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Brain sites involved in the antinociceptive effect of bradykinin in rats

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1 The localization of brain sites where bradykinin (BK) induces its antinociceptive effect in rats, was studied using as index the threshold for the jaw-opening reflex elicited by the dental pulp electrical stimulation test (DPEST).

2 The microinjection of BK into the lateral or fourth cerebral ventricles induced an antinociceptive effect, with Index of Antinociception (IA) of 0.51 ± 0.03 and 0.68 ± 0.05, respectively. However, microinjections of the peptide into the third ventricle induced a less marked antinociception ($IA = 0.28 \pm 0.08$).

3 The brain sites where the microinjection of BK caused an antinociceptive effect were: *locus coeruleus*, principal nucleus, oral part of the spinal sensorial trigeminal nucleus, and the sensory root of the trigeminal nerve.

4 The antinociceptive effect was more intense when BK (4–16 nmol) was injected into the *locus coeruleus*. Microinjection of BK (4 nmol) into the fourth ventricle, but not into the *locus coeruleus*, induced an increase in blood pressure. The microinjection of the peptide into the *nucleus tractus solitarius*, a site that is also involved in the pressor effect of BK, did not induce an antinociceptive effect. These results indicate that the antinociceptive effect of BK is not related to blood pressure changes.

5 The microinjection of BK into some of the sites involved in the mechanisms of analgaesia, including the periaquenductal gray matter (dorsal, lateral and ventrolateral) and the dorsal raphe nucleus did not induce an antinociceptive effect.

6 The results suggest that the most likely brain sites involved in the antinociceptive effect of BK are the *locus coeruleus* and the principal sensory trigeminal nucleus. The present results did not exclude the involvement of other brain sites surrounding the lateral and the third ventricles.

Keywords: Bradykinin; antinociceptive effect; *locus coeruleus*; principal sensory trigeminal nucleus; rats

Introduction

Bradykinin (BK), a nonapeptide released by proteolytic cleavage of the precursor proteins kininogens, was initially identified in the plasma. The presence of peptidases involved in BK synthesis or degradation was previously demonstrated in the brain by Chao *et al*. (1983; 1987), Richoux *et al*. (1991; 1992) and Raidoo *et al*. (1996). Furthermore, the peptide itself as well as BK-immunoreactive neurons and specific high-affinity binding sites for BK have also been demonstrated in the brain (Corrêa *et al*., 1979; Perry & Snyder, 1984; Karya *et al*., 1985; Fujiwara *et al*., 1988; Raidoo & Bhoola, 1997; Murone *et al*., 1997) and in neuronal cell cultures (Lewis *et al*., 1985; Boehm & Huck, 1997).

BK induces many effects when microinjected into the lateral ventricles or different brain sites, such sedation and catatonia (Graeff *et al*., 1969; Da Silva & Rocha e Silva, 1971), noradrenaline depletion (Graeff *et al*., 1969; Purkiss *et al*., 1995), hyperthermia (Almeida e Silva & Pelá, 1978; Mohan Rao & Bhattacharya, 1988; Coelho *et al*., 1997), hypertension (Corrêa & Graeff, 1975; Lindsey *et al*., 1997; 1989; Fior *et al*., 1993), antidiuretic hormone release (Rocha e Silva Jr & Malnick, 1964) and antiaversive effect (Burdin *et al*., 1992).

There is also evidence of the involvement of BK in central antinociceptive mechanisms. Microinjection of BK into the lateral ventricle induced a dose-dependent antinociceptive effect in rabbits (Ribeiro *et al*., 1971; Ribeiro & Rocha e Silva, 1973), while intrathecal microinjection of BK induced an antinociceptive effect in rats *via* a noradrenergic mechanism within the spinal cord (Laneuville & Couture, 1987; Laneuville *et al*., 1989). Previously, we have shown that intracerebroventricular (i.c.v.) injections of BK increased the threshold for dental pulp electrical stimulation in rats (Pelá *et al*., 1996). This effect was dose-dependent and mediated by kinin B_2 receptors.

