 and 2.04 ± 0.4 times of those recorded before BK treatment, respectively (Fig. 4, A and B). BK shifted the cumulative distribution of interevent interval to the left and that of amplitude to the right (Fig. 4C). The differences were significant in Kolmogorov-Smirnov test (P < 0.0001). Pretreatment of BK for 60 s gave

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**Fig. 3.** Effects of nicotinic acetylcholine receptor antagonist on excitatory postsynaptic currents (EPSCs) in the 30-mM K⁺ external solution. A: representative record showing the effect of 10⁻⁵ M mecamylamine on EPSCs. At first, the 30-mM K⁺ external solution was applied for 1 min as a precontrol. After a washout with the 5-mM K⁺ external solution for 4 min, the neuron was pretreated with 10⁻⁵ M mecamylamine for 20 s in the 5-mM K⁺ external solution and then treated with 10⁻⁵ M mecamylamine for 1 min in the 30-mM K⁺ external solution. B: cumulative probability plot for EPSC amplitude. Plots were obtained from the record partly shown in A. Circle and triangle indicate the plots obtained from the records in the absence and presence of 10⁻⁵ M mecamylamine, respectively. C: effect of 10⁻⁵ M mecamylamine on amplitude and frequency of spontaneous transient inward current. The amplitude and frequency of spontaneous currents in the presence of 10⁻⁵ M mecamylamine were normalized to the mean amplitude and the frequency recorded in precontrol, respectively. D: effect of mecamylamine on activation and deactivation kinetics of transient inward current. a: Averaged current traces recorded in the absence and presence of 10⁻⁵ M mecamylamine. Representative traces were obtained from the records partly shown in A. Activation phases were well fitted with single exponential function, respectively (white traces). On the other hand, deactivation phase in the presence of mecamylamine was fitted with double exponential functions (gray trace). b: Time constants of rising and decaying kinetics of averaged traces in the absence and presence of 10⁻⁵ M mecamylamine. Data are shown as means ± SE (n = 3). *P < 0.05, **P < 0.01 vs. individual control in the absence of mecamylamine.
similar effects on the EPSCs (data not shown). At 10^{-7} M BK, an increase in EPSC amplitude was similar to that at 10^{-8} M BK. However, its frequency was a little more increased (Fig. 4D). In the presence of BK, both the activation and deactivation phases of EPSC were well fitted with the function in Eqs. 1 and 2, respectively. BK did affect neither the activation nor deactivation kinetics of EPSC (Fig. 4E). Activation and deactivation time constants were 1.52 \pm 0.07 and 14.49 \pm 0.83 ms, respectively, in the absence of BK and 1.60 \pm 0.24 and 14.90 \pm 0.86 ms in the presence of BK.

\[ \text{[Hyp}^3\text{]-BK, a B}_2\text{ kinin receptor agonist, had similar effects on EPSC at 10}^{-6}\text{ M (Fig. 5). In the presence of [Hyp}^3\text{]-BK, relative mean amplitude and frequency of the EPSC were 1.49 \pm 0.36 and 2.16 \pm 0.44 times of those recorded before [Hyp}^3\text{]-BK treatment, respectively. [Hyp}^3\text{]-BK also did not affect the activation and deactivation kinetics of EPSC (Fig. 5C). In the normal solution, the activation and deactivation time constants of EPSCs were 1.70 \pm 0.21 and 17.19 \pm 0.47 ms, respectively and 1.53 \pm 0.01 and 18.66 \pm 0.28 ms in the presence of [Hyp}^3\text{]-BK.]

On the other hand, the potentiation of EPSCs by BK was abolished by 10^{-6} M HOE 140, a B_2 kinin receptor antagonist (Fig. 6). HOE 140 itself did affect neither EPSC amplitude nor its frequency. Relative mean EPSC amplitude and its frequency in the presence of HOE 140 and BK were 0.95 \pm 0.08 and 1.04 \pm 0.03 times of those recorded before treatment of them, respectively. These values were not significantly different from the control in the absence of BK and HOE 140.

**DISCUSSION**

In this study, we treated PTG with enzyme at a low concentration and then gently trituated. This protocol led to a success in preparing single PTG neurons attached with intact presynaptic buttons (terminals). Furthermore, we succeeded in recording EPSCs from the synaptic button-attached single PTG neurons by the patch-clamp technique. To our knowledge, this is the first documentation about physiology and pharmacology of the synaptic button-attached single PTG neurons, although a dissociation technique of the single PTG neurons with synaptic buttons has been briefly described by Ishibashi and Umezu (2003 in Japanese).

