Neuropeptides 75 (2019) 65–74

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Excitatory effect of bradykinin on intrinsic neurons of the rat heart

Shiho Arichi[a,b], Sachie Sasaki-Hamada[a], Yuichi Kadoya[c], Masanori Ogata[a,b], Hitoshi Ishibashi[a,b,*]

[a] Department of Physiology, School of Allied Health Sciences, Kitasato University, Sagamihara 252-0373, Japan
[b] Department of Brain Science, Kitasato University Graduate School of Medical Sciences, Sagamihara 252-0373, Japan
[c] Department of Anatomical Science, School of Allied Health Sciences, Kitasato University, Sagamihara 252-0373, Japan

ARTICLE INFO

Keywords:
Acutely dissociated neuron
Cardiac plexus
Cation channel
Electrophysiology
M-current
Perforated patch-clamp technique

ABSTRACT

The heart receives sympathetic and parasympathetic innervation through the intrinsic cardiac nervous system. Although bradykinin (BK) has negative inotropic and chronotropic properties of cardiac contraction, the direct effect of BK on the intrinsic neural network of the heart is still unclear. In the present study, the effect of BK on the intracardiac ganglion neurons isolated from rats was investigated using the perforated patch-clamp technique.

Under current-clamp conditions, application of 0.1 μM BK depolarized the membrane, accompanied by repetitive firing of action potentials. When BK was applied repeatedly, the second responses were considerably less intense than the first application. The BK action was fully inhibited by the B_2 receptor antagonist Hoe-140, but not by the B_1 receptor antagonist des-Arg^9-[Leu^8]-BK. The BK response was mimicked by the B_2 agonist [Hyp^3]-BK. The BK-induced depolarization was inhibited by the phospholipase C inhibitor U-73122. BK evoked inward currents under voltage-clamp conditions at a holding potential of −60 mV. Removal of extracellular Ca^{2+} markedly increased the BK-induced currents, suggesting an involvement of Ca^{2+}-permeable non-selective cation channels. The muscarinic agonist oxotremorine-M (OxoM) also elicited the extracellular Ca^{2+}-sensitive cationic currents. The OxoM response did not exhibit rundown with repeated agonist application. The amplitude of current evoked by 1 μM OxoM was comparable to that induced by 0.1 μM BK. Co-application of 0.1 μM BK and 1 μM OxoM elicited the current whose peak amplitude was almost the same as that elicited by OxoM alone, suggesting that BK and OxoM activate same cation channels. BK also reduced the amplitude of M-current, while the M-current inhibitor XE-991 affected neither resting membrane potential nor the BK-induced depolarization. From these results, we suggest that BK regulates excitability of intrinsic cardiac neurons by both an activation of non-selective cation channels and an inhibition of M-type K^+ channels through B_2 receptors.

1. Introduction

Bradykinin (BK) is an endogenous nonapeptide released from cleavage of kininogens by tissue plasma kallikrein enzymes in response to a variety of physiological and pathological stimuli, including ischemia and tissue injury (Baxter and Ebrahim, 2002). BK plays an important role in the regulation of circulatory system such as blood pressure and cardiac functions (Regoli et al., 2012). It is well known that angiotensin-converting enzyme (ACE) not only converts angiotensin I to angiotensin II, but also catalyzes the breakdown of BK to inactive metabolites (Su et al., 1999). ACE inhibitors, which are often used to treat high blood pressure, are reported to increase BK levels in normal human subjects (Pellacani et al., 1994) and in dogs with heart failure (Barbe et al., 1996).

The injection of BK into the left coronary artery of anesthetized dogs produces a simultaneous decrease in blood pressure and heart rate, which might be initiated by stimulation of vagal afferent fibers for the reflex responses (Neto et al., 1974; Kaufman et al., 1980). On the other hand, epicardium application of BK evokes a reflex increase in blood pressure by stimulating sympathetic afferent nerve endings in the heart in dogs (Kaufman et al., 1980). It has been reported that BK is increased in cardiac ischemia (Kimura et al., 1973; Shimamoto et al., 1992) and myocardial infarction (Hashimoto et al., 1978). BK has also been shown to have cardioprotective effects in ischemic conditions (Yang et al., 1997; Ito et al., 2003). BK exerts its effects via B_1 and B_2 receptors. The B_2 receptors are widely distributed and constitutively expressed in the

Abbreviations: ACE, angiotensin-converting enzyme; BK, bradykinin; DMSO, dimethylsulfoxide; HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid; IP_3, inositol trisphosphate; NMDG, *N*-methyl-D-glucamine; OxoM, oxotremorine-M; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C; Tris-OH, tris (hydroxymethyl)aminomethane; SGCs, satellite glial cells; TRP, transient receptor potential; TTX, tetrodotoxin; XeC, xestospongin-C

* Corresponding author at: Department of Physiology, School of Allied Health Sciences, Kitasato University, Sagamihara, Kanagawa 252-0373, Japan.
E-mail address: isibasih@kitasato-u.ac.jp (H. Ishibashi).

https://doi.org/10.1016/j.npep.2019.04.002
Received 7 January 2019; Received in revised form 7 April 2019; Accepted 23 April 2019
Available online 25 April 2019
0143-4179/

vascular, cardiac and nervous system, while B_1 receptors are expressed at a very low level in healthy tissues and induced in pathophysiological conditions such as inflammation and pain (Prado et al., 2002). The mice lacking B_2-receptors exhibit an accelerated heart rate under basal conditions (Emanueli et al., 1999), suggesting an important role for the B_2 receptor in cardiovascular function.

The heart is under control of autonomic nervous system. The intracardiac ganglia are innervated by the vagus nerve, and post-ganglionic neurons within the cardiac ganglia send their projections to discrete regions of the heart. These ganglion neurons form neuronal network on the surface of the heart, termed ganglionated plexuses. It has been assumed that the individual cardiac ganglia may serve as complex integrative centers from multiple synaptic, hormonal and sensory inputs (Ashton et al., 2018; Durães Campos et al., 2018). In addition, the intrinsic cardiac nervous system plays a significant role in the initiation and maintenance of atrial fibrillation (Scherlag et al., 2005; Nakagawa et al., 2009). Thus, the cardiac plexus plays a prominent role in the modulation of cardiac function in physiological and pathological conditions. Previous studies have shown that intracardiac neurons possess receptors for a variety of putative neuromodulators, including muscarinic receptors (Allen and Burnstock, 1990; Hirayama et al., 2015), adrenergic receptors (Ishibashi et al., 2003) and neuropeptide receptors (Cuevas and Adams, 1996; Braas et al., 1998). In addition, BK has been reported to modulate rabbit heart function via intrinsic cardiac nervous system (Izrailtyan and Kresh, 1997). However, it remains unclear how BK modulates the excitability of the intrinsic cardiac neurons. In the present study, therefore, the effect of BK was investigated in neurons acutely isolated from rat intracardiac ganglia using an amphotericin B perforated patch-clamp recording configuration.

2. Materials and methods

All experiments were conducted under the 'Guiding Principle for the Care and Use of Laboratory Animals' approved by the Japanese Physiological Society and were approved by the Animal Experimentation Ethics Committee of the Kitasato University School of Allied Health Sciences.

2.1. Preparation

Intracardiac neurons were isolated from cardiac plexuses located on the hearts of 2–3- or 8-week-old Wistar rats. Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium, after which the heart was excised in cold normal external solution. Then, the atria were isolated and pinned to the Sylgard-covered base of a culture dish in cold normal external solution. The dorsal ganglionated plexuses from vicinity of the pulmonary vein were removed under a stereomicroscope, using spring scissors and forceps with very thin, sharp tips. The plexuses were incubated for 45 to 60 min at 35 °C in normal external solution containing 0.3% collagenase (type I, Sigma-Aldrich, St. Louis, MO, USA) and 0.3% trypsin (type I, Sigma-Aldrich). Finally, single neurons were obtained by gentle trituration of tissue pieces with fire-polished Pasteur pipettes. For electrophysiological recordings, the dissociated neurons were placed on the bottom of culture dishes (#353801, Corning Inc., Corning, NY, USA) containing normal external solution.

2.2. Electrophysiological recordings

The isolated intracardiac ganglion neurons were visualized on an inverted microscope with phase-contrast equipment (DMIRB, Leica, Nussloch, Germany). Membrane potential and current were monitored with the amphotericin B perforated-patch recording mode (Akaike and Harata, 1994; Ishibashi et al., 2012). Patch pipettes were made from borosilicate glass tubes in two stages on a vertical pipette puller (PC-10, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with the internal solution and the reference electrode in normal external solution was 4 to 8 MΩ. For perforated patch-clamp experiments, a stock solution of 100 mg ml^{-1} of amphotericin B in dimethylsulfoxide (DMSO) was prepared the day of the experiment and kept in the dark. Immediately prior to use, the amphotericin B stock solution was diluted in pipette solution to yield a final concentration of 500 μg ml^{-1} amphotericin B in 0.5% DMSO. Following gigaseal formation, the neurons were held at −60 mV. Amphotericin B incorporation into the membrane patch resulted in an increase in a fast capacitive transient and an appearance of a slow capacitive transient. After stable perforated-patch formation, the series resistance ranged from 8 to 22 MΩ and was compensated in the same manner as previously described (Ishibashi et al., 2003). The membrane potential and current signals were measured by a patch-clamp amplifier (AXOPATCH 200B, Molecular Devices, Sunnyvale City, CA, USA). Before digitization (sampling rate 10 kHz), the signals were filtered at 2 kHz with a three-pole low-pass Bessel-type filter. Data were stored on a microcomputer hard disk for subsequent analysis with the pClamp9 system (Molecular Devices). The membrane potential is given without a liquid junction potential correction of −3 mV. All experiments were carried out at room temperature (21–24 °C).