Therefore, the aim of this study was to identify the brain sites where BK acts to induce its antinociceptive effect in rats. The approach used to address this question was the microinjection of BK into the ventricles and at different brain sites and the nociceptive method used was the dental pulp electrical stimulation test (DPEST).

Methods

Animals

Adult male Wistar rats weighing 180–200 g were used. Animals were housed under identical conditions with free access to food and water and maintained on a 12 h light/dark cycle (light on at 06:00). Two days before the experimental session each rat was handled and habituated to the testing procedures, except that neither dental pulp electrical stimulation nor the microinjection of substances was performed.

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Implantation of intracerebral and intracerebroventricular cannula

Under pentobarbital anesthesia (45 mg kg^{-1}, i.p.), a stainless steel guide cannula direct to the ventricles or different brain sites was implanted stereostatically according to the coordinates taken from the atlas of Paxinos & Watson (1986).

After the surgery, the animals received an intramuscular injection of veterinary penicillin. Animals that showed weight loss, signs of infection or a misplaced cannula were excluded from the study.

Surgery for blood pressure measurement

Animals with cannulas implanted into the *locus coeruleus* or the fourth ventricle were anaesthetized with tribromoethanol (2.5 mg kg^{-1}, i.p.) and after local anaesthesia with lidocaine with epinephrine (1 : 200,000), a polyethylene catheter (PE-10) was implanted into the femoral artery for blood pressure (BP) recording. The catheter was filled with 0.3% heparin in sterile saline and extruded at the dorsum of the animals. Twenty four hours after this procedure, BP was measured for 35 min, starting 5 min before BK microinjection into the *locus coeruleus* or the fourth ventricle.

Procedures

The DPEST was carried out as described by Pelá *et al*. (1996). Briefly, 2 days before the experiments, the animals were anaesthetized with sodium pentobarbital (30 mg kgt^{-1}, i.p.) and two cavities of 1.0 mm diameter were made laterally, one in each of the two upper incisors, 2.0 mm below the level of the labial gingival margin. These cavities were deepened just for the placement of electrodes. The forceps-shaped electrode consisted of a platinum wire with a diameter of 1.0 mm at the end and insulated with enamel, except at the tip, to avoid short circuiting by saliva. This procedure was carried out because stimulation of extradental afferents also elicit a jaw-opening reflex. In order to determine the threshold of the DPEST, the electric current, delivered by a stimulator was increased stepwise from a subthreshold intensity until eliciting a response characterized by the jaw-opening reflex. Stimulation parameters were: duration of 2 ms, frequency of 2 Hz and variable current (μA) applied during 1 s.

Animals were tested every 5 min, until a stable baseline DPEST was obtained and then for up to 60 min following microinjection of drug or saline. Each DPEST was normalized as an index of antinociception (IA) using the formula:

$$\text{IA} = \frac{(\text{test DPEST}) - (\text{average baseline DPEST})}{(\text{average baseline DPEST})}$$

The results were expressed as means $\pm$ s.e.mean of IA values against time of readings.

Drugs

BK was obtained from Fundap-SP, Brazil and Sigma, U.S.A. The peptide was dissolved in artificial cerebrospinal fluid (CSF) ($NaCl = 8.1$ g; $KCl = 0.25$ g; $CaCl_2 = 0.14$ g; $MgCl_2 = 0.11$ g; $NaHCO_3 = 1.0$ g for 1 l of solution).

BK or CSF were injected i.c.v. in a volume of 2 μl over a 1 min period using a glass needle (70 μm o.d.) inserted into the guide cannula immediately before the microinjection. The intracerebral (i.c.) microinjection was carried out by inserting the microinjection system into the guide cannula, according to the technique described by Azami *et al*. (1980). Briefly, a glass needle ($\leqslant 90$ μm in diameter) protected by a telesystem of a stainless tube, was inserted through the guide canulla. The tip of the glass needle was positioned 1 mm below the end of the guide cannula. BK was dissolved in a final volume of 0.5 μl CSF and injected over a 30 s period and the needle was held in place for 15 s following the injection.

