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Short communication

Dose-depending effect of intracerebroventricularly administered bradykinin on nociception in rats

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Abstract:
Background: The effect of small and high doses of intracerebroventricularly (*icv*) applied bradykinin (BK) on nociception produced by mechanical stimuli and the participation of B_1 and B_2 receptors in this nociception were investigated in rats.
Results: BK at the lowest dose (0.06 μg) produced hyperalgesia whereas at the higher doses (6 and 12 μg) antinociception. This effect was abolished by B_1 or B_2 receptor antagonists, des-Arg^{10}-HOE140 and HOE140 (1 pmol *icv*), respectively.
Conclusion: Depending on the dose used, BK produces pro- or anti-nociceptive action. Both B_1 and B_2 receptors are involved in the action of *icv* applied BK.

Key words:
bradykinin, intracerebroventricular, B_1/B_2 receptor antagonists, antinociception, nociception, dose-response relationship, rats

Introduction

Bradykinin (BK) as well as kallidin, T-kinin and their active metabolites, des-Arg^9-kinin, belong to a group of autacoids named kinins. The role of BK in the peripheral formation of pain is well known. Tissue damage, allergic reactions, and other inflammatory events, including bacterial or viral infection, activate a series of proteolytic reactions and generate BK formation in the tissues, which in turn stimulates primary afferent fibres. Moreover, BK can sensitize nociceptors following the release of prostaglandins, cytokines and nitric oxide, either from sensory neurons, endothelial and immune cells or fibroblasts in addition to its interaction with mast cell mediators [4]. Kinins exert their biological effect through the activation of two receptors, B_1 and B_2, which belong to G protein-coupled metabotropic receptors [5]. B_2 receptors are present constitutively in most tissues, including nociceptive neurons. These receptors are responsible for most BK actions associated with nociceptor activation and the induction of acute pain [4]. B_1 receptors are not physiologically present in most tissues, but, as a result of inflammation, are induced (*de novo* synthesis) under the influence of cytokines and other inflammatory factors on sensory neurons and/or other cells, e.g., macrophages, fibroblasts, and endothelial cells. It was suggested, that B_1 receptors are especially responsible for long-term hyperalgesia [5]. However, it is currently known that B_1 receptors are also present constitutively on peripheral endings of primary afferent fibre terminals [28].

The role of BK in the central nervous system, especially in the brain, has been much less studied. The presence of B_2 receptors, BK itself, and other members of the kallikrein-kinin system have been found in various areas of the brain, e.g., brain stem, cortex, cerebellum, hypothalamus, and the pituitary gland [7, 16]. Moreover, data available in the literature indicate that the B_1 receptor can also be present constitutively in the nervous tissue [23].

The proalgesic action of BK in peripheral tissues is well documented. However, several observations indicated that BK can also exert an antinociceptive effect when it is administered either intrathecally or intracerebroventricularly at higher doses [11, 17, 19]. Therefore, it was of interest to investigate the effect of both a small and a high dose of intracerebroventricularly applied BK on nociception produced by mechanical stimuli and the participation of B_1 and B_2 receptors in this nociception.

Materials and Methods

Laboratory animals

The study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw. The aforementioned Committee approved the experimental protocols. Male Wistar rats (220–280 g) were housed in a room maintained at a temperature of 20 ± 2°C, under 12 h light-dark cycle (lights on at 7:00 a.m.). Experiments started at 10:00 a.m. Experimental groups consisted of at least six rats. Animals had free access to food and water. The individual animals were used in only one experiment.

Drug administration

Bradykinin acetate salt was purchased from Bachem AG (Bubendorf, Switzerland), HOE 140 and des-Arg^{10}-HOE 140 from Research Biochemicals International (Natick, MA, USA).

Bradykinin, HOE 140, and des-Arg^{10}-HOE 140 were dissolved in distilled water immediately before injection. BK was administered at a dose of 0.06, 0.12, 6, and 12 μg, while HOE 140 at a dose of 1 pmol and des-Arg^{10}-HOE 140 at a dose of 1 pmol. All drugs were administered intracerebroventricularly (*icv*) in a 3-μl volume over a 30 s period.

Icv injections of peptides were based on the method described by Noble and Wurtman [18] as modified by Robinson et al. [22] and Strada et al. [25]. The animals were anesthetized with pentobarbital (Vetbutal, Biowet Puławy Sp. z o.o., Puławy, Poland) at a dose of 30 mg/kg. The cannula was implanted into the right lateral ventricle. The animals were then placed in separate cages. *Icv* administration began on the fifth day after cannulation. Administration was performed using a Hamilton syringe.