2.3. Immunofluorescence analysis

Six Wistar rats (2–3 weeks old) were used for immunofluorescence analysis. Cells isolated from intracardiac ganglia were plated on 35 mm glass-bottomed culture dishes coated with poly-D-lysine (D11131H, Matsunami, Tokyo, Japan). The isolated cells were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS, pH 7.4) for 15 min at 4 °C and washed 3 times with PBS. Subsequently, they were incubated in a blocking buffer containing 0.1% Triton-X-100 and 1% BSA for 2 h at room temperature. After the cells were washed 3 times with PBS, polyclonal rabbit antibodies against B_1- or B_2-receptors (ABR-011 or ABR-012, Alomone Labs, Jerusalem, Israel; 1:2000 dilution) were loaded and kept at 4 °C overnight (Kawaguchi et al., 2015). The cells were then washed 3 times with PBS and incubated with Alexa Fluor 488 donkey anti-rabbit IgG (A-21206, Thermo Fisher Scientific; 1:1000 dilution) at room temperature for 1 h. The fluorescence image was taken with confocal laser scanning microscope (Nikon C2si, Nikon Instech Co., Ldt., Tokyo, Japan). At the periphery, small satellite glial cells (SGCs) surround the cell bodies of neurons. The round or fusiform cell bodies of SCGs are approximately < 10 μm (Zhang et al., 2009). In the present study, therefore, neurons with cell bodies larger than 20 μm in diameter were counted, and the percentage of positive immunolabeled neurons were calculated.

2.4. Solutions and chemicals

The ionic composition of the normal external solution was (mM): NaCl 150, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2, HEPES 10 and glucose 10. The pH was adjusted to 7.4 with tris (hydroxymethyl) aminomethane (Tris-OH). The nominally Ca^{2+}-free solution was made by simply omitting Ca^{2+} from the normal external solution. The composition of the patch pipette (internal) solution was (mM): NaCl 10, KCl 60, potassium methanesulfonate 80, and HEPES 10. The pH of this patch-pipette solution was adjusted to 7.3 with Tris-OH. Amphotericin B was dissolved in DMSO, resulting in a 100 mg ml^{-1} stock solution. Drugs were topically applied with the 'Y-tube' solution exchange device (Murase et al., 1989).

The drugs used in the present experiments were BK, des-Arg^{10}-kallidin, des-Arg^9-[Leu^8]-BK, [Hyp^3]-BK, Hoe-140 (Peptide Institute, Osaka, Japan), tetrodotoxin (TTX) (Wako, Tokyo, Japan), xestospongin-C (XeC) (Enzo Life Sciences, Inc., NY, USA), thapsigargin (nacalai tesque, Kyoto, Japan), BAPTA-AM (Tokyo Kasei, Tokyo, Japan), amphotericin B, collagenase, HEPES, KB-R7943, ML204, oxotremorine-M (OxoM), trypsin, U-73122, U-73343, *N*-methyl-D-glucamine (NMDG),

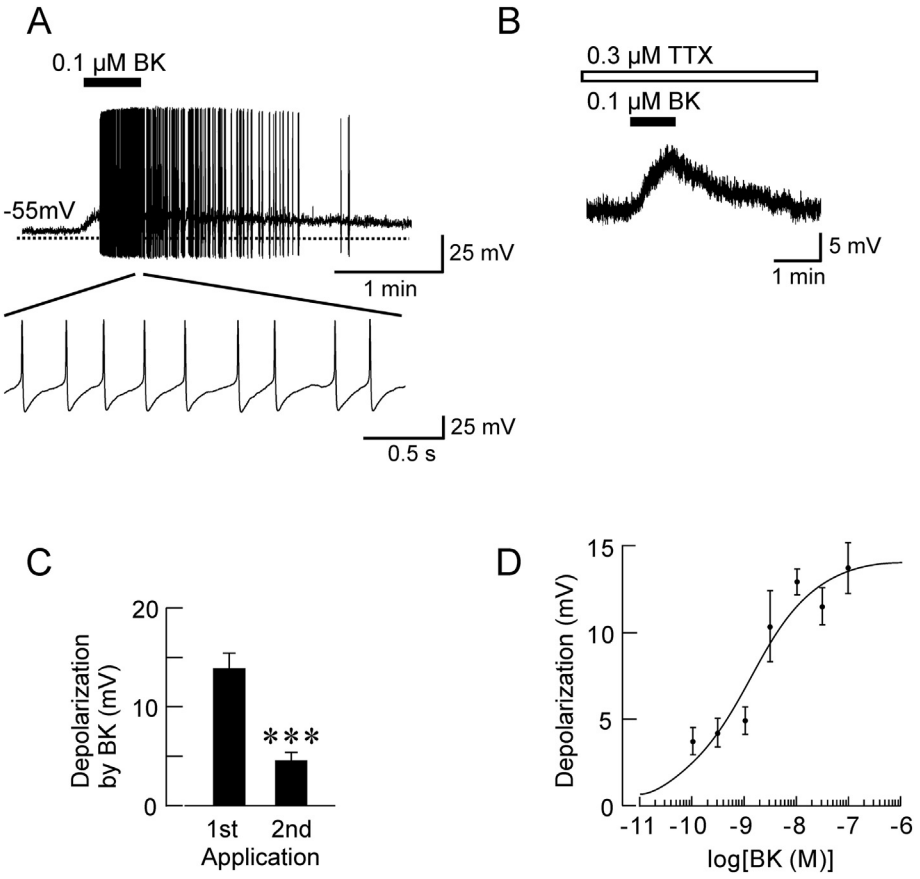

Fig. 1. Effect of bradykinin on rat intracardiac ganglion neurons. Recordings were performed under current-clamp conditions. Horizontal bars above the traces indicate the application of agents. (A) Depolarization of a rat intracardiac neuron by 0.1 μM bradykinin (BK). Dotted line corresponds to −55 mV. Lower panel shows the trace in an expanded time scale. (B) Effect of BK on membrane potential in the presence of TTX. The trace was representative from six experiments. (C) Desensitization of BK-induced depolarization. BK (0.1 μM) was applied for 1 min at 15–20 min interval. ***$P < 0.001$ vs first application. (D) Concentration-dependent action of BK. Each point represents the average value ± S.E.M. from five to eight neurons.

XE-991 (Sigma-Aldrich, St. Louis, MO, USA) and pentobarbital sodium (Dainippon Seiyaku, Tokyo, Japan).

Drugs that are not soluble in water were first dissolved in DMSO and then diluted in normal external solution. The final concentration of DMSO never exceed 0.1%.

2.5. Statistical analysis

All statistical analyses were performed in ORIGIN9 software (OriginLab Corporation, Northampton, MA, USA). The depolarizing responses induced by first and second applications of BK (Fig. 1C) were compared by Student's two-tailed paired *t*-test.

The M-current inhibition by BK and XE-991 (Fig. 7) were also compared by Student's two-tailed paired *t*-test. Unless otherwise noted, only one recording of the BK response was performed in each culture dish of neurons to ensure that recordings were not made from cells which had been inadvertently exposed to BK. The BK response was determined by pooling data obtained from single responses to BK of several neurons. Experiments involving 2 groups were compared using unpaired, 2-tailed *t*-tests. Multiple comparisons were made using one-way ANOVA, followed by Tukey multiple comparisons test. Statistical significance was assumed when the *P* value was below 0.05. All data are presented as mean ± S.E.M of *n* experiments, where *n* corresponds to the number of recorded cells. For constructing the concentration-response curve, the data were fitted to a modified Michaelis-Menten equation using least squares fitting: $V = (V_{max}C^h)/(C^h + EC_{50})$, where V is the BK-induced depolarization, Vmax is the maximum response, C is the BK concentration, EC_{50} is the concentration that causes a half-maximum response, and h is the Hill coefficient.