Histology

After the experiments, the microinjection sites were identified by an i.c.v. or i.c. injection of a dye (Evan's blue 1%) followed by histologic analysis. Briefly, after the microinjection of the dye, the animals were sacrificed under deep anaesthesia and perfused through the heart with saline followed by 10% formalin. The brains were removed and fixed for at least 3 days in 10% formalin. Frozen sections of 50 μm were placed on a glass slide and later examined microscopically. Injection sites were localized in diagrams from a rat brain atlas (Paxinos & Watson, 1986).

Statistics

Data are presented as means $\pm$ s.e.mean. Variables were analysed with one-way ANOVA followed by the Scheffe's test using the SPSS statistical software. $P \leqslant 0.05$ was considered statistically significant.

Results

The increase in the DPEST induced by the microinjection of BK (4 nmol) into the lateral ventricle of the rat brain is shown in Figure 1a. The maximum response ($IA = 0.51 \pm 0.03$) was observed 15 min after the injection and IA returned to baseline level after 45 min. These results are similar to those we have previously reported (Pelá *et al*., 1996).

I.c.v. injection of the same dose of BK into the third ventricle also induced an antinociceptive effect. However, the effect was not as intense as that induced by the microinjection into the lateral ventricle, with maximum response ($IA = 0.28 \pm 0.08$) 20 min after the injection and a duration of 40 min (Figure 1b). Greater antinociceptive effect was observed after the injection of BK into the fourth ventricle (Figure 1c). The maximum response ($IA = 0.68 \pm 0.05$) was observed 10 min after microinjection and IA returned to baseline level after 40 min.

To determine which brain sites are involved in the antinociceptive effect of BK, microinjection of the peptide was carried out into different sites. The first brain site investigated was the periaqueductal gray matter, because this area is involved in many aspects of the physiology of pain and antinociception. No statistically significant effects were observed after the microinjection of 4 nmol of BK into different portions of the periaqueductal gray matter. The IA values were 0.11 ± 0.04; 0.19 ± 0.06 and -0.09 ± 0.05 for the dorsal, lateral and ventrolateral periaqueductal gray matter, respectively (Figure 2a, b and c).

Microinjection of BK (4 nmol) into the dorsal raphe nucleus did not cause antinociceptive effect (data not shown).

Located adjacent to fourth ventricle, the *locus coeruleus* has been demonstrated to be involved with antinociception. Figure 3 shows that microinjection of BK into the *locus coeruleus* caused a dose-related antinociceptive effect, with a significant linear regression [$y = 0.69$ (log x) $+ 0.37$, $r = 0.99$, $P = 0.002$]. One-way ANOVA test between doses showed a significant

influence of the treatment ($F_{4,30}=15.40$, $P=0.001$). The maximum response was obtained 10–20 min after microinjection. The antinociceptive effect induced by the low doses of BK (2 and 4 nmol) lasted about 40 min, while the effect induced by the high doses (8 and 16 nmol) declined slowly during the experimental period.

Central injection of BK is known to cause blood pressure increase in the rat. Among the proposed areas involved in this pressor effect, those surrounding the fourth ventricle are the most sensitive (Lindsey *et al*., 1988). To investigate if pressor responses were concomitant to the antinociceptive effect, BK (4 nmol) was injected into the fourth ventricle or the *locus coeruleus* and blood pressure recorded. An increase of BP was only observed after the microinjection of BK into the fourth ventricle (Figure 4).

Another brain site investigated was the *nucleus tractus solitarius*, an area involved in blood pressure regulation. Microinjection of BK into this area did not cause an antinociceptive effect (data not shown).