It was impossible to confirm whether or not dissociated single neurons have synaptic buttons under stereoscope with 400-fold magnification. However, spontaneously inwardly fluctuating currents were recorded from the neurons in the normal external solution possessing 5 mM K^+ when a patch electrode was applied to the neurons. The frequency of the
currents was increased with increasing external $K^+$ concentration from 5 to 20 mM. Since presynaptic terminals are out of voltage clamp, the adherent terminals should be depolarized in the high-$K^+$ external solution, followed by an increase in $Ca^{2+}$ concentration in the terminal, facilitating the release of neurotransmitters from the terminal. In support of this insistence, $Cd^{2+}$, an inorganic $Ca^{2+}$ channel blocker, which blocks $Ca^{2+}$ influx to the presynaptic terminals, reduced the frequency of this transient current without affecting its amplitude. $Cd^{2+}$ is known to inhibit other receptor/channel/exchanger proteins including nicotinic receptor. However, much higher concentrations are needed to do so (Nutter and Adams 1995; Rychkov et al. 1997; Hobai et al. 1997; Tomaselli et al. 1995; Waters and Allen 1998). The concentration of $Cd^{2+}$ used in the present study was comparative with that for the inhibition of high-voltage-activated $Ca^{2+}$ channels (Kasai and Neher 1992) and rather similar to that for the inhibition of acetylcholine release from presynaptic terminals of skate electric organ (Richardson et al. 1996). Remarkably, $Cd^{2+}$ and the high-$K^+$ solution did not affect the activation and deactivation kinetics of the transient current at all. Time constants of the activation and deactivation kinetics were similar to those in other ganglion neurons (Skok et al. 1998; Mac Dermott et al. 1980; Shen and Horn 1998; Kertser et al. 1998; Ullian and Barres 1998; Zhang et al. 1996; Selyanko et al. 1979). Taken altogether, it is confirmed that the currents recorded from the single PTG neurons attached with synaptic buttons are spontaneous EPSCs. It has been reported that the activities of spontaneous EPSCs reflect not only transmitter release and postsynaptic responses but also the conduction of impulses in the neuronal network formed in the preparations (Kitamura et al. 2003). Since $10^{-5}$ M mecamylamine significantly inhibited EPSCs, the EPSCs must be nicotinic. According to the previous report, mecamylamine ($10^{-8}–10^{-4}$ M) inhibits nicotine-induced current with an IC$_{50}$ of $1.7 \times 10^{-6}$ M in dissociated PTG neurons (Kanemoto et al. 2002), and the currents are fully blocked by mecamylamine at a concentration larger than $10^{-4}$ M. In addition, 50 $\mu$M mecamylamine reduced cholinergic EPSC amplitudes by 33% in central nicotinic receptors (Chu et al. 2000), and 10–20 $\mu$M mecamylamine blocked 62–79% of the EPSC in other ganglionic nicotinic receptors (Nong et al. 1999). Thus the inhibitions by mecamylamine on EPSCs and nicotine-induced currents are comparative.

Both activation and deactivation kinetics of the EPSCs recorded were well fitted with single exponential function, suggesting that the subunit composition contributing synaptic transmission might be monoclass. Although double exponential decay is common in many parasympathetic ganglion neurons (Bobryshev and Skok 2002; Zhou et al. 1999; Kerster et al. 1998; Ullian et al. 1997; Minota et al. 1989; Yawo 1989; Dun et al. 1986; Rang 1981), such single exponential decay has been reported in several other ganglion neurons (Taylor et al. 1995; Marshall 1986; Connor and Parsons 1983). This difference might be caused by the difference of subunit composition or the number of receptor subtypes existing in the postsynaptic site (Ullian et al. 1997). Rapid component of the current recorded in the presence of mecamylamine (Fig. 3) seems to reflect channel block, because mecamylamine acts as an open channel blocker (Shen and Horn 1998; Nelson and Lindstrom 1999). Single channel analysis is needed to prove this interpretation in the future.

In the current study, we found that BK potentiates the spontaneous EPSCs. BK potentiation of the EPSCs was found both in the amplitude and frequency. Interestingly, BK discriminatively potentiated both the amplitude and frequency of the EPSCs. BK at $10^{-8}$ M potentiated the amplitude of the EPSCs, although the increased concentration failed to potenti-
ate the amplitude more. On the other hand, BK at $10^{-8}$ to $10^{-7}$ M concentration dependently increased the frequency of the EPSCs. Previously, we reported that BK augmented nicotine-induced currents ($I_{\text{Nic}}$) in PTG neurons, suggesting the post-synaptic augmentation of $I_{\text{Nic}}$ by bath-applied BK. The current results suggest that very low concentrations of BK potentiated the spontaneous EPSC at not only the postsynaptic site but also the presynaptic site in synaptic-button attached single PTG neurons, favoring more at the presynaptic site.

The effect of BK on airway nerves is thought to be mediated by B2 kinin receptors (Reynolds et al. 1999). In our study, both the effects of BK on the amplitude and frequency of EPSCs were blocked by HOE 140, a B2 kinin receptor antagonist and mimicked by [Hyp3]-BK, a B2 kinin receptor agonist. Therefore, it is likely that the B2 kinin receptors mediate the BK-induced potentiation of cholinergic transmission at both pre- and postsynaptic site. We previous reported that the B2 kinin receptors in PTG neurons may activate dual signal transduction pathways, the PTX-sensitive pathway for BK-induced potentiation of nicotinic current (Zhou et al. 2006), and the PTX-insensitive pathway for BK-induced slow inward current or the inhibition of the M current (Mochidome et al. 2001). In addition, phospholipase C (PLC) is involved in both the potentiation of nicotinic current and the inhibition of the M current by BK. Thus the postsynaptic effect of BK on cholinergic EPSC seems to correspond to the effect described on the nicotine-induced current. Concerning the presynaptic effect of BK, the relevance of PTX and PLC still require further exploration.

The effect of BK on the EPSC frequency may have any pathological significance. However, the effect on the EPSC amplitude needs to be discussed. In discussing this result, it is important to consider morphological characteristics of PTG neurons. They have many long dendrites and the majority of synapses are axo-dendritic (Myers 2000). This indicates that the cable property of plasma membrane functions as an important factor for passive spread of the local membrane potential generated at the synaptic sites, resulting in a high filter function for signal integration. Further, BK strongly increases the membrane resistance of PTG neurons via inhibition of the M current (Mochidome et al. 2001; Jones et al. 1995). Therefore, even though BK-induced potentiation of EPSC amplitude was only $\sim 40\%$ increased, it might have biological significance in the integration of signal transduction.

In discussing the pathophysiological significance of the present results in the airway diseases, it is also important to consider the negative feedback control of acetylcholine release from the postganglionic nerve terminal (Coulson and Fryer 2003). This feedback regulation via $M_2$ muscarinic acetylcholine receptors at the terminal becomes dysfunctional under condition of airway inflammation (Jacoby et al. 1998; Yost et al. 1999). Consequently, an augmentation in the excitability of PTG neurons should increase acetylcholine release from the postganglionic nerve terminals under pathological conditions.

Fig. 6. Effects of B2 kinin receptor antagonist on EPSCs potentiated by BK in the 30-mM K+ external solution. A: representative record showing the effect of $10^{-8}$ M HOE 140 on the potentiation of EPSCs by BK. At first, the 30-mM K+ external solution was applied for 1 min as a precontrol. After a washout for 4 min with the 5-mM K+ solution, the neuron was pretreated with the mixture of $10^{-8}$ M BK and $10^{-6}$ M HOE 140 for 20 s in 5 mM K+ solution, and then treated with them in the 30-mM K+ solution for 1 min. After control was recorded in the 30-mM K+ solution after a 5-min washout with the 5-mM K+ solution. B: normalized EPSC amplitude and its frequency in the absence or presence of BK and HOE 140. The amplitude and frequency of EPSCs in the presence of $10^{-8}$ M BK and $10^{-6}$ M HOE 140 were normalized to the mean amplitude and frequency recorded in precontrol, respectively. Data are shown as means $\pm$ SE ($n = 3$).

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such as airway inflammation. Apart from the above, there are cumulated findings that BK may be involved in pathogenesis of airway diseases including bronchitic asthma. Although normal subjects do not show any changes in pulmonary functions, even after high concentrations of BK are inhaled asthmatic subjects respond to BK inhalation concentration dependently (Berman et al. 1996; Fuller et al. 1987). It is also reported that airway inflammation markedly enhances the airway reactivity of asthmatic subjects to BK (Berman et al. 1995). Furthermore, the BK level in the lower airway is increased under airway inflammation probably as a result of reduced activities of BK-degrading enzymes such as neutral endopeptidase. On the other hand, in human asthmatic airway bronchoconstriction by BK is largely neuronally mediated, involving cholinergic nerves, and products of arachidonic acid metabolism by cyclooxygenase (COX) are involved (Ellis and Fozard 2002). However, in guinea pig inhaled BK causes bronchoconstriction predominantly via neural mechanisms involving cholinergic pathways (Ichinose et al. 1990; Turner et al. 2000), and the COX pathway is not involved in the bronchoconstrictor response (Ichinose et al. 1990). Thus it might be necessary to evaluate the effect of BK on synaptic transmission in rat PTG in the presence of COX blockade. Taken altogether, it is likely that BK-induced augmentation of the cholinergic transmission in PTG may be involved at least partly in pathogenesis of airway diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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