

Activation of Ca²⁺-dependent K⁺ Channels Is Essential for Bradykinin-induced Microglial Migration

M. Ifuku, B. Wang and M. Noda

Laboratory of Pathophysiology, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Fukuoka, Japan

Summary

Bradykinin (BK) is produced and acts at the site of injury and inflammation not only in periphery but also in the brain. In the central nervous system, migration of microglia towards damaged tissue plays a role in regeneration under pathological condition. In the present study, we found that bradykinin (BK) induced migration of cultured microglia, which was blocked by charybdotoxin, a blocker of large conductance Ca²⁺-dependent K⁺ channels, but not by pertussis toxin (PTX). These results indicate that activation of large conductance Ca²⁺-activated K⁺ channel is required for BK-induced microglial migration, while activation of PTX-sensitive G protein is not. Our findings may help to understand the function of kinins in the brain and the role of microglia in response to brain injury.

Introduction

Activation of microglial cells, the resident macrophages of the brain, occurs rapidly following brain injury. Activated microglia migrate rapidly to the affected sites of the CNS. Bradykinin (BK), a mediator of inflammation and a vasodepressor, is produced in the brain during trauma and stroke, and is thought to open the blood-brain barrier. BK receptors are couple to multiple signal transduction (Noda *et al.* 2004). One is mobilization of intracellular Ca²⁺ induced by inositol 1,4,5-trisphosphate (IP₃) and activation of Ca²⁺-dependent K⁺ channels. BK receptor was also reported to couple to G_{i/o} protein (Liebmann, 2001).

Recently, a variety of factor stimulating microglial motility has been identified, such as ATP (Honda *et al.* 2001), cannabinoid (Franklin *et al.* 2003, Walter *et al.* 2003) and morphine (Takayama *et al.* 2005). It was reported that ATP-induced migration was inhibited by treatment of microglial cells with pertussis toxin (PTX), suggesting that activation of $G_{i/o}$ protein induced by P2Y receptor was important (Honda *et al.* 2001). On the other hand, the importance of Ca^{2+} -activated K^+ channels was reported for lysophosphatidic acid (LPA)-induced migration of microglia (Schilling *et al.* 2004).

In the present study, we tested whether BK induces migration of microglia, because BK induced Ca^{2+} -dependent K^+ currents (Noda *et al.* 2003). Furthermore, we investigated the mechanism of BK-induced migration in comparison with that of ATP-induced chemotaxis.

Materials and Methods

Cell culture

Microglial cells were isolated from the mixed cultures of cerebrocortical cells from postnatal day 3 Wistar rats (Kyudo, Kumamoto, Japan) as described previously (Noda *et al.* 1999).

Migration experiments

Migration of microglia was monitored with time lapse video microscopy. The video camera (Nikon inverted microscope, TE-2000-E) was controlled by Luminavision software (O-kumashokai, Fukuoka, Japan). Images were acquired in 1-min intervals for 1 hour and stored on a computer and analyzed by Dipp-Motion 2D (DITECT, Tokyo, Japan)

Results

Bradykinin increased migration of cultured rat microglia

Bradykinin (100 and 300 nM) increased migration of microglia in a concentration-dependent manner. In the presence of 300nM BK, the total distance of microglial migration for 1 hour was increased 2-fold (Fig.1A) and it continued to increase for 18 hours (data not shown).

The mechanism of bradykinin-induced microglial migration

Since functional importance of Ca^{2+} -activated K^+ channels was reported in LPA-induced microglial migration (Schilling *et al.* 2004), charybdotoxin (CTX), a blocker of large conductance Ca^{2+} -activated K^+ channels, was tested whether or not to block BK-induced microglial