Stimuli originated in the dental pulp reach the trigeminal nucleus, an area that contains excitatory interneurons involved in the jaw-opening reflex (Hu *et al*., 1986). Furthermore, the oral and interpolar parts of the spinal trigeminal nucleus receive neural input from brain centers involved in the

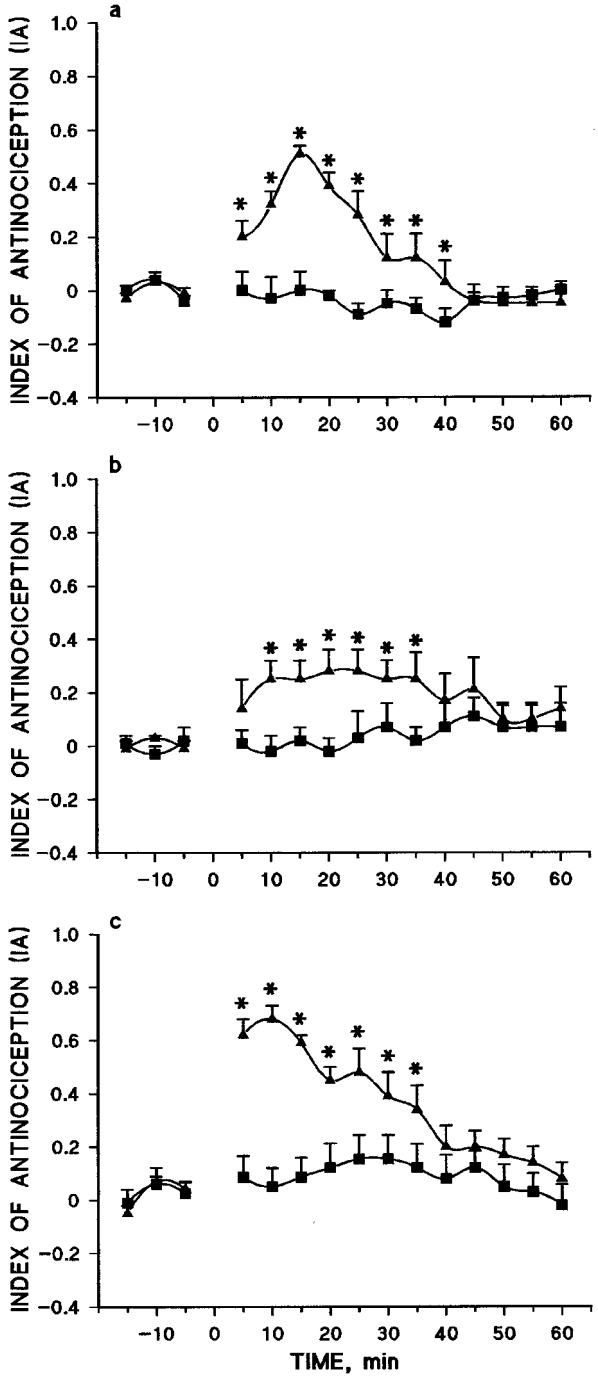

Figure 1 Time course of the increase in DPEST, represented by the index of antinociception, induced by the microinjection of BK (4 nmol) into: (a) lateral ventricle, (b) third ventricle and (c) fourth ventricle. *Significantly different from CSF ($P<0.05$).

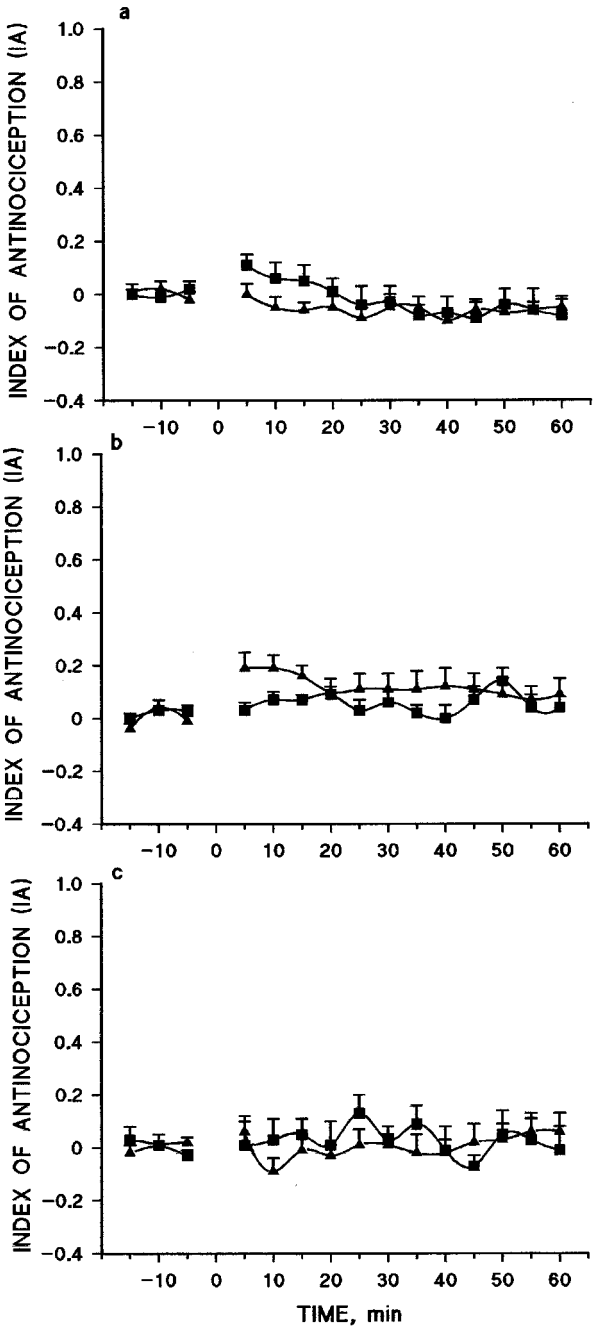

Figure 2 Time course of the increase in DPEST, represented by the index of antinociception, induced by the microinjection of 4 nmol of BK or CSF into: (a) dorsal; (b) lateral and (c) ventrolateral area of the periaqueductal gray matter.

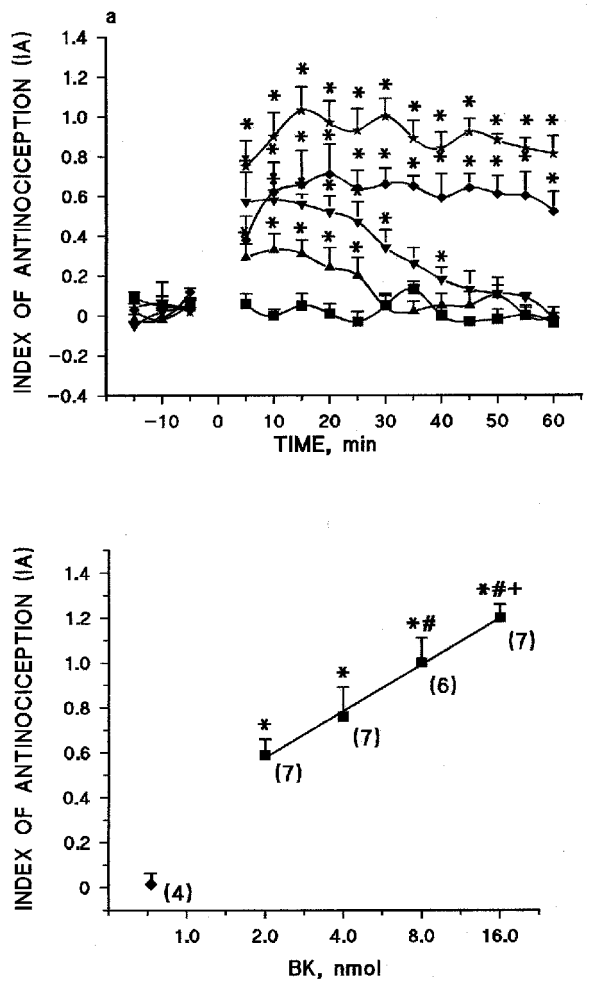

Figure 3 BK-induced antinociception after microinjection into the *locus coeruleus*. (a) shows the time-course of DPEST induced by 2, 4, 8 and 16 nmol of BK, represented by the index of antinociception (b) represents the dose-response curve to BK. Points in the curve represent the mean of the maximum responses for each dose. Number of animals in each group is indicated in parentheses. *Significantly different from CSF; # from BK 2 nmol; + from BK 8 nmol ($P<0.05$).