Measurement of the nociceptive threshold

The changes in nociceptive thresholds were estimated using mechanical stimuli (the modification of the Randall-Selitto paw withdrawal test) [21]. Analgesimeter, progressively increasing pressure stimulus (type 7200, Ugo-Basile Biological Research Apparatus, Comerio-Varese, Italy), was used. For mechanical stimulation, progressively increasing pressure was applied to the dorsal surface of the rat's paw using an analgesimeter. The instrument increased force on the paw at a rate of 32 g/s. The nociceptive threshold was defined as force in grams at which point the rat attempted to withdraw its hindpaw, and values of pressure were recorded at this moment. The nociceptive threshold was measured in duplicate and the mean was taken for further calculations. At least two observers controlled the response. HOE 140 and des-Arg^{10}-HOE 140 were administered 5 min before BK. Control animals were injected *icv* with distilled water in accordance with the same time schedule.

Nociceptive thresholds (average of two trials) were measured for each animal before administration of the investigated drugs (A). Measurements of the activity of the investigated drugs were determined at 5, 15, 30, 45, 60, 90, 120, and 180 min after administration of BK, HOE 140 + BK, or des-Arg^{10}-HOE 140 + BK (B).

In all experimental sessions, thresholds obtained (B) were compared to the baseline (A).

Changes in pain threshold were calculated as a percentage of baseline value according to the following formula:

$$\%\ \text{of analgesia} = \left(\frac{B}{A} \times 100\%\right) - 100\%$$

A – pressure (in g), baseline pain threshold, B – pressure (in g) in consecutive measurements.

Percentages of analgesia values calculated as above for individual animals were subsequently used to calculate average values in particular experimental groups and for statistical analysis [6, 10].

Statistical analysis

The results are expressed as the means ± standard error of the mean (SEM). The statistical significance of differences between groups was evaluated by a one-way analysis of variance and Student's paired data *t*-test; $p < 0.05$ was accepted as statistically significant. All statistical calculations were performed using the computer software (Pharm/PCS ver. 4.2) described by Tallarida and Murray [26].

Results

Effect of BK administered *icv* on nociception

The effect of *icv* administered BK on the threshold to the mechanical stimuli was dose dependent. BK was administered at increasing doses in the range of 0.06–12 μg. The lowest 0.06 dose produced hyperalgesia during the entire measurement period. This effect occurred at 5 min (with maximums at 5 and 15 min) and then gradually returned to the baseline values (at 120 min). BK administered at a dose of 0.12 μg not only decreased the nociceptive threshold at 5 min but also induced gradual antinociception. This effect reached a nadir at 45 min and then diminished by 90 min. The application of higher doses (6 and 12 μg) resulted in a dose dependent antinociceptive effect with a maximum at 5 min. Starting from 15 min to 120 min, a statistically gradual decrease of analgesia was observed.

Influence of the B_2 receptor antagonist HOE 140 on BK-induced antinociception and hyperalgesia

As shown in Figure 1, the selective B_2 receptor antagonist HOE 140, administered *icv,* only slightly, but significantly, increased the nociceptive threshold. This effect was observed at measurements from 5 to 45 min, inclusively. Pretreatment with HOE 140 almost completely abolished the antinociception induced by the high doses of BK, 12 μg (Fig. 1) and 6 μg (data not shown). HOE 140 administered before the low dose 0.06 μg (Fig. 1) and 0.12 μg of BK (data not shown) reversed BK hyperalgesia and a clear antinociceptive effect was observed.

Influence of the B_1 receptor antagonist des-Arg^{10}-HOE 140 on hyperalgesia and antinociception induced by BK

des-Arg^{10}-HOE 140 administered separately, slightly but significantly increased the nociceptive threshold to mechanical stimuli (from 5 to 45 min).