3. Results

3.1. Depolarizing response to bradykinin

Perforated patch-clamp recordings were performed in neurons freshly isolated from intracardiac ganglia of 2- to 3-week-old rats. Under current-clamp conditions, the neurons exhibited no spontaneous action potentials, and had resting membrane potential of −55.4 ± 1.2 mV (n = 14), which is in accordance with reported values in previous studies (Whyte et al., 2009; Hirayama et al., 2015). Application of 0.1 μM BK depolarized the membrane with repetitive firing of action potentials (Fig. 1A). The excitability gradually ceased after washout of BK. The depolarizing response was also observed without action potentials in the presence of 0.3 μM TTX (n = 6, Fig. 1B). In five neurons tested, membrane input resistance before and after application of 0.1 μM BK were 733 ± 53 and 732 ± 55 MΩ, respectively. The relatively high membrane input resistance of isolated intracardiac neurons was consistent with previous studies (Xu and Adams, 1992; Cuevas et al., 1997). No net change in input resistance produced by BK might suggest that this neuropeptide is probably exerting its effects by simultaneously opening and closing multiple membrane channels. The BK-induced depolarization without changing input resistance was also reported in bronchial parasympathetic ganglion neurons (Kajekar and Myers, 2000). A second identical BK application 15–20 min later resulted uniformly in a substantially smaller depolarization (Fig. 1C). Because of the desensitization, it was difficult to obtain the concentration-response relationship by applying different concentrations of the peptide to a single neuron. Therefore, concentration-dependent actions of BK were determined by pooling data obtained from several cells to a given concentration of the peptide. This yielded a single concentration-response curve (Fig. 1D). The EC_{50} value for the response, calculated from the concentration-response curve, was 1.2 nM.

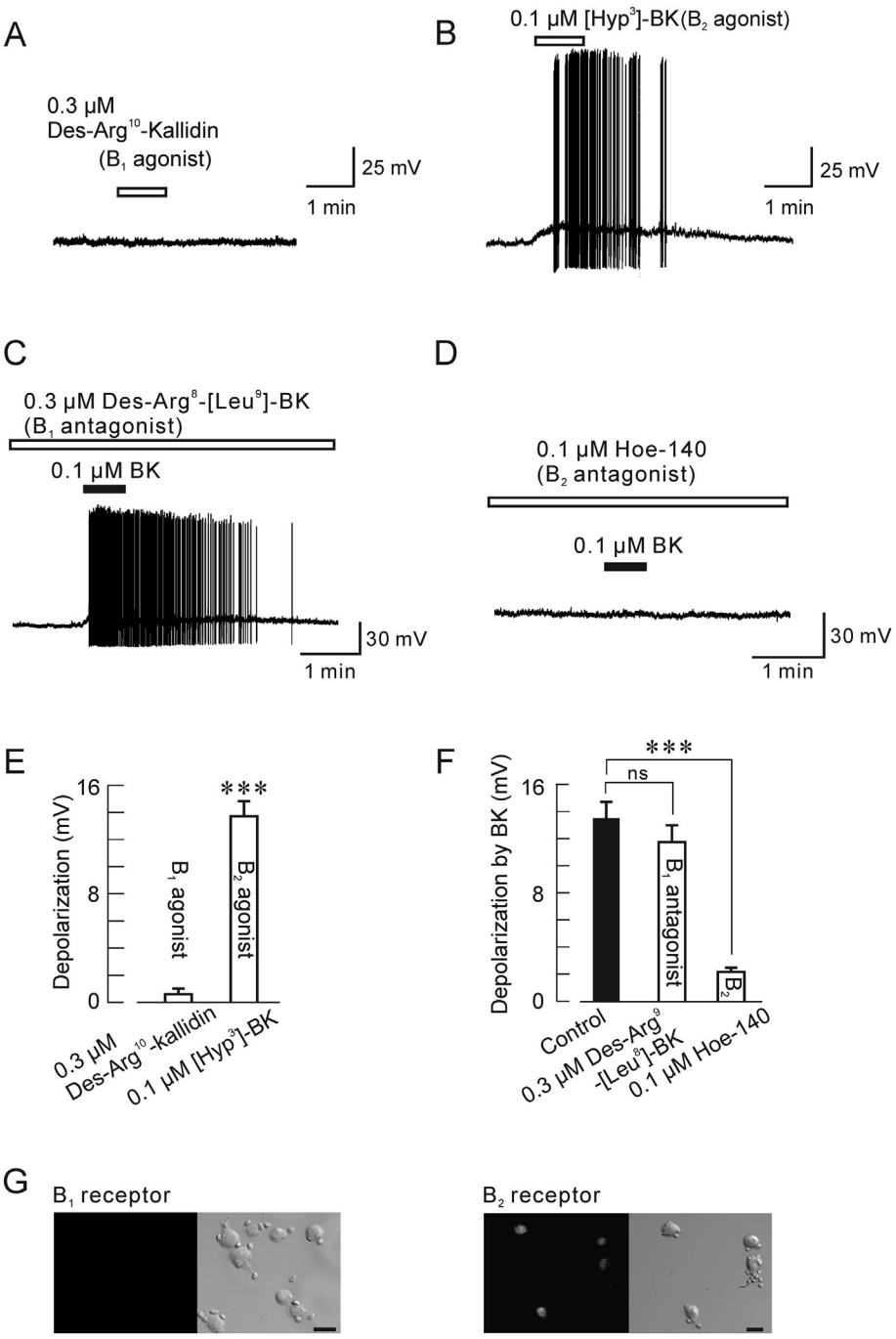

Fig. 2. Involvement of B_2 receptor. (A) Effect of the B_1 receptor agonist des-Arg^{10}-kallidin on resting membrane potential. (B) Depolarizing effect of [Hyp^3]-BK, a B_2 receptor agonist. (C) Effect of des-Arg^9-[Leu^8]-BK, a B_1 antagonist. (D) Effect of the B_2 antagonist Hoe-140. (E) Depolarization by agonists of BK receptors. ***$P < 0.001$ vs B_1 receptor agonist. (F) Effects of BK receptor antagonists on the BK-induced depolarization. Each column shows the average ± S.E.M. from five to six neurons. ***$P < 0.001$ vs control. (G) Immunofluorescent analysis of B_1- and B_2-receptors of the rat intracardiac ganglion cells. Phase contrast and fluorescence views of the same microscopic field are shown. Scale bar = 25 μm.

BK is reported to fail to activate superior cervical ganglion neurons acutely isolated from 7- to 12-week-old rats, while these neurons cultured for 48 h with nerve growth factor showed significant depolarization and action potential firings after BK application (Vivas et al., 2014). In the present study, on the other hand, application of BK to the intracardiac ganglion neurons acutely dissociated from 8-week-old rats produced the membrane depolarization (11.8 ± 1.2 mV, n = 5) with repetitive action potentials (data not shown), as observed in the ganglion neurons isolated from immature rats. Thus, the following experiments were carried out in neurons acutely isolated from 2 to 3-week-old rats.

3.2. Subtype of BK receptors

To clarify the subtype of BK receptors, the effects of BK receptor agonists and antagonists including des-Arg^{10}-kallidin (B_1 agonist, EC_{50} = 4 nM), [Hyp^3]-BK (B_2 agonist, EC_{50} = 1 nM), des-Arg^9-[Leu^8]-BK (B_1 antagonist, IC_{50} = 0.1 μM) and Hoe-140 (B_2 antagonist, IC_{50} = 1 nM) were studied (Rabito et al., 1996; Zubakova et al., 2008). As shown in Fig. 2, the B_2 receptor agonist [Hyp^3]-BK (0.1 μM) mimicked the BK response, while the B_1 receptor agonist des-Arg^{10}-kallidin (0.3 μM) failed to depolarize the membrane. The depolarization evoked by BK was fully inhibited by the B_2 receptor antagonist Hoe-140 (0.1 μM), while the B_1 receptor antagonist des-Arg^9-[Leu^8]-BK (0.3 μM) had little effect. Fig. 2G shows immunofluorescent imaging of B_1- and B_2-receptors in the isolated intracardiac ganglion cells. All of the isolated neurons were positive for B_2 receptors (n = 42), although subcellular distribution of B_2 receptors remains elusive. On the other hand, 15.3% neurons were positive for B_1 receptors (n = 72). These results suggest the involvement of B_2 receptors in the BK response.

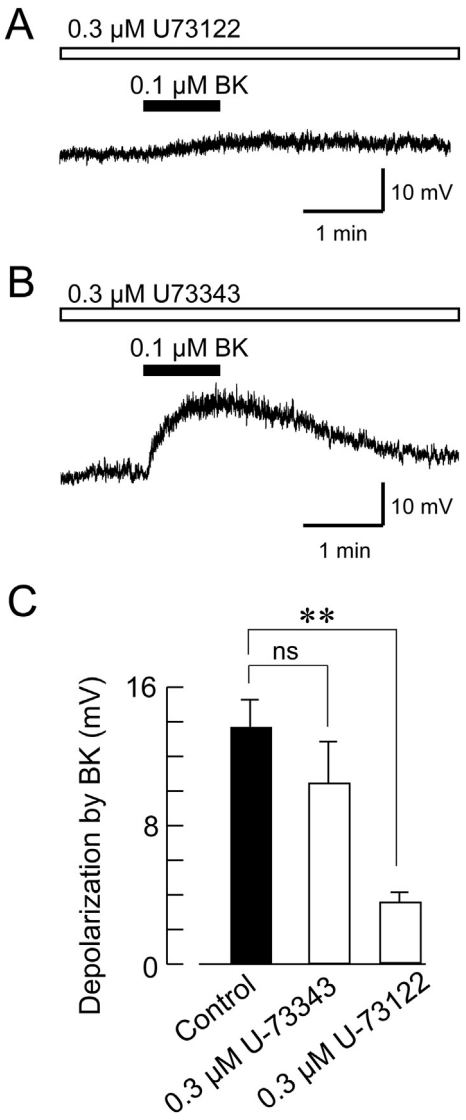

Fig. 3. Contribution of PLC. (A and B) Effects of U-73122 and U-73343 on the BK-induced depolarization. (C) Summary of the data. Each column represents the average ± S.E.M. from five to eight neurons. $^{**}P < 0.01$ vs control.

3.3. Contribution of phospholipase C

The B_2 receptor couples to the GTP-binding protein $G_{q/11}$, and activates phospholipase C (PLC), causing phosphoinositide hydrolysis, that leads to generation of inositol trisphosphate (IP_3) and to subsequent mobilization of intracellular Ca^{2+} from the endoplasmic reticulum (Regoli et al., 2012). To clarify the role of PLC in the BK response, the effect of the PLC inhibitor U-73122 was examined in the presence of 0.3 μM TTX. U73122 was reported to inhibit the PLC-mediated response with an IC_{50} of 0.18 μM (Okamoto et al., 2004). U-73122 at a concentration of 0.3 μM had no effect on membrane potential. A five minutes preincubation of rat intracardiac neurons with 0.3 μM U-73122 significantly inhibited the BK-induced depolarization (Fig. 3). The inactive analogue U-73343 (0.3 μM), used often as a control compound for U-73122, did not significantly alter the BK response. These results suggest that PLC plays a crucial role in the BK action.

3.4. Activation of cation channels

The $G_{q/11}$-mediated activation of PLC couples to regulation of intracellular Ca^{2+} signaling via IP_3 receptors. The PLC pathway is well known to be main modulator of cation channels including transient receptor potential (TRP) channels and Orai channels (Mercer et al., 2006; Majewski and Kuznicki, 2015). In the present study, therefore, the effect of ML204, a specific inhibitor of TRPC4 and TRPC5 (IC_{50} = 4 μM; Miller et al., 2011), was examined. ML204 itself had no effect on the resting membrane potential (−53.0 ± 1.5 mV and − 54.7 ± 1.7 mV respectively in the absence and presence of 10 μM ML204, n = 6). As shown in Fig. 4A and C, ML204 (10 μM) significantly inhibited the BK-induced depolarization. We also investigated the effect of KB-R7943 on the BK-induced depolarization. Although KB-R7943 was introduced as a selective inhibitor of the Na^+/Ca^{2+} exchanger in the reverse mode (Iwamoto et al., 1996), this agent is reported to be able to potently block the receptor-operated TRP channels (IC_{50} = 1 μM; Kraft, 2007). KB-R7943 at a concentration of 3 μM produced small depolarization of 4.5 ± 1.4 mV (n = 6, data not shown). In the presence of 3 μM KB-R7943, the BK-induced depolarization was markedly inhibited (Fig. 4B and C). These results suggest an involvement of cation channels in the BK action.

To further investigate the role of ion channels in the BK response, the effect of BK was examined under voltage-clamp conditions. At a holding potential of −60 mV, BK evoked small inward currents (10.3 ± 1.4 pA, n = 5) in the normal external solution containing 2 mM Ca^{2+} (Fig. 4D). The inward current was fully inhibited by replacement of extracellular Na^+ with NMDG (Fig. 4E). On the other hand, removal of extracellular Ca^{2+} markedly potentiated the BK-induced currents (Fig. 4F). It should be noted that the current amplitude of second BK response in the absence of extracellular Ca^{2+} was 24.0 ± 4.5% of that induced by the first BK application (n = 7). Fig. 4G summarizes the current responses to the first application of BK. Since extracellular Ca^{2+} is well known to reduce the monovalent cation currents of various Ca^{2+}-permeable channels (Helliwell and Large, 1996; Owsianik et al., 2006), the present results suggest the participation of Ca^{2+}-permeable non-selective cation channels in the BK response.

3.5. Involvement of intracellular Ca^{2+} release in activation of cation channels

In order to reveal the contribution of the intracellular Ca^{2+} release to the BK-induced currents, we next investigated the effect of XeC, a membrane permeable inhibitor of IP_3 receptors (IC_{50} = 0.4 μM; Gafni et al., 1997). As shown in Fig. 5B, the BK-induced current in the absence of extracellular Ca^{2+} was markedly inhibited in the presence of 2 μM XeC, which was pretreated for 5 min. Pretreatment for 10 min with the sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase inhibitor thapsigargin (IC_{50} = 30 nM; Thastrup et al., 1990), significantly inhibited the BK-induced currents (Fig. 5C and E). In addition, the membrane permeable Ca^{2+} chelator BAPTA-AM (20 μM), that was pretreated for 10 min, also significantly inhibited the BK-induced currents (Fig. 5D and E). On the other hand, thapsigargin and BAPTA-AM themselves did not cause any detectable inward currents (data not shown). These results suggest that Ca^{2+} released from intracellular Ca^{2+} stores via IP_3 receptors is involved in the cation channel activation.

3.6. Comparison of cation currents mediated by BK- and muscarinic-receptors

In the rat intracardiac neurons, activation of muscarinic receptors induces the extracellular Ca^{2+}-sensitive cationic currents which are mediated by intracellular Ca^{2+} release via PLC-IP_3 pathway (Hirayama et al., 2015). Although this activation mechanism is similar to that of the BK-induced currents observed in the present study, the muscarinic receptor-mediated current does not show any marked rundown with repeated agonist application. In the present study, therefore, the inward currents elicited by submaximal concentrations of BK and

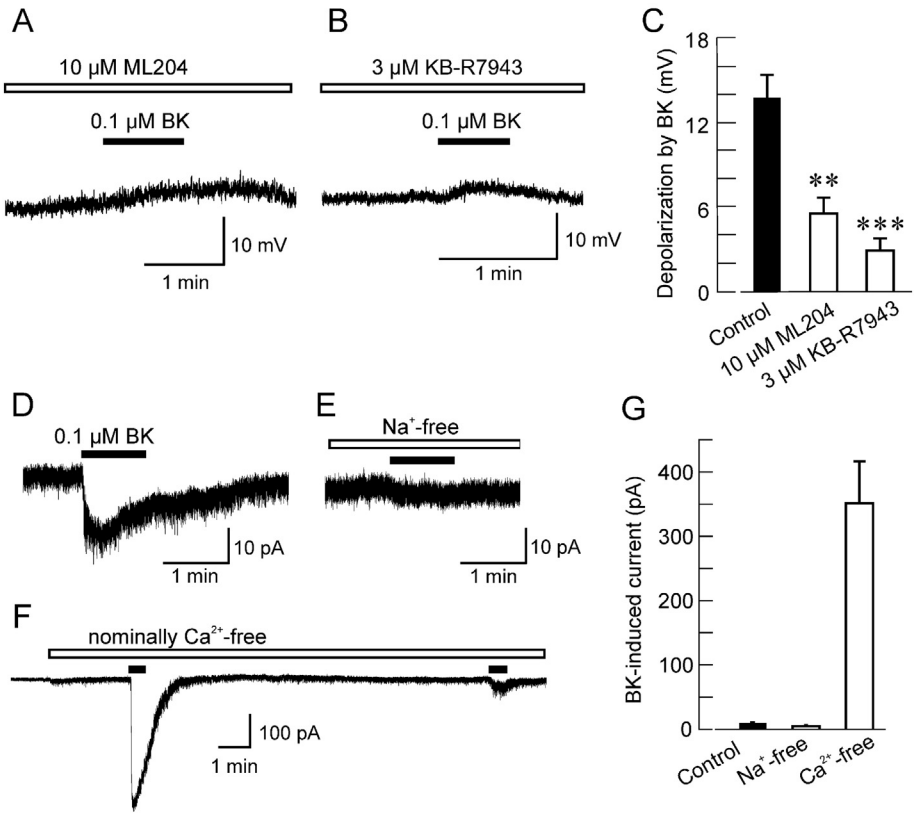

Fig. 4. Activation of cation channels. (A-C) Effects of the cation channel blocker ML204 and KB-R7943 on the BK-induced depolarization. Each column represents the average ± S.E.M. from six to eight neurons (C). $^{**}P < 0.01$, $^{***}P < 0.001$ vs control. (D) Inward current induced by BK. Recordings were performed at a holding potential of −60 mV. (E) The BK response under Na^+-free conditions. (F) The BK-induced current in the absence of extracellular Ca^{2+}. (G) Summary of the data. Each column represents the average ± S.E.M. from five to seven experiments.

oxotremorine-M (OxoM), a muscarinic agonist, were compared. As shown in Fig. 6A and B, OxoM (1 μM) evoked an inward current whose amplitude was comparable to that evoked by 0.1 μM BK. Interestingly, with the concomitant application of both agonists, the resultant inward currents have almost the same amplitude as that produced by OxoM alone (Fig. 6C). In addition, the cationic channels could be fully activated by 1 μM OxoM even after recording the BK response.

3.7. Inhibition of M-currnets

The M-type K^+ channels are voltage-gated K^+ channels that regulate the excitability of many neurons. It is well known that activity of M-type K^+ channels is maintained by membrane phosphatidylinositol-4,5-bisphosphate (PIP_2). Local depletion of PIP_2 induced by PLC activation can suppress the M-currents (Suh and Hille, 2002). In addition, the rat intracardiac neurons possess M-currents (Xi-Moy and Dun, 1995). BK has been reported to be able to inhibit M-currents in NG108-15 cells (Higashida and Brown, 1986) and in rat paratracheal neurons (Mochidome et al., 2001). To clarify the contribution of M-type K^+ channels, we investigated whether or not BK affects the M-currents in the intracardiac neurons. The M-current inhibition was measured as the time-dependent inward current relaxation during hyperpolarizing voltage steps from a holding potential of −20 mV to −60 mV. As shown in Fig. 7A, BK suppressed the amplitude of the M-current by 60.2 ± 6.3% (n = 5). To estimate to what extent the M-current inhibition may contribute to the BK-induced change in membrane potential, the effect XE-991, an M-current inhibitor (I_{C50} = 2 μM; Schwarz et al., 2006), was investigated. As shown in Fig. 7B, XE-991 (10 μM) inhibited the M-current by 76.3 ± 3.9% (n = 6). Under current-clamp conditions, on the other hand, neither resting membrane potential nor the BK-induced depolarization was significantly affected by 10 μM XE-991 (Fig. 7C and D).

4. Discussion

The present study was performed in dissociated intracardiac ganglion neurons, and we observed that BK depolarizes the resting membrane potential and elicits repetitive firing of action potentials. The physiological actions of BK are mediated by activation of B_1 and/or B_2 receptors. The B_2 receptors are expressed in a wide range of tissues, whereas the B_1 receptors are expressed mainly under pathological conditions that are related to inflammation and tissue damage (for review, see Marceau et al., 1998). In the present study, the BK-induced depolarization was mimicked by the B_2 receptor agonist [Hyp^3]-BK and inhibited by the selective B_2 receptor antagonist Hoe-140 (Fig. 2). In addition, immunofluorescence analysis revealed that all of the isolated neurons expressed B_2 receptors. These results indicate that the depolarizing response of BK is likely to be mediated by B_2 receptors.

It has been reported that stimulation of B_2 receptors by BK triggers rapid internalization of the agonist-receptor complex and loss of cell surface receptors (Munoz and Leeb-Lundberg, 1992; Haasemann et al., 1998). By contrast, B_1 receptors show almost no ligand internalization or receptor sequestration upon stimulation (Faussner et al., 1999). In the present study, a second application of BK 15–20 min after the first one induced a smaller response in all cells tested (Fig. 1C), indicating a homologous desensitization. Thus, it is possible to assume that the functional desensitization of BK response observed in the present study may result from internalization of B_2 receptors, whereas further studies are needed to clarify the desensitization mechanisms.

It is well established that B_2 receptors couple to $G_{q/11}$ and activate PLC (Couture et al., 2001). As a consequence of the PLC activation, generation of diacylglycerol and IP_3, and release of Ca^{2+} from intracellular Ca^{2+} stores could occur (Kadamur and Ross, 2013). These PLC-mediated pathways are well known to activate Ca^{2+}-permeable cation channels including TRP and Orai channels (Smyth et al., 2006). In the present study, as shown in Fig. 4, removal of extracellular Ca^{2+} markedly potentiated the BK-induced inward currents. This Ca^{2+} action

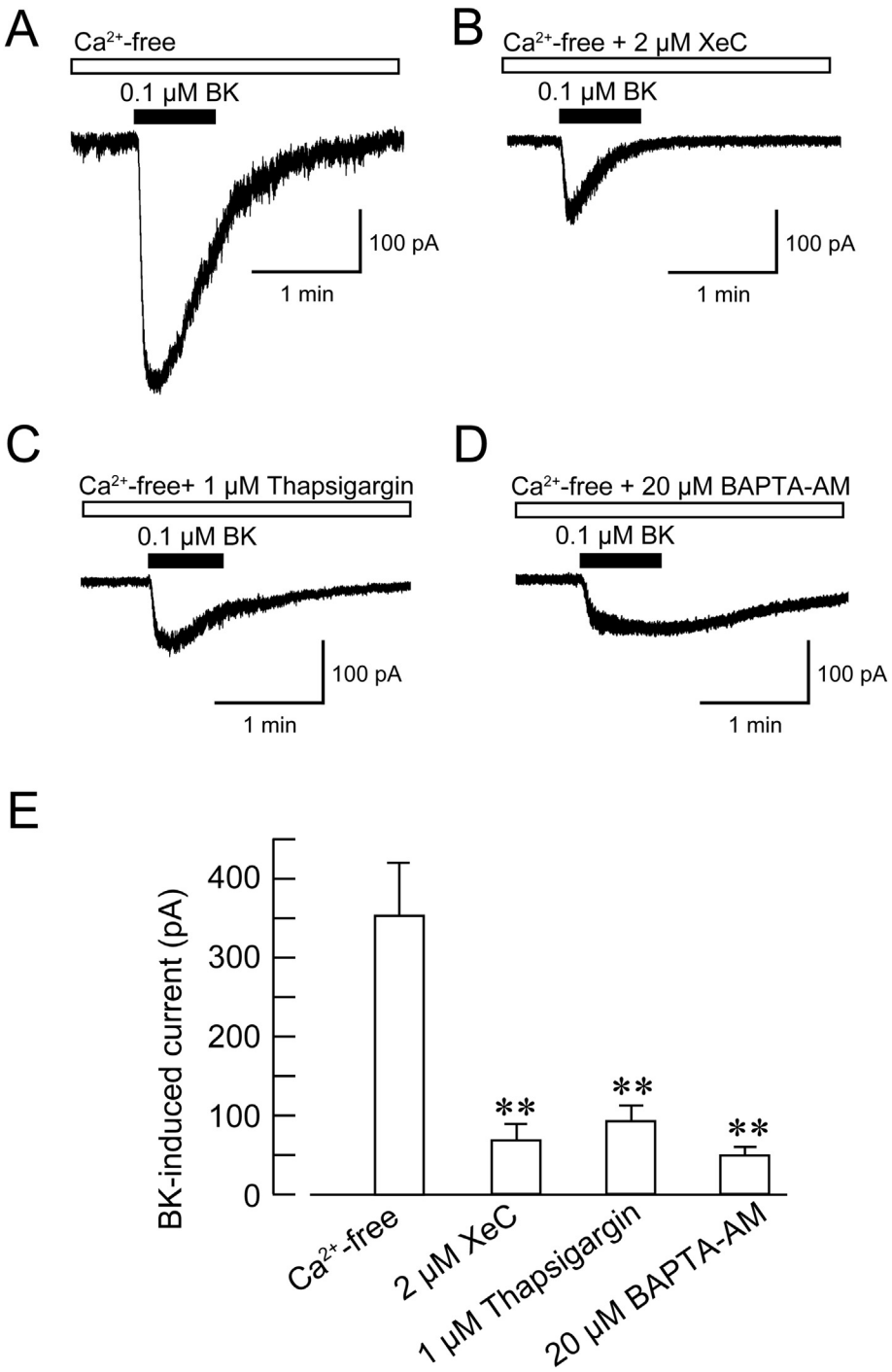

Fig. 5. Involvement of Ca^{2+} release from intracellular Ca^{2+} stores. Recordings were performed at a holding potential of −60 mV in the Ca^{2+}-free extracellular solution. (A and B) Representative current traces in the absence (A) and presence (B) of XeC, a membrane permeable IP_3 receptor blocker. (C and D) Effect of thapsigargin and BAPTA-AM on the BK-induced current. These agents were pretreated for 10 min. (E) Effects of XeC, thapsigargin and BAPTA-AM on the BK-mediated inward currents. Each column represents the average ± S.E.M. from five to six experiments. $^{**}P < 0.01$ vs Ca^{2+}-free.

has been reported to result from Ca^{2+} binding to sites in the ion-conducting pathway (Owsianik et al., 2006). In Ca^{2+} permeating channels, Ca^{2+} selectivity is attributed to a high-affinity Ca^{2+} binding site in the channel pore, which reduces monovalent cation conductance.

As for the activation mechanism of the cation channels, the BK-induced current was inhibited by the membrane-permeable IP_3 receptor inhibitor XeC (Fig. 5), thereby indicating the contribution of IP_3 receptors. Furthermore, the BK-induced current was suppressed by thapsigargin and BAPTA-AM (Fig. 5). Thapsigargin is known to inhibit the Ca^{2+} pump of internal Ca^{2+} stores, leading to depletion of the stores. BAPTA-AM is a membrane-permeable analogue of a Ca^{2+} chelator BAPTA. Thus, the present result suggests that Ca^{2+} released from intracellular Ca^{2+} stores via IP_3 receptors is involved in the BK action. The store-operated cation channels are well known to be activated by depleting internal Ca^{2+} stores. It has been reported that BAPTA-AM, which depletes internal Ca^{2+} stores by chelating intracellular free Ca^{2+}, activates cation channels (Albert and Large, 2002). In the present study, on the other hand, thapsigargin and BAPTA-AM themselves did not activate the cationic conductance. Therefore, it could be concluded that the activation of cation channels by BK did not depend on the

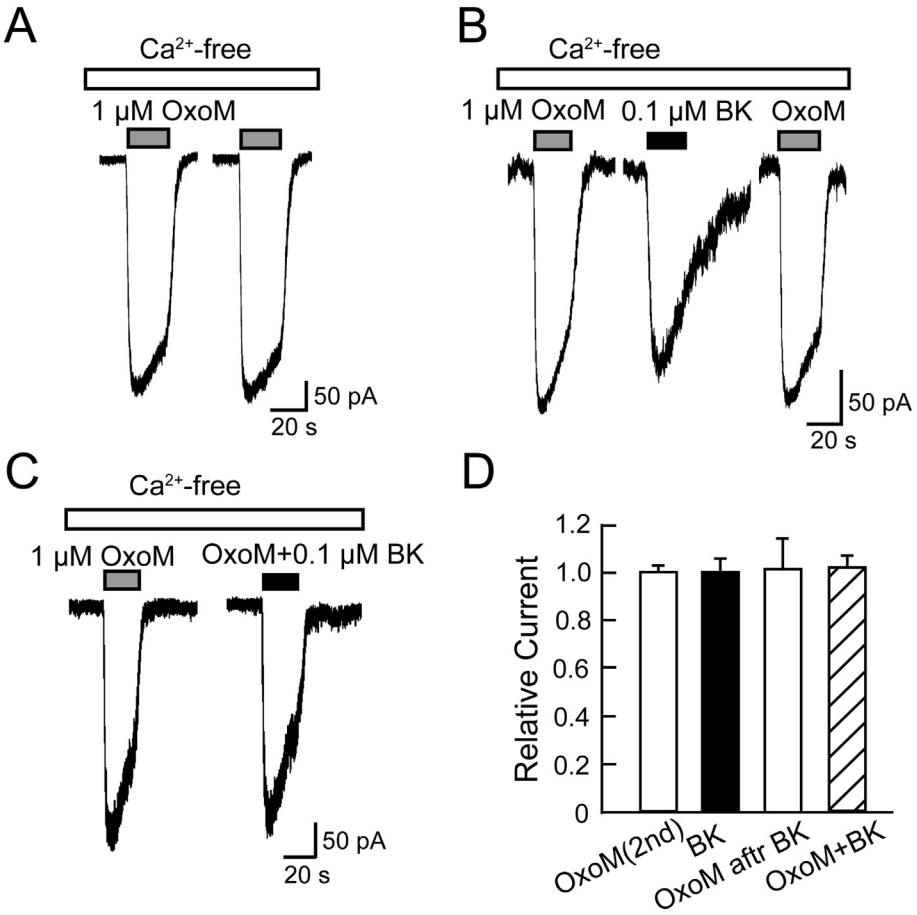

Fig. 6. Comparison of inward currents mediated by BK- and muscarinic-receptors. Recordings were performed at a holding potential of −60 mV in the Ca^{2+}-free extracellular solution. (A) Consecutive current traces obtained by repetitive application of OxoM. (B) The representative traces showing repeated inward currents induced by successive application of the muscarinic receptor agonist Oxo-M (1 μM) or BK (0.1 μM). (C) The representative current traces evoked by Oxo-M alone or concomitant application of Oxo-M and BK. (D) Bar graphs illustrate the comparison of the inward currents elicited by Oxo-M, BK and co-application of OxoM + BK. Each column represents the average ± S.E.M. from six experiments.

depletion of the Ca^{2+} store per se.

There are five distinct muscarinic receptor subtypes designated M_1-M_5 (Caulfield and Birdsall, 1998). Among these subtypes, M_1 and M_3 muscarinic receptors participate in the activation of extracellular Ca^{2+}-sensitive non-selective cation channels in rat intracardiac neurons (Hirayama et al., 2015). These subtypes of muscarinic receptors couple to $G_{q/11}$ and activate PLC, thus raising the possibility that BK and the muscarinic agonist OxoM activate the same cationic channels. As shown

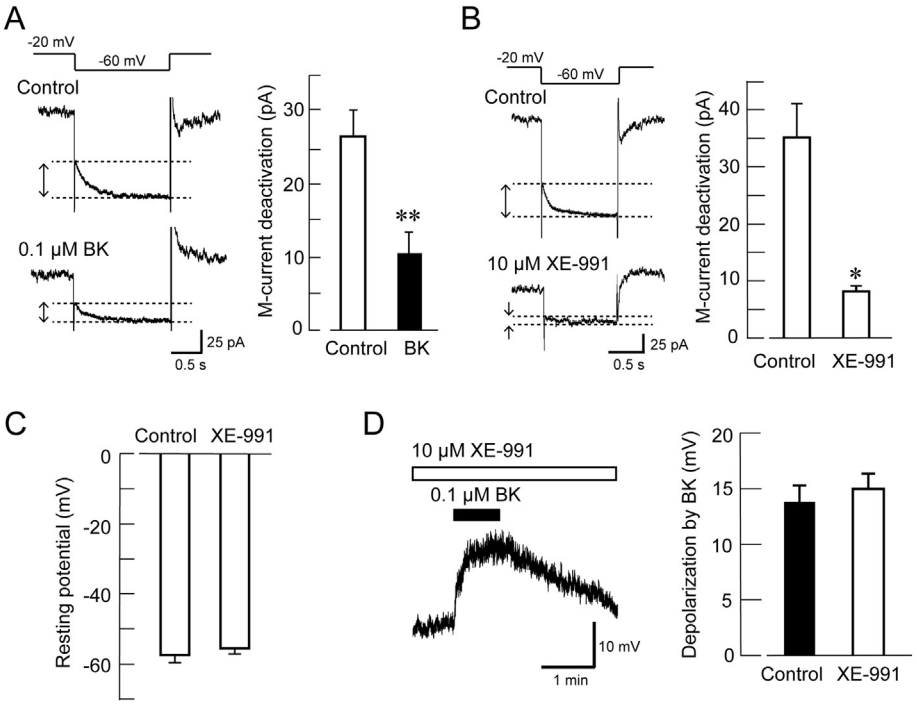

Fig. 7. Inhibition of M-currents. (A) Inhibition of M-current by BK. Left panel shows the representative M-current recordings for control (upper trace) and during BK application (lower trace). Right panel shows the amplitudes of M-current deactivation which are shown in left panel as double-headed arrow. Each column represents the average ± S.E.M. from five experiments. $^{**}P < 0.01$ vs control. (B) Effect of XE-991 on the M-current deactivation. $^{*}P < 0.05$ vs control. (C) Effect of XE-991 on resting membrane potential. (D) Effect of XE-991 on the BK-induced depolarization. Each column represents the average ± S.E.M. from five to eight experiments.

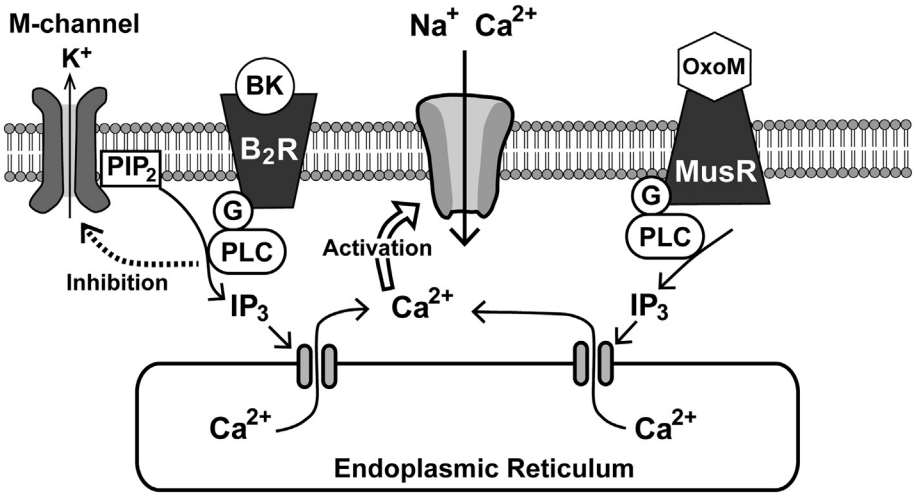

Fig. 8. Summary of the activation mechanisms proposed to explain the BK action. BK activates PLC via B_2 receptor and the PLC-mediated hydrolysis of PIP_2 inhibits M type K^+-channels. The hydrolysis of PIP_2 causes formation of IP_3 which evokes release of Ca^{2+} from endoplasmic reticulum via IP_3 receptors. Activation of muscarinic receptors is known to increase intracellular Ca^{2+} via PLC-IP_3 pathway. Increase in intracellular Ca^{2+} concentration is able to activate non-selective cation channels. B_2R: B2 receptor, G: G-protein, MusR: muscarinic receptor.

in Fig. 6, the inward currents elicited by submaximal concentrations of BK and OxoM are not additive, suggesting that the B_2 receptors and muscarinic receptors share a common mechanism of action (Fig. 8). Thus, the extracellular Ca^{2+}-sensitive cation channels may play a role in integrating multiple inputs from various types of receptors. Further studies will be required to establish the intracellular mechanism of the cation channel activation.

The M-type K^+ channels are voltage-dependent K^+ channels and activated at sub-threshold potentials (Hernandez et al., 2008). As shown in Fig. 7, BK reduced the deactivation of the M-current in isolated intracardiac neurons. The M-current inhibitor XE-991 mimicked the BK action on the M-current. Unexpectedly, neither the resting membrane potential nor the BK-induced depolarization were affected by XE-991. This may suggest that most of the M-type K^+ channels expressed in intracardiac ganglion neurons are inactive near the resting membrane potential and show greater activity upon depolarization. The M-current lacks voltage-dependent inactivation even at depolarized membrane potentials (Hernandez et al., 2008). These properties allow the firing of a single action potential under physiological conditions but make it difficult to repetitively fire action potentials at high frequencies. An increased M-current activity in the efferent parasympathetic ganglion cause an increased heart rate in spontaneous hypertensive rats (Berg, 2016). On the other hand, the dysfunction of M-currents is reported to be associated with neuronal hyperexcitability (Greene and Hoshi, 2017). In addition, Qi et al. (2014) reported that hyper-SUMOylation of Kv7 K^+ channels, which diminishes the M-current, causes severe bradycardia and sudden death. Thus, the BK-induced depolarization together with inactivation of M-currents may cause hyperexcitability of cardiac neurons, resulting in increased acetylcholine release in the heart.

In conclusion, the results of the present study indicate that BK activates rat intracardiac ganglion neurons. It is known that a number of patients experience bradycardia after myocardial infarction or during coronary occlusion (Airaksinen, 1999). Such conditions are responsible for sudden cardiac death. Kinins including BK and kallidin are generated and released during myocardial ischemia, and this can perpetuate further ischemic damage (Hashimoto et al., 1978). Thus, activation of intracardiac neurons by BK could play an important role in such cases of bradycardia.

Acknowledgements

The authors wish to thank Ms. Minatsu Inagawa for her assistance of immunocytochemical analysis. This work was supported by JSPS KAKENHI Grant Numbers 15H03046, 16K13050 and 19K07287. This study was also supported by grant from the Kitasato University School of Allied Health Sciences (Research project No. 2018-1004).

References

Airaksinen, K.E., 1999. Autonomic mechanisms and sudden death after abrupt coronary occlusion. Ann. Med. 31, 240–245.
Akaike, N., Harata, N., 1994. Nystatin perforated patch recording and its applications to analyses of intracellular mechanisms. Jpn. J. Physiol. 44, 433–473.
Albert, A.P., Large, W.A., 2002. A Ca^{2+}-permeable non-selective cation channel activated by depletion of internal Ca^{2+} stores in single rabbit portal vein myocytes. J. Physiol. 538, 717–728.
Allen, T.G., Burnstock, G., 1990. M1 and M2 muscarinic receptors mediate excitation and inhibition of guinea-pig intracardiac neurones in culture. J. Physiol. 422, 463–480.
Ashton, J.L., Burton, R.A.B., Bub, G., Smaill, B.H., Montgomery, J.M., 2018. Synaptic plasticity in cardiac innervation and its potential role in atrial fibrillation. Front. Physiol. 9, 240.
Barbe, F., Su, J.B., Guyene, T.T., Crozatier, B., Ménard, J., Hittinger, L., 1996. Bradykinin pathway is involved in acute hemodynamic effects of enalaprilat in dogs with heart failure. Am. J. Phys. 270, H1985–H1992.
Baxter, G.F., Ebrahim, Z., 2002. Role of bradykinin in preconditioning and protection of the ischaemic myocardium. Br. J. Pharmacol. 135, 843–854.
Berg, T., 2016. M-currents (Kv7.2-7.3/KCNQ2-KCNQ3) are responsible for dysfunctional autonomic control in hypertensive rats. Front. Physiol. 7, 584.
Braas, K.M., May, V., Harakall, S.A., Hardwick, J.C., Parsons, R.L., 1998. Pituitary adenylate cyclase-activating polypeptide expression and modulation of neuronal excitability in guinea pig cardiac ganglia. J. Neurosci. 18, 9766–9779.
Caulfield, M.P., Birdsall, N.J., 1998. International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacol. Rev. 50, 279–290.
Couture, R., Harrisson, M., Vianna, R.M., Cloutier, F., 2001. Kinin receptors in pain and inflammation. Eur. J. Pharmacol. 429, 161–176.
Cuevas, J., Adams, D.J., 1996. Vasoactive intestinal polypeptide modulation of nicotinic ACh receptor channels in rat intracardiac neurones. J. Physiol. 493, 503–515.
Cuevas, J., Harper, A.A., Trequattrini, C., Adams, D.J., 1997. Passive and active membrane properties of isolated rat intracardiac neurons: regulation by H- and M-currents. J. Neurophysiol. 78, 1890–1902.
Durães Campos, I., Pinto, V., Sousa, N., Pereira, V.H., 2018. A brain within the heart: a review on the intracardiac nervous system. J. Mol. Cell. Cardiol. 119, 1–9.
Emanueli, C., Maestri, R., Corradi, D., Marchione, R., Minasi, A., Tozzi, M.G., Salis, M.B., Straino, S., Capogrossi, M.C., Olivetti, G., Madeddu, P., 1999. Dilated and failing cardiomyopathy in bradykinin B_2 receptor knockout mice. Circulation 100, 2359–2365.
Faussner, A., Bathon, J.M., Proud, D., 1999. Comparison of the responses of B_1 and B_2 kinin receptors to agonist stimulation. Immunopharmacology 45, 13–20.
Gafni, J., Munsch, J.A., Lam, T.H., Catlin, M.C., Costa, L.G., Molinski, T.F., Pessah, I.N., 1997. Xestospongins: potent membrane permeable blockers of the inositol 1,4,5-trisphosphate receptor. Neuron 19, 723–733.
Greene, D.L., Hoshi, N., 2017. Modulation of Kv7 channels and excitability in the brain. Cell. Mol. Life Sci. 74, 495–508.
Haasemann, M., Cartaud, J., Mu¨ller-Esterl, W., Dunia, I., 1998. Agonist-induced redistribution of bradykinin B_2 receptor in caveolae. J. Cell Sci. 111, 917–928.
Hashimoto, K., Hamamoto, H., Honda, Y., Hirose, M., Furukawa, S., Kimura, E., 1978. Changes in components of kinin system and hemodynamics in acute myocardial infarction. Am. Heart J. 95, 619–626.
Helliwell, R.M., Large, W.A., 1996. Dual effect of external Ca^{2+} on noradrenaline-activated cation current in rabbit portal vein smooth muscle cells. J. Physiol. 492, 75–88.
Hernandez, C.C., Zaika, O., Tolstykh, G.P., Shapiro, M.S., 2008. Regulation of neural KCNQ channels: signalling pathways, structural motifs and functional implications. J. Physiol. 586, 811–1821.
Higashida, H., Brown, D.A., 1986. Two polyphosphatidylinositide metabolites control two

K^+ currents in a neuronal cell. Nature 323, 333–335.
Hirayama, M., Ogata, M., Kawamata, T., Ishibashi, H., 2015. Muscarinic receptor-mediated excitation of rat intracardiac ganglion neurons. Neuropharmacology 95, 395–404.
Ishibashi, H., Umezu, M., Jang, I.S., Ito, Y., Akaike, N., 2003. Alpha 1-adrenoceptor-activated cation currents in neurones acutely isolated from rat cardiac parasympathetic ganglia. J. Physiol. 548, 111–120.
Ishibashi, H., Moorhouse, A.J., Nabekura, J., 2012. Perforated whole-cell patch-clamp technique: user's guide. In: Okada, Y. (Ed.), Patch Clamp Techniques: From Beginning to Advanced Protocols, Springer Protocols Handbooks. Springer, pp. 71–83. https://doi.org/10.1007/978-4-431-53993-3_4.
Ito, H., Hayashi, I., Izumi, T., Majima, M., 2003. Bradykinin inhibits development of myocardial infarction through B_2 receptor signalling by increment of regional blood flow around the ischaemic lesions in rats. Br. J. Pharmacol. 138, 225–233.
Iwamoto, T., Watano, T., Shigekawa, M., 1996. A novel isothiourea derivative selectively inhibits the reverse mode of Na^+/Ca^{2+} exchange in cells expressing NCX1. J. Biol. Chem. 271, 22391–22397.
Izrailtyan, I., Kresh, J.Y., 1997. Bradykinin modulation of isolated rabbit heart function is mediated by intrinsic cardiac neurons. Cardiovasc. Res. 33, 641–649.
Kadamur, G., Ross, E.M., 2013. Mammalian phospholipase C. Annu. Rev. Physiol. 75, 127–154.
Kajekar, R., Myers, A.C., 2000. Effect of bradykinin on membrane properties of guinea pig bronchial parasympathetic ganglion neurons. Am. J. Physiol. Lung Cell Mol. Physiol. 278, L485–L491.
Kaufman, M.P., Baker, D.G., Coleridge, H.M., Coleridge, J.C., 1980. Stimulation by bradykinin of afferent vagal C-fibers with chemosensitive endings in the heart and aorta of the dog. Circ. Res. 46, 476–484.
Kawaguchi, A., Sato, M., Kimura, M., Yamazaki, T., Yamamoto, H., Tazaki, M., Ichinohe, T., Shibukawa, Y., 2015. Functional expression of bradykinin B1 and B2 receptors in neonatal rat trigeminal ganglion neurons. Front. Cell. Neurosci. 9, 229.
Kimura, E., Hashimoto, K., Furukawa, S., Hayakawa, H., 1973. Changes in bradykinin level in coronary sinus blood after the experimental occlusion of a coronary artery. Am. Heart J. 85, 635–647.
Kraft, R., 2007. The Na^+/Ca^{2+} exchange inhibitor KB-R7943 potently blocks TRPC channels. Biochem. Biophys. Res. Commun. 361, 230–236.
Majewski, L., Kuznicki, J., 2015. SOCE in neurons: signaling or just refilling? Biochim. Biophys. Acta 1853, 940–1952.
Marceau, F., Hess, J.F., Bachvarov, D.R., 1998. The B_1 receptors for kinins. Pharmacol. Rev. 50, 357–386.
Mercer, J.C., Dehaven, W.I., Smyth, J.T., Wedel, B., Boyles, R.R., Bird, G.S., Putney Jr., J.W., 2006. Large store-operated calcium selective currents due to co-expression of Orai1 or Orai2 with the intracellular calcium sensor, Stim1. J. Biol. Chem. 281, 24979–24990.
Miller, M., Shi, J., Zhu, Y., Kustov, M., Tian, J.B., Stevens, A., Wu, M., Xu, J., Long, S., Yang, P., Zholos, A.V., Salovich, J.M., Weaver, C.D., Hopkins, C.R., Lindsley, C.W., McManus, O., Li, M., Zhu, M.X., 2011. Identification of ML204, a novel potent antagonist that selectively modulates native TRPC4/C5 ion channels. J. Biol. Chem. 286, 33436–33446.
Mochidome, T., Ishibashi, H., Takahama, K., 2001. Bradykinin activates airway parasympathetic ganglion neurons by inhibiting M-currents. Neuroscience 105, 785–791.
Munoz, C.M., Leeb-Lundberg, L.M., 1992. Receptor-mediated internalization of bradykinin. DDT1 MF-2 smooth muscle cells process internalized bradykinin via multiple degradative pathways. J. Biol. Chem. 267, 303–309.
Murase, K., Ryu, P.D., Randic, M., 1989. Excitatory and inhibitory amino acids and peptide-induced responses in acutely isolated rat spinal dorsal horn neurons. Neurosci. Lett. 103, 56–63.
Nakagawa, H., Scherlag, B.J., Patterson, E., Ikeda, A., Lockwood, D., Jackman, W.M., 2009. Pathophysiologic basis of autonomic ganglionated plexus ablation in patients with atrial fibrillation. Heart Rhythm. 6 (12), S26–S34 Suppl.
Neto, F.R., Brasil, J.C.F., Antonio, A., 1974. Bradykinin-induced coronary chemoreflex in the dog. Naunyn Schmiedeberg's Arch. Pharmacol. 283, 135–142.
Okamoto, H., Unno, T., Arima, D., Suzuki, M., Yan, H.-D., Matsuyama, H., Nishimura, M., Komori, S., 2004. Phospholipase C involvement in activation of the muscarinic receptor-operated cationic current in guinea pig ileal smooth muscle cells. J. Pharmacol. Sci. 95, 203–213.
Owsianik, G., Talavera, K., Voets, T., Nilius, B., 2006. Permeation and selectivity of TRP channels. Annu. Rev. Physiol. 68, 685–717.
Pellacani, A., Brunner, H.R., Nussberger, J., 1994. Plasma kinins increase after angiotensin-converting enzyme inhibition in human subjects. Clin. Sci. 87, 567–574.
Prado, G.N., Taylor, L., Zhou, X., Ricupero, D., Mierke, D.F., Polgar, P., 2002. Mechanisms regulating the expression, self-maintenance, and signaling-function of the bradykinin B2 and B1 receptors. J. Cell. Physiol. 193, 275–286.
Qi, Y., Wang, J., Bomben, V.C., Li, D.P., Chen, S.R., Sun, H., Xi, Y., Reed, J.G., Cheng, J., Pan, H.L., Noebels, J.L., Yeh, E.T., 2014. Hyper-SUMOylation of the Kv7 potassium channel diminishes the M-current leading to seizures and sudden death. Neuron 83, 1159–1171.
Rabito, S.F., Minshall, R.D., Nakamura, F., Wang, L.X., 1996. Bradykinin B_2 receptors on skeletal muscle are coupled to inositol 1,4,5-trisphosphate formation. Diabetes 45 (Suppl. 1), S29–S33.
Regoli, D., Plante, G.E., Gobeil Jr., F., 2012. Impact of kinins in the treatment of cardiovascular diseases. Pharmacol. Ther. 135, 94–111.
Scherlag, B.J., Nakagawa, H., Jackman, W.M., Yamanashi, W.S., Patterson, E., Po, S., Lazzara, R., 2005. Electrical stimulation to identify neural elements on the heart: their role in atrial fibrillation. J. Interv. Card. Electrophysiol. 13 (Suppl. 1), 37–42.
Schwarz, J.R., Glassmeier, G., Cooper, E.C., Kao, T.C., Nodera, H., Tabuena, D., Kaji, R., Bostock, H., 2006. KCNQ channels mediate IKs, a slow K^+ current regulating excitability in the rat node of Ranvier. J. Physiol. 573, 17–34.
Shimamoto, K., Miura, T., Miki, T., Iimura, O., 1992. Activation of kinins on myocardial ischemia. Agents Actions Suppl. 38, 90–97.
Smyth, J.T., DeHaven, W.I., Jones, B.F., Mercer, J.C., Trebak, M., Vazquez, G., Putney Jr., J.W., 2006. Emerging perspectives in store-operated Ca^{2+} entry: roles of Orai, Stim and TRP. Biochim. Biophys. Acta 1763, 1147–1160.
Su, J.B., Barbe, F., Crozatier, B., Campbell, D.J., Hittinger, L., 1999. Increased bradykinin levels accompany the hemodynamic response to acute inhibition of angiotensin-converting enzyme in dogs with heart failure. J. Cardiovasc. Pharmacol. 34, 700–710.
Suh, B., Hille, B., 2002. Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. Neuron 35, 507–520.
Thastrup, O., Cullen, P.J., Drøbak, B.K., Hanley, M.R., Dawson, A.P., 1990. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. Proc. Natl. Acad. Sci. U. S. A. 87, 2466–2470.
Vivas, O., Kruse, M., Hille, B., 2014. Nerve growth factor sensitizes adult sympathetic neurons to the proinflammatory peptide bradykinin. J. Neurosci. 34, 1959–11971.
Whyte, K.A., Hogg, R.C., Dyavanapalli, J., Harper, A.A., Adams, D.J., 2009. Reactive oxygen species modulate neuronal excitability in rat intrinsic cardiac ganglia. Auton. Neurosci. 150, 45–52.
Xi-Moy, S.H., Dun, N.J., 1995. Potassium currents in adult rat intracardiac neurons. J. Physiol. 486, 15–31.
Xu, Z.J., Adams, D.J., 1992. Resting membrane potential and potassium currents in cultured parasympathetic neurones from rat intracardiac ganglia. J. Physiol. 456, 405–424.
Yang, X.P., Liu, Y.H., Scicli, G.M., Webb, C.R., Carretero, O.A., 1997. Role of kinins in the cardioprotective effect of preconditioning: study of myocardial ischemia/reperfusion injury in B_2 kinin receptor knockout mice and kininogen-deficient rats. Hypertension 30, 735–740.
Zhang, H., Mei, X., Zhang, P., Ma, C., White, F.A., Donnelly, D.F., LaMottea, R.H., 2009. Altered functional properties of satellite glial cells in compressed spinal ganglia. Glia 57, 1588–1599.
Zubakova, R., Gille, A., Faussner, A., Hilgenfeldt, U., 2008. Ca^{2+} signalling of kinins in cells expressing rat, mouseand human B_1/B_2-receptor. Int. Immunopharmacol. 8, 276–281.