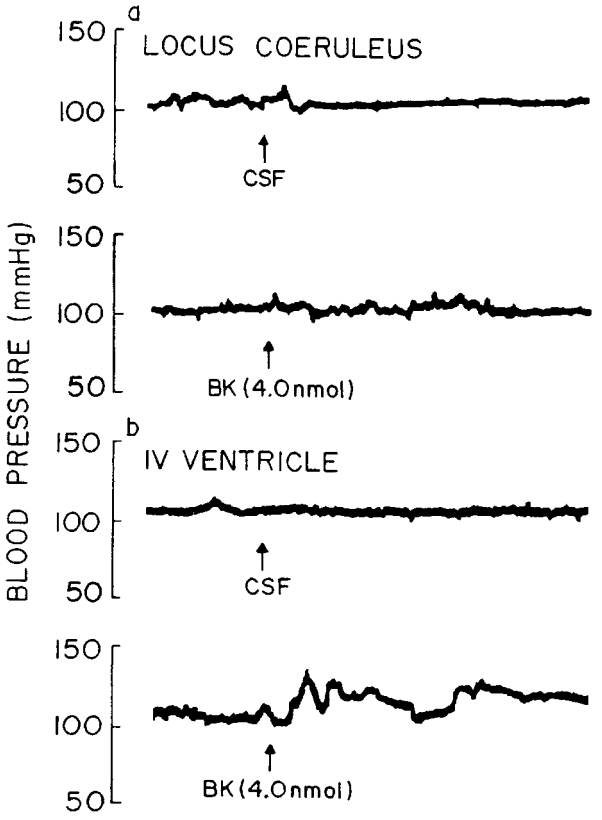

Figure 4 Blood pressure changes induced by the microinjection of CSF into the fourth ventricle or *locus coeruleus* (a). Blood pressure changes induced by microinjection of BK (4 nmol) into fourth ventricle or *locus coeruleus* (b).

nociceptive control (Matthews *et al*., 1984; Sessle, 1987). Microinjection of BK (4 nmol) into the principal trigeminal nucleus induced an antinociceptive effect with a maximum response (IA = 0.59 ± 0.19) 25 min after (Figure 5a). Similar effect was observed after the BK microinjection into the spinal trigeminal nucleus, with a maximum response (IA = 0.46 ± 0.1) 20 min after (Figure 5b). The last trigeminal area analysed was the sensory root of the trigeminal nerve, where 4 nmol of BK induced a maximum antinociceptive effect (IA = 0.70 ± 0.07) 20 min after the microinjection (Figure 5c).

Figure 6 shows the histological identification of brain sites where BK was microinjected.

Discussion

The present results confirm the observation that the i.c.v. microinjection of 2–4 nmol BK induces antinociception in rats (Pelá *et al*., 1996) and further demonstrate that BK may be involved in the antinociceptive mechanisms in the brain.

The low density of bradykinin-like containing nerve cell bodies and terminals in the central nervous system (Corrêa *et al*., 1979) associated to the short half life of the peptide, 26 s in the cerebral ventricular space (Kariya *et al*., 1982) and the high angiotensin-converting enzyme (kininase II) activity in the medulla oblongata (Kariya *et al*., 1981), may account for the relatively high doses of BK necessary to cause antinociceptive effects.