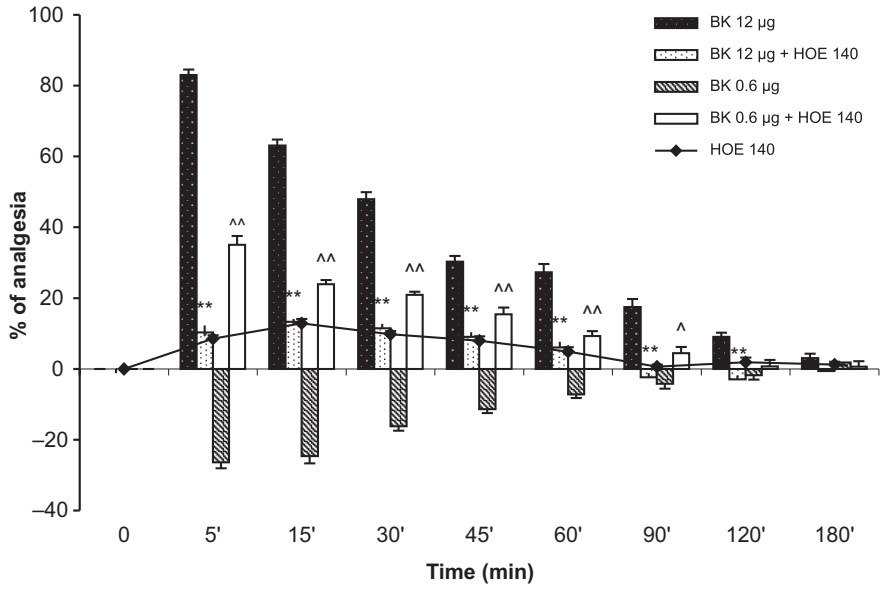

Fig. 1. Effect of HOE 140 at a dose of 1 pmol *icv* on the hyperalgesia induced by bradykinin (BK) at a dose 0.06 μg and on the analgesia induced by BK at a dose of 12 μg *icv*. Values are the means ± SEM. ** $p < 0.01$ * $p < 0.05$ BK 12 μg + HOE140 *vs.* BK 12 μg; ^^ $p < 0.01$ ^ $p < 0.05$ BK 0.06 μg + HOE140 *vs.* BK 0.06 μg

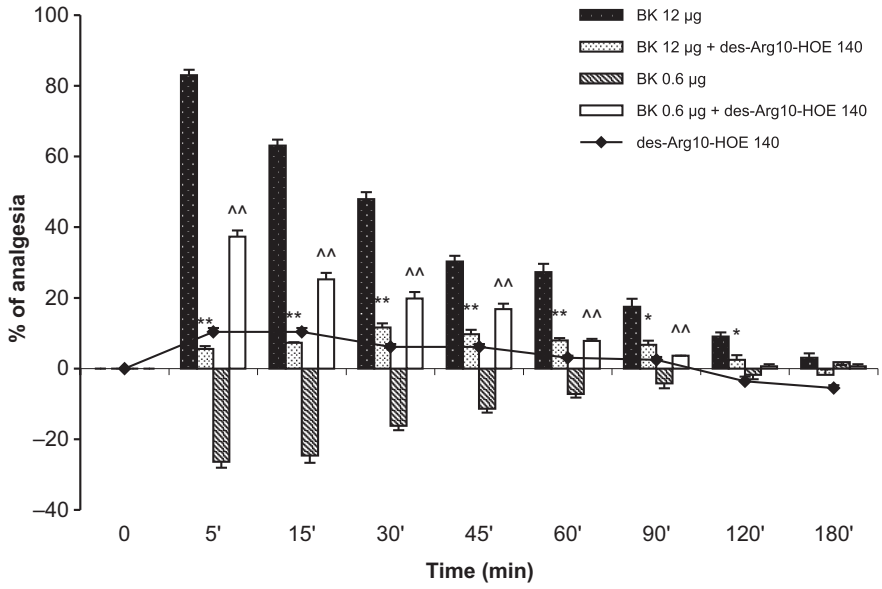

Fig. 2. Effect of des-Arg^{10}-HOE140 at a dose of 1 pmol *icv* on the hyperalgesia induced by bradykinin (BK) at a dose 0.06 µg and on the analgesia induced by BK at a dose of 12 µg *icv*. Values are the means ± SEM. ** $p < 0.01$ * $p < 0.05$ BK 12 µg + desArgHOE140 *vs.* BK 12 µg; ^^ $p < 0.01$ ^ $p < 0.05$ BK 0.06 µg + desArgHOE140 *vs.* BK 0.06 µg

However, pretreatment with des-Arg^{10}-HOE 140 prevented the antinociceptive action of the higher dose of BK, 12 µg (Fig. 2) and 6 µg (data not shown). The hyperalgesic action of the low doses of BK, 0.06 µg (Fig. 2) and 0.12 µg (data not shown) after premedication with des-Arg^{10}-HOE 140 was not only prevented, but a clear antinociceptive effect was observed.