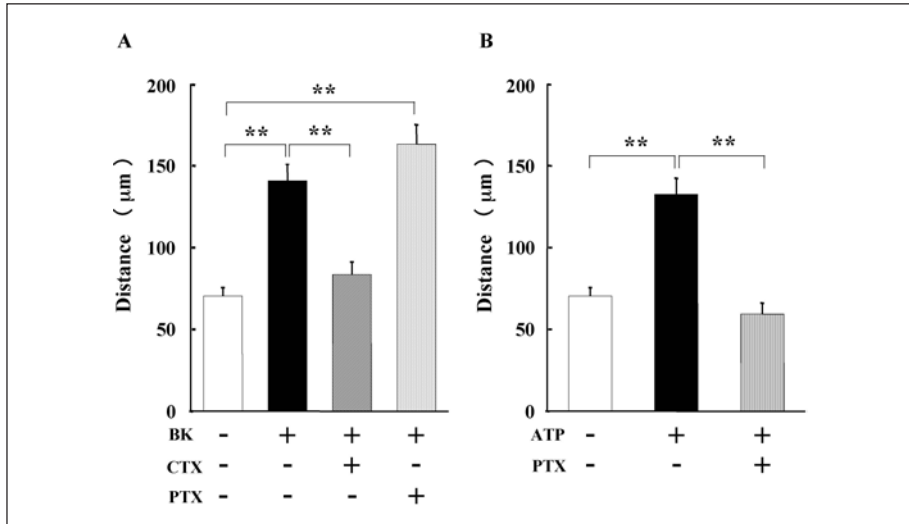


Figure 1. Bradykinin (BK)- and ATP-induced microglial migration and the effects of charybdotoxin (CTX) and pertussistoxin (PTX). **A**, Total migration distance (μm) for 1 hour of microglial cells was increased by BK (300 nM). CTX (0.1 μM), applied together with BK, inhibited BK-induced migration. PTX (50 ng/ml), pretreated for 12 hr with addition of 50 ng/ml PTX before the measurement of migration, did not have significant effect. **B**, Migration distance (μm) of microglial cells was increased by ATP (50 μM), which was inhibited by pretreatment of the cells with PTX. Values are mean \pm SEM (control, $n=48$; BK, $n=20$; BK+CTX, $n=28$; BK+PTX, $n=20$; ATP, $n=15$; ATP+PTX, $n=17$). ** $p<0.01$.

migration. BK-induced migration of microglia was completely inhibited by 0.1 μM of CTX (Fig.1A).

Next, we tested the effect of pertussis toxin (PTX). BK-induced migration of microglia was not affected by pretreatment with PTX for 12 hr (Fig.1A), while ATP-induced migration was inhibited significantly by PTX (Fig. 1B), as reported previously (Honda *et al.* 2001). These results suggest that activation of large conductance Ca^{2+} -activated K^+ channel was essential for BK-induced migration of microglia but PTX-sensitive G protein ($G_{i/o}$) was not.

Discussion

In the present study, we provide the first evidence that BK induces migration of microglia. In addition, we demonstrated that BK-induced microglial migration was completely inhibited by CTX, suggesting that BK-induced migration was totally dependent on the activation of Ca^{2+} -dependent K^+ channels. On the other hand, BK-induced microglial migration

was not inhibited by PTX, suggesting that it was not dependent on activation of $G_{i/o}$ protein. Since ATP-induced microglial chemotaxis was mediated by $G_{i/o}$ -coupled P2Y receptors (Honda *et al.* 2001), these results indicate that the mechanism of BK-induced migration of microglia is similar to that of LPA but is different from that of ATP.

The BK receptors belong to the family of receptors with seven transmembrane domains coupled to G-proteins. It was reported that BK receptors couple to $G_{q/11}$ protein, activate phospholipase C, increase IP_3 and consequently induce Ca^{2+} release from intracellular Ca^{2+} store. On the other hand, BK receptors are also reported to couple to $G_{i/o}$ protein and mitogen-activated protein (MAP) kinase, leading to cell proliferation (Liebmann, 2001). It was interesting that activation of $G_{i/o}$ protein by BK was not coupled to the signaling for migration unlike to ATP-induced chemotaxis. It may be because BK receptors in microglia couple to mainly $G_{q/11}$ protein.

There are two subtypes of BK receptors, B_1 and B_2 receptor. In microglia, B_2 receptor mRNA is constitutively expressed and B_1 receptor mRNA is expressed at very low levels under normal conditions but is up-regulated by treatment with BK (Noda *et al.* 2003). Most biological actions of BK are mediated by B_2 receptor. However, it would be interesting to see which BK receptor subtype is responsible for microglial migration.

Though physiological functions of BK in the CNS still remain to be investigated, our finding will help to understand the physiological and pathophysiological role of BK in the brain via microglia.

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