The microinjection of BK in sites involved with the descending system of endogenous analgesia indicated that the *locus coeruleus* was the most sensitive site responsive to the microinjection of the peptide. The *locus coeruleus* consists of a bilateral and distinct cluster of noradrenergic neurons located near the wall of the fourth ventricle, at the level of the dorsal pontine tegmentum (Guyenet, 1980; Van Bockstaele *et al*., 1996). Extensive projections from the *locus coeruleus* reach the diencephalon, telencephalon, cerebellum and spinal cord of cats (Nakasato, 1987) and rats (Guyenet, 1980; Westlund *et al*., 1983; Arvanitogiannis *et al*., 1997). Furthermore, Laneuville & Couture (1987) and Laneuville *et al*. (1989) observed that after intrathecal microinjection of BK in rats, this peptide acts presynaptically on kinin B_2 receptors, located on spinal noradrenergic terminals. These results support the suggestion that BK may stimulate spinal noradrenergic neurons, which may originate in the *locus coeruleus*.

Another effect induced by i.c.v. injection of BK in rats is a persistent increase of the blood pressure. Corrêa & Graeff (1975) observed this effect after the microinjection of BK into the lateral septal area, whereas Lewis & Phillips (1984) reported the same effect after the microinjection of the peptide

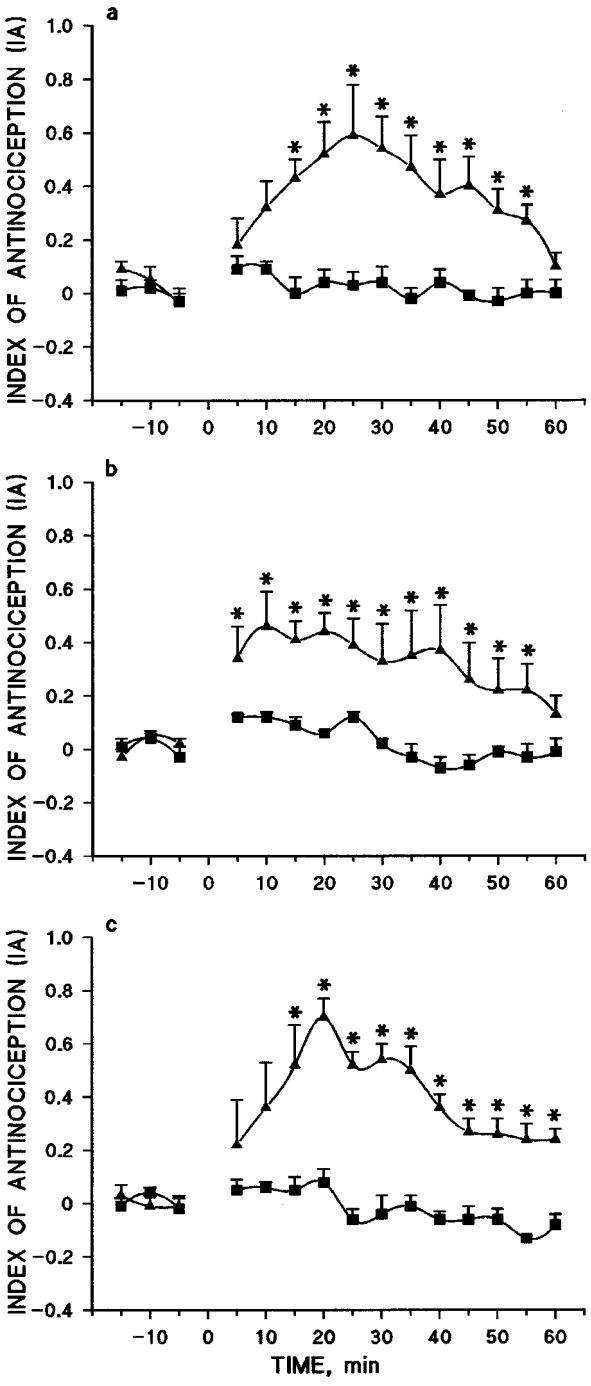

Figure 5 Time course of the increase in DPEST induced by microinjection of BK (4 nmol) into the; (a) Principal trigeminal nucleus; (b) Spinal sensorial trigeminal nucleus, oral part and (c) Sensory root of the trigeminal nerve. *Significantly different from CSF ($P<0.05$).

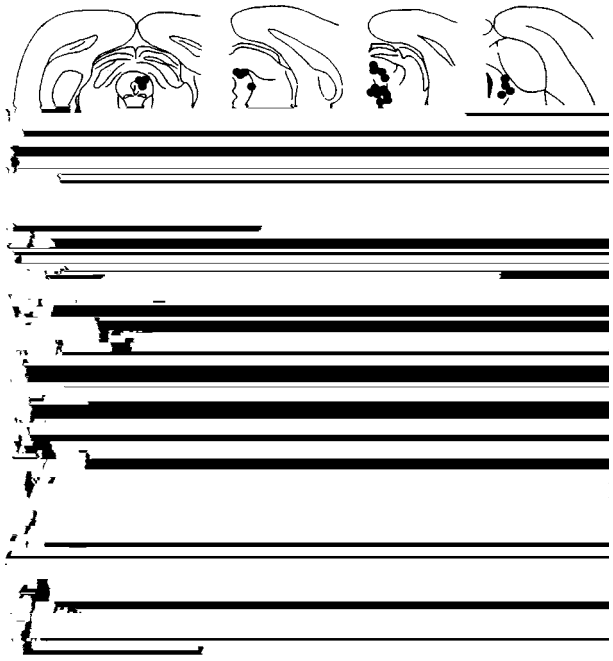

Figure 6 Sites of microinjection of BK. The number of points in the Figure is less than the total number of rats used because of several overlaps. The number beside each Figure represents the section posterior to the interaural line.

into areas adjacent to the ventral portion of the third ventricle. Fior *et al.* (1993) also reported that BK injection into dorsal or dorsal lateral surfaces of the medulla induced an increase of mean arterial pressure, with the highest pressor effect observed after the injection of the peptide into the medial part of the *nucleus tractus solitarius*. In the present study, the microinjection of BK into this area did not induce an antinociceptive effect. Nevertheless, the microinjection of the peptide into the fourth ventricle or the *locus coeruleus* induced marked antinociceptive effect. However, the pressor effect was only observed when BK was injected into the fourth ventricle. These results suggest that the BK antinociceptive effect is not secondary to its pressor effect.

Microinjection of BK into the principal nucleus, the oral part of the spinal sensorial trigeminal nucleus or the sensory root of the trigeminal nerve also caused a marked antinociceptive effect. The maximum antinociceptive effect induced by the microinjection of BK into these areas was only observed 20–25 min after the injection of the peptide. The delay in the antinociceptive effect when BK was microinjected into these areas indicates that the sites involved in these responses are not the actual injections coordinates or that a neuronal network must be recruited.

Sasa & Takaori (1973) and Sasa *et al.* (1979) described in cats the existence of direct noradrenergic connection from *locus coeruleus* to the spinal trigeminal nucleus. Otherwise, there are no reports of a neuronal connection between the trigeminal areas and the *locus coeruleus*, but unpublished results from our laboratory indicate that this is possible, because labelled cell bodies were identified in trigeminal areas after microinjection of a retrograde neuromarker (nuclear yellow) into the *locus coeruleus*.

Microinjection of BK into other areas involved with the pain inhibitory descending system, such as the periaqueductal gray matter and the dorsal raphe nucleus did not induce an antinociceptive effect. Additionally, Burdin *et al.* (1992) reported the observation of moderate nociceptive and antiaversive effects of microinjection of BK into the dorsal periaqueductal gray matter.

In summary, the present results indicate the *locus coeruleus* and the principal sensory trigeminal nucleus as important brain sites involved in the antinociceptive effect of BK. The present results did not exclude the involvement of other brain sites surrounding the lateral and third ventricles.

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