Discussion

BK is an endogenous nonapeptide that, when administered peripherally, directly stimulates nociceptors and induces pain and hyperalgesia to heat and mechanical stimulation. Numerous studies have proved that BK and BK receptors are present not only in peripheral tissue but also in the dorsal root ganglia, on the endings of primary afferent fibers, on the dorsal horn interneurons of the spinal cord and in the brain [5, 14]. Such a distribution of BK receptors suggests that BK is an important modulator and/or transmitter of nociceptive information in the central nervous system (CNS). The peripheral pronociceptive effect of BK was revealed in other kinds of pain [1, 2, 5, 13, 15, 27]. However, the role of BK in the transduction of pain stimuli at the level of the CNS is much less understood. In the literature, there are available data indicating that this compound administered intrathecally (*it*) revealed an antinociceptive effect [9, 20, 24]. Sot et al. [24] demonstrated antinociception when bradykinin was administered at a higher dose (6.0 µg) while the lower dose of BK (0.15 µg) produced hyperalgesia. BK hyperalgesia was also observed by Kamei et al. [12]. However, in this study, the decrease of the nociceptive pain threshold was preceded by a 30-min antinociceptive action of *it* administered BK. In the study of Ferreira et al. [9], kinins acting at both B_1 and B_2 receptors at the spinal level exerted a critical role in controlling the nociceptive processing mechanisms. Also Pesquero et al. [20] suggested that the action of B_1 receptors in the spinal cord underlies, at least in part, the central component of pain. In other studies, an *icv* injection of BK at doses of 4, 8, and 16 nmol induced a dose-dependent antinociceptive effect in rats, as indicated by an increase in the dental pulp electrical stimulation threshold [19]. Also Germany at al. [11] observed antinociception after *icv* administration of BK at doses of 4, 8, and 16 µg in mice in the hot-plate test. Couto et al. [8] demonstrated that microinjection of BK in the principal sensory trigeminal nucleus caused a statistically significant long-lasting antinociception. Moreover, this antinociception was antagonized by the damage of locus coeruleus-noradrenergic neuronal fibres. The author suggests that serotonin- and noradrenaline-containing nuclei (especially locus coeruleus) of the endogenous pain inhibitory system exert a key role in the antinociceptive mechanisms of bradykinin. In a study of Burdin et al. [3], BK antinociceptive activity was also observed when BK was injected into the periaqueductal grey matter.

In the present study, it was shown that BK administered *icv* produced either hyperalgesia or antinociception in response to mechanical stimuli and these effects were dose-dependent. Whereas a low dose of *icv* administered BK (0.06 μg) resulted in a significant decrease in nociceptive threshold, higher doses (6.0 and 12.0 μg) produced marked antinociception. A dose of 0.12 μg initially induced hyperalgesia and then analgesia. These results, therefore, not only support the antinociception noted in other studies [3, 8, 11, 19], but also indicate the possibility of hyperalgesia after *icv* application of BK.

On the basis of immunohistochemical studies, the constitutive presence of not only B_2, but also B_1 receptors in the supraspinal structures associated with sensation of pain was demonstrated. [7, 23]. These observations suggest that both B_1 and B_2 receptors can be involved in proalgesic and/or antialgesic actions of BK.

The results obtained in this study indicated that BK-induced modifications in nociception at the brain level were due to the stimulation of both B_1 and B_2 receptors. Both pretreatments with the selective B_1 antagonist, des-Arg^{10}-HOE 140 and the selective B_2 antagonist, HOE 140, strongly limited the action of BK regardless of whether the drug was administered at low (proalgesic) or high (antialgesic) doses. The time of activation of B_1 and B_2 receptors after separate or concomitant administration of BK and B_1 or B_2 receptor antagonists is similar, which seems to confirm that the B_1 and B_2 receptors are constitutively present in the pathways of pain transmission.

The results of this study are in line with those previously obtained in our laboratory [24] in which both B_1 and B_2 receptors participated in the proalgesic and antialgesic activity of *it* administered BK, in response to acute thermal stimulation.

In the literature, a small amount of data are available that indicate a participation of B_1 and B_2 receptors in the supraspinal analgesic action of BK. Mortari et al. [17] showed that BK B_2 receptor activation in the brain by the BK analogue, Trh^6-bradykinin, potently reduced acute, noxious heat-evoked reflex responses in naive rats. Pelá et al. [19] suggested that a blockage of BK antinociceptive effect by administered *icv* B_2 antagonist (D-Arg^0-Hyp^3-Thi^{5,8}-D-Phe^7-BK) indicates that the antinociceptive effect of this peptide can be mediated by the activation of B_2 receptors. The B_1 agonist, des-Arg^9-BK, also induced a significant antinociceptive effect, but not as intensive as that induced by BK. This may lead to the suggestion that part of the BK-antinociceptive effect may also involve the activation of B_1 receptors, since BK is rapidly cleaved to des-Arg^9-BK in the CNS. Pesquero et al. [20] showed that in the behavioral tests of chemical and thermal nociception, B_1 receptor knockout mice showed significant analgesia compared with wild-type controls. By using two electrophysiological preparations, the authors showed that the action of B_1 receptors in the spinal cord underlies, at least in part, the function of this receptor in baseline nociception.

In conclusion, the results of the present study appear to confirm that BK analgesia on the supraspinal level depends on high doses of BK and the activation of B_1 as well as B_2 receptors. We also showed that the doses of *icv* administered BK can evoke nociceptive action. Both B_1 and B_2 receptors are also engaged at the supraspinal level in the proalgesic effect of BK.

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