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Possible role of nitric oxide in the antinociceptive action of intraventricular bradykinin in mice

Aniza Germany [a], Paulo González [b], Enrique Contreras [a,*]

[a] *Department of Pharmacology, Faculty of Biological Sciences, University of Concepción, P.O. Box 152-C, Concepción, Chile*
[b] *Department of Pharmacy, Faculty of Pharmacy, University of Concepción, P.O. Box 152-C, Concepción, Chile*

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Abstract

The i.c.v. administration of bradykinin (4, 8 and 16 μg) induced antinociception in mice which was resistant to naloxone; furthermore, the induction of tolerance to morphine by a single s.c. injection (100 mg/kg, 24 h before test doses of the peptide) did not affect antinociception. Since bradykinin is known to increase nitric oxide (NO) in peripheral tissues, we studied the possibility that its antinociceptive action may be related to NO effects in the central nervous system. Bradykinin effects were antagonized by previous treatment with N^G-nitro-L-arginine or concomitant i.c.v. administration of bradykinin and methylene blue. The immediate precursor of NO, L-arginine, which by itself produces analgesia, also reduced bradykinin effects; moreover, tolerance to L-arginine significantly decreased the response to the peptide. These results suggest that NO is involved in antinociception induced by i.c.v. administration of bradykinin.

Keywords: Bradