Korean J Physiol Pharmacol
Vol 3: 615−621, December, 1999

Effects of Bradykinin on Intracellular Calcium Transients in Cardiac Myocytes

Choon-Ok Park, Yang Mi Kim, Jae Hee Han, David G. Allen[1], and Seong Geun Hong

Department of Physiology, College of Medicine Gyeongsang National University, Jinju 660−751, Korea; [1]Department of Physiology, University of Sydney, NSW, Australia

In spite many evidences has supported the cardioprotective effect of bradykinin, its direct effects at the cell level are still under question. We investigated the both effects of bradykinin (BK) on Ca^{2+}-related ionic currents using whole cell voltage clamp technique in rabbit cardiomyocytes and on the intracellular Ca^{2+} transient using calcium sensitive fluorescence dye, indo-1AM. Simultaneously with recording intracellular Ca^{2+} transients, cell contractility was estimated from the changes in length of the electrical stimulated rat cardiac myocytes. L-type Ca^{2+} current decreased by bradykinin at the entire voltage range. Inward tail current increased initially up to its maximum about 4 min after exposing myocytes to BK, and then gradually decreased again by further exposure to BK. This tail current decreased remarkably at washing BK off but slowly recovered ca. 20 min later. The change in cell contractility was similar to that in tail current showing initial increase followed by gradual decrease. Removal of BK brought remarkable decrease in contractility, which was recovered 15~20 min after cessation of electrical stimulation. Bradykinin increased Ca^{2+} transient initially but after some time Ca^{2+} transient also decreased coincidentally with contractility. From these results, it is suggested that bradykinin exerts directly its cardioprotective effect on the single myocytes by decreasing the intracellular Ca^{2+} level followed by an initial increase in Ca^{2+} transient.

Key Words: Bradykinin, Cardioprotection, Intracellular calcium transient, Calcium current, Inward tail current, Contractility

INTRODUCTION

The mechanism of cardioprotection after cardiac ischemia and reperfusion has long been of interest because of the clinical importance of myocardial infarction. Since the first report about the beneficial effect of the increased level of blood kinin following ischemia on the survival rate and prognosis in man (Hashimoto et al, 1978), the role of bradykinin (BK) related with the cardioprotection after ischemia has been widely studied. In isolated rat hearts subjected to ischemia with reperfusion, treatment of bradykinin improved myocardial function and reduced ventricular fibrillation (Linz et al, 1989, 1992). Pathohistological examination showed that BK reduced infarct size (Maratorana et al, 1990). The recent molecular studies revealed the close relationship between the genetic defect in the expression of a special enzyme linked to blood kinin system and the incidence and prognosis of myocardial infarction (Cambien et al, 1992). In spite of these evidences supporting cardioprotective effect of bradykinin, a direct action of BK on cardiac myocytes remains unclear, which is essential to understand its cardioprotection mechanism.

It is well known that the elevation of intracellular free calcium concentration ($[Ca^{2+}]_i$) during cardiac ischemia and reperfusion causes an ischemic arrhythmia and cell damage (Murphy et al, 1987; Lee & Allen, 1991). Since several reports showed that BK induced the mobilization and elevation of $[Ca^{2+}]_i$ in some tissues (Luckhoff et al, 1988), BK may serve

Corresponding to: Choon-Ok Park, Department of Physiology, College of Medicine, Gyeongsang National University, Jinju 660-280, Korea. (Tel) 0591-751-8721 (Fax) 0591-759-0169, (E-mail) annap@zipworld.com.au

as a regulator of intracellular Ca^{2+}. Therefore, it is probable that BK modulates $[Ca^{2+}]_i$ in heart cell, finally leading to a cardioprotective effect against ischemia and reperfusion injury. In this study, we investigated the effects of BK on the factors which affect intracellular Ca^{2+}, such as Ca^{2+} current, Ca^{2+} activated-tail currents, intracellular Ca^{2+} transient and contractility, to elucidate the role of BK in the regulation of intracellular Ca^{2+}.

METHODS

Single cardiac cells from rabbit (either sex, ca. 1 kg) or rat (male Wistar rats of 250 g) were enzymatically isolated at the Langendorff column at 37°C for coronary perfusion. Rabbit cells were used in ionic currents measurement and rat cells were used when intracellular calcium transient and contractility were measured.

Ionic currents were recorded at room temperature (25°C) by using whole cell voltage clamp method with a patch-clamp amplifier (EPC-7, LIST, Germany). To measure the calcium current, the pipette solution contained (mM): Cs-asparate, 110; Mg-ATP, 5; di-Tris creatine phosphate, 5; $MgCl_2$, 1; HEPES, 5; tetraethylammonium chloride, 20; EGTA, 5, pH 7.4 with CsOH. To measure the inward tail current, 10 mM, KCl, 0.1 mM EGTA were used instead of Cs-aspartate and 5 mM EGTA. These chemicals were obtained from Sigma (St. Louis, MO, USA).

Intracellular Ca^{2+} transient was measured using Ca^{2+} sensitive fluorescence dye, indo 1-AM (Molecular Probe, USA). Isolated rat ventricular cells were loaded in indo-1 AM 5 μM for 20 min and then washed out. Illuminating light was 360 nm and dual emission light of 400 nm and 500 nm were collected to each photomultiplier tubes. The ratio of the intensity at 400 : 500 nm were obtained with spectrofluorimeter and stored in the computer. To minimize the bleaching ND 1.5 filter was used. Field electrical stimulation was applied at 0.5 Hz using stimulator (model SD9, Grass).

Cell contractility was estimated from the changes in cell length during field stimulation. Contraction of the cell was recorded by spectrofluorimeter-attatched video camera and the length change was obtained by video monitor rater line. All signals were stored in computer and analyzed using Acknowledge and Sigma Plot program.

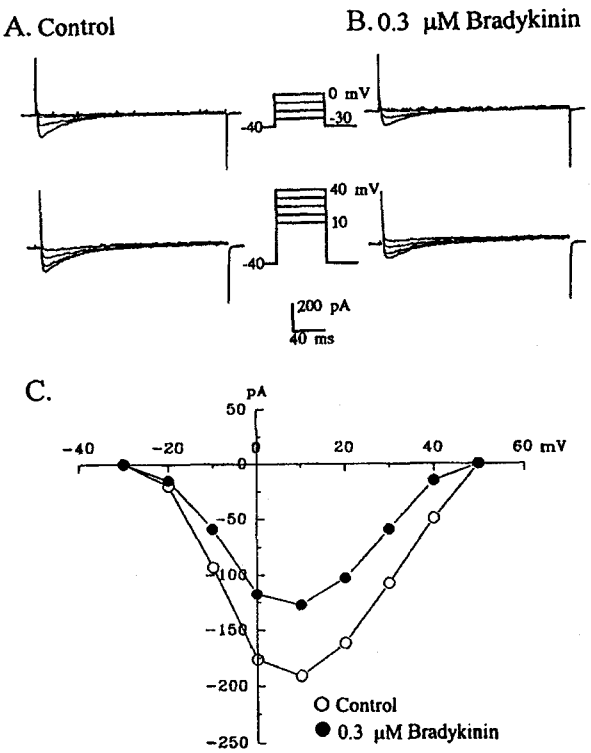

Fig. 1. The effect of bradykinin on the current-voltage relationship of the calcium current. A: Calcium currents in normal Tyrode solution. B: Calcium currents of the same cell as A after application of 0.3 μM bradykinin. C: The current-voltage relationship for the calcium currents obtained from A and B. Calcium currents were decreased by bradykinin in the whole range of potential.

RESULTS

The effect of bradykinin (BK) on the Ca^{2+} current, which is the major Ca^{2+} influx from cell exterior, was examined in rabbit atrial myocytes (Fig. 1). Peak Ca^{2+} currents were recorded at various depolarizing pulses from a holding potential of −40 mV and they decreased over the entire voltage range without changing voltage-dependency by 0.3 μM BK (n=4).

Hyperpolarization of −70 mV following the brief depolarizing step pulse (+40 mV) of 2 ms activated an inward current. This tail current has been known to be activated by increased intracellular Ca^{2+} transient and to be dependent upon sodium concentration gradient across the cell membrane (Earm et al, 1989; 1990). That is, a current reflecting the Na/Ca exchanger activity. Fig. 2 shows the effects of BK on the inward tail currents of the single cell of rabbit

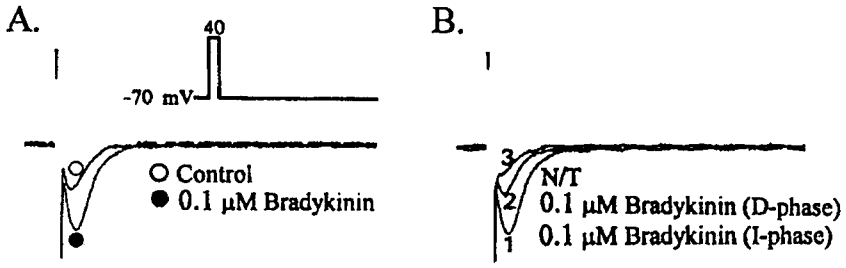

Fig. 2. Effects of bradykinin on the inward tail currents of the single rabbit atrial cell. A: The tail current was increased remarkably at the initial phase of superfusing 0.1 μM bradykinin. B: The tail current was decreased progressively (trace 2: 10 min after the exposure) following the initial increasing phase (trace 1: 4 min after exposure). When the superfusing solution was returned to normal Tyrode solution, tail current was decreased abruptly (trace 3).

atria. The magnitude of the current increased initially reached a peak level at 5±2 min by 0.1 μM bradykinin (n=6) (Fig. 2A), and then progressively decreased. This alteration due to sustained exposure to BK could be arbitrarily divided into two phases, *I-phase* as an increasing phase and *D-phase* as a decreasing phase (Fig. 2B). The magnitude of the current got smaller than the control at about 10 min later after the BK exposure. The current decreased remarkably by washing off (trail 3, Fig. 2B) but recovered after 20 min of resting period (n=4).

Fig. 3 shows that these effects of BK on the inward tail current were reproducible. The Ca^{2+} sensitive inward tail currents initially increased by each exposure to 0.3 μM BK followed by gradual decrease with sustained exposure. In response to the repeated application of BK (0.3 μM) in a same cell, the current maximums in the *I-phase* were gradually reduced (e.g. closed circles in first trial *vs.* those in third trial in Fig. 3). This implies that BK eventually leads to decrease in tail currents although the tail current amplitude was enhanced at the beginning of the BK application (trace a *vs* c in Fig. 3). Application of isoprenaline (ISP, 2 nM) increased tail currents (see trace d) and further addition of BK increased the currents almost twice (trace e). However methylene blue (MB; 10 μM) reversed a current portion increased by BK to a level of current amplitude before adding BK (trace f in Fig. 3), suggesting that MB selectively blocked the effect of BK. Therefore it implies that an underlying mechanism of BK on intracellular Ca^{2+} transient is different from that of ISP.

If BK brought some changes in intracellular calcium status, it would affect contractility of the cell. Fig. 4 shows the effect of the repeated exposures (for 1 min) of 1 μM BK in single rat ventricular cell. The contractility was estimated from the cell length shortening during field stimulation at 0.5 Hz. The

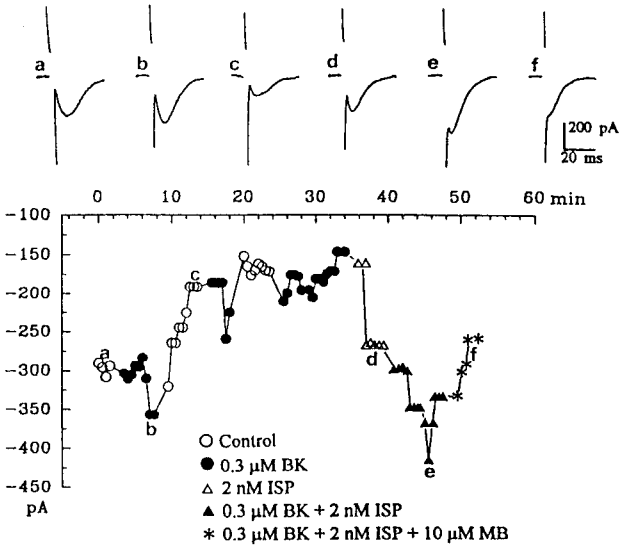

Fig. 3. Effects of the repetitive exposure to bradykinin (BK, 0.3 μM) and isoproterenol on the Ca^{2+} sensitive inward tail currents. Each episode of the BK application shows an initial increase (I-phase) in the inward tail current (closed circles, ●). The current amplitudes after washing BK (open circles, o) were more reduced than the previous trial (*compare open circles in between b and c, c and d in the lower panel*). Gradual decreases in currents (D-phase) was shown in third trial of the long-lasted BK application (● between c and d). MB stands for methylene blue. Tail currents were obtained at every 30 s in a same cell.

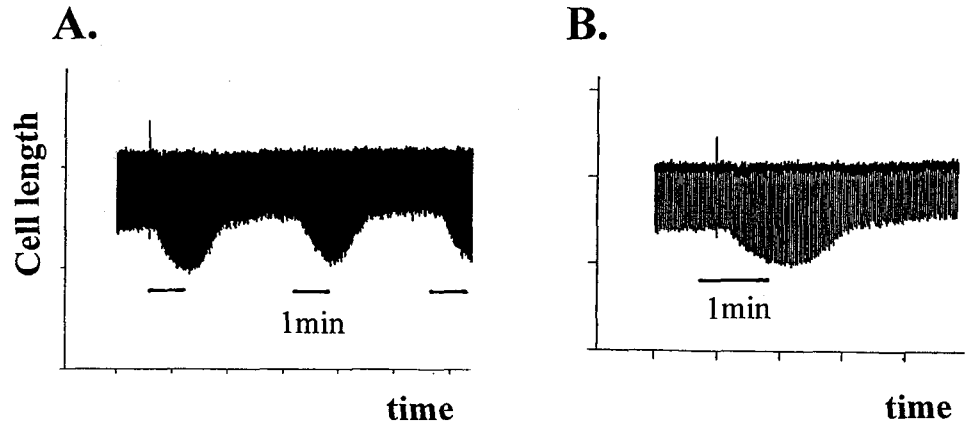

Fig. 4. Effect of bradykinin (BK, 1 μM) on the contraction in single ventricular myocyte of the rat. Shortening of the cell length indicates the increase of contractility. The contractility increased during the short exposure to BK. But after washing off BK, the contractility was smaller than that of the previous ones before exposure. With repeated exposures, contractility became smaller (A).

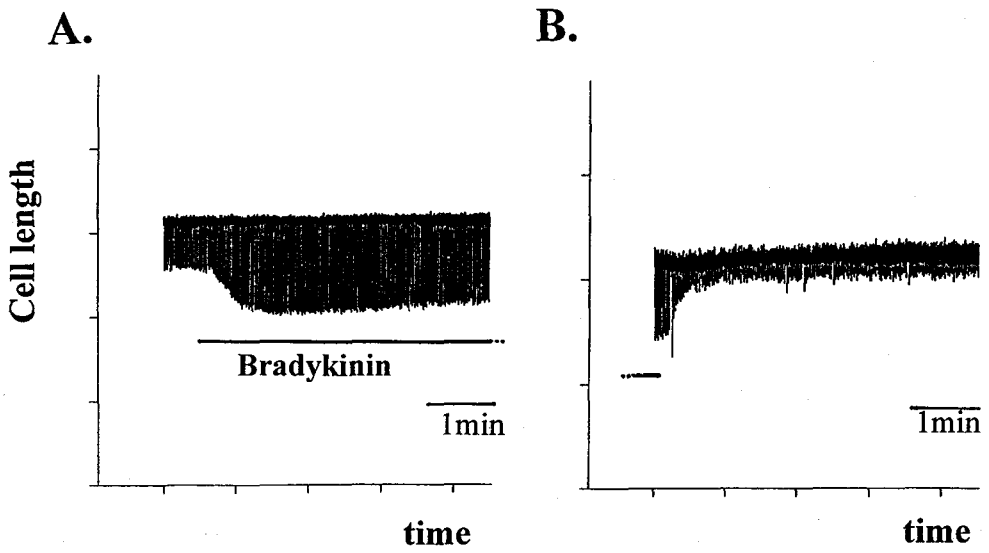

Fig. 5. Changes in contractility during long exposure to bradykinin (BK; 1 μM). A: The contractility of cell was increased by BK but it gradually decreased. B: When the cell was washed off after exposure to BK for 20 min, the contractility remarkably decreased. Panel A and B partially adopted from a recording in a same cell.

contractility increased during 1 min's exposure of BK and then decreased by washing it off. The contractility became smaller than the pre-exposure control after 3 min (n=7) (Fig. 4B). During sustained exposure to bradykinin for 20 min, the changes in contractility was similar to that of inward tail current showing initial increase followed by gradual decrease (n=5) (Fig. 5A). On washing off BK, the contractility decreased remarkably (Fig 5B). But it was recovered after the rest of 15~20 min (n=4) without stimulation.

Fig. 6 shows the effects of BK on the intracellular Ca^{2+} transient recorded simultaneously with cell contractility. Calcium transient was represented by the indo-1 AM fluorescence ratio of the intensity at 400 : 500 nm and cell contractility was estimated by the changes in cell length during field stimulation.

Dotted line in Fig. 6A, B, shows the contractility

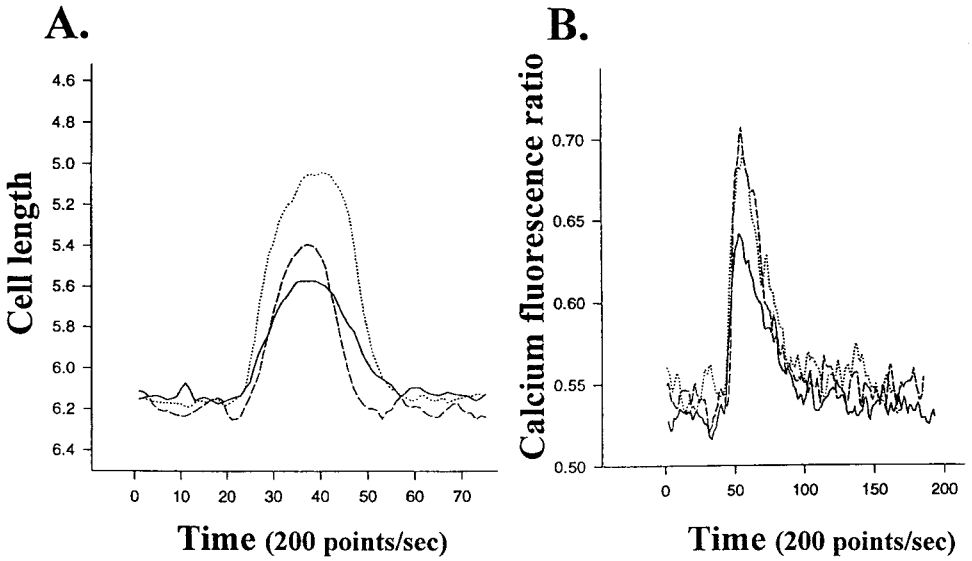

Fig. 6. Effect of bradykinin (BK; 1 μM) on cell length and fluorescence ratio. Ca^{2+} fuorescence ratio at 400 : 500 nm with indo-1 AM indicates the changes in intracellular Ca^{2+} transient. Records drawn as solid line were measured before the exposure to BK, dotted lines were at 3 min, and semi-dotted lines were 10 min of exposure to BK, respectively. BK increased both contractility and Ca^{2+} transient. However at 10 min exposure to BK, there is a difference between cell contractility and Ca^{2+} transient (semi-dotted lines in A and B), comparing to those (dotted lines) recorded 3 min later.

and the Ca^{2+} transient 3 min later BK exposure and those of semi-dotted lines were recorded 10 min later, respectively. They both increased 3 min later at the beginning of BK exposure (dotted line). However 10 min later, the contractility clearly decreased (semi-dotted line), while intracellular Ca^{2+} transient kept still high. However, longer treatment of BK for more than 10 min, both Ca^{2+} transient and cell contractility decreased (Data not shown).

DISCUSSION

In spite of growing evidences for the cardio-protective effect of bradykinin (Linz et al, 1997), its direct effects on cardiac cells are still under question. However, our study with single cardiac cells strongly suggested that bradykinin can directly affect on the cardiac cells.

Intracellular Ca^{2+} level is generally determined by the dynamic balance of Ca^{2+} influx, intracellular Ca^{2+} release from sarcoplasmic reticulumn (SR), Ca^{2+} efflux and the affinity with intracellular buffers. Extracellular Ca^{2+} enters into the cell via calcium channels and stimulates Ca^{2+} release from SR. Our result showed that BK restricts calcium influx to the cell by decreasing Ca^{2+} current. However, the BK effect on the other factors requires a careful interpretation because of their reciprocal effects on the others. Na/Ca exchanger is the major mechanism of Ca^{2+} efflux from the cell, which is activated by the increased Ca^{2+} transient mainly released from intracellular Ca^{2+} store, such as SR. The inward tail current recorded on repolarization after brief depolarization pulse has been suggested as the indicator of Na/Ca exchanger activity with its close relation to the changes in intracellular calcium transient (Earm et al, 1989; 1990). This study showed the biphasic effect of BK on the inward tail current (*I-phase* and *D-phase*). The *I-phase* in the inward tail current is likely that BK facilitates Ca^{2+} release from intracellular store. Our result that BK elevates both cell contractility and Ca^{2+} transient (Fig. 6) can support this implication. This is also supported by evidence that BK mobilized 9.4% of intracellular Ca^{2+} stores in cardiomyocytes of the neonatal rat, as assessed by chlortetracycline-based fluorometry (Nakamura et al, 1996). For the BK effect in *D-phase*, it was reasonable to be explained by a negative staircase phenomenon reported in heart cells (Hilgemann, 1986).

After some resting period following post-stimulatory potentiation in rabbit atrium, the contractile force generated by a first stimulus (first post-rest stimulation) was largest, but gradually decreased again by subsequent train of stimuli. This negative staircase might reflect changes in extracellular Ca^{2+} transients showing the maximal increase by the first stimuli after resting interval. Hilgemann (1986) suggested that the reduction in the contractile force by subsequent stimuli followed by the first post-rest stimulation be due to Ca^{2+} efflux out of cell, leaving intracellular Ca^{2+} unavailable to mobilize for the next stimulation. This Ca^{2+} efflux altered extracellular Ca^{2+} transients, which affects to the Na/Ca exchange. This alteration in Na/Ca exchange linked to extracellular Ca^{2+} transients caused the shape of action potential (AP) to be changed, especially in the late plateau phase of cardiac AP. The inward tail current, indicating the Na/Ca exchange activity, contributes to the late plateau phase and this current shows the negative staircase phenomenon by the post-rest stimulation (Earm et al, 1989; 1990). Accordingly, a negative staircase phenomenon can be applied to explain our experiment that the magnitude of the current gradually decreased by repeated or sustained BK exposure.

If BK enhanced Ca^{2+} mobilisation, Na/Ca exchanger activity might be increased, which reflected an increase in inward tail current. At the same time, an increased activity of the Na/Ca exchanger facilitated further Ca^{2+} efflux out of the cell without influx Ca^{2+} through channel (*refer to inhibitory effect of BK on the Ca^{2+} current in Fig. 1*). Due to imbalance between influx and efflux of Ca^{2+}, the amount of released Ca^{2+} from SR might be gradually depleted in spite of the BK-induced facilitation of Ca^{2+} release from SR. This accounts for the *D-phase*. The sudden decrease in the inward tail current with washing off BK suggested that intracellular calcium have been decreased by the sustained increase in calcium efflux. This idea was supported by the fact that the current was recovered after washing off BK if enough time was given (more than 20 min, n=4).

Responses of cell contractility and intracellular Ca^{2+} transient to BK were similar to those of inward tail currents showing *I-phase* and *D-phase*. However there was a difference between the contractility and the Ca^{2+} transient. As shown in Fig. 6, the reduction in cell contractility was rapid, comparing to that Ca^{2+} transient. This suggested that the affinity of Ca^{2+} to myofilaments, one of the important Ca^{2+} buffers, becomes low. This could provide more chance of Ca^{2+} efflux by an enhanced Na/Ca exchange, resulting in the lowered intracellular Ca^{2+}. Shah et al (1994) observed similar results in rat cardiac myocytes by 8-Br-cGMP to those by BK in our experiment, which the contractility increased initially and then decreased by the sustained exposure. They also observed reduced the myofilament response to Ca^{2+} in rat cardiac myocytes. They found that contractility was decreased faster than Ca^{2+} level did.

The cardioprotective effect of BK seems to be mediated by NO signalling system (Balligand et al, 1993). This suggestion was further supported by that NO, cGMP were formed by BK, and NO inhibitors abolished effect of BK (Linz et al, 1989). We also tested this hypothesis using nitroprusside and 8-Br cGMP (Data not shown). These effects on the Ca^{2+} current and inward tail current were very similar to those of BK and those effects were blocked by L-NNA and methylene blue. Therefore, the mechanism of cardioprotective effect of BK related to NO signalling system will further to be studied.

In conclusion, BK does exert bimodal action on Ca^{2+} channels and SR. That is, restriction of Ca^{2+} influx by reducing Ca^{2+} current and facilitation of Ca^{2+} mobilisation from SR. This brings out more Ca^{2+} efflux by increasing activity of Na/Ca exchanger, which could deplete intracellular Ca^{2+} store. This might be beneficial to cardiac cells to be protected from Ca^{2+} overload encountered on reperfusion after ischemia.

ACKNOWLEDGEMENT

This study was supported by a grant (KOSEF 971-0704-033-1) from the Korea Science and Engineering Foundation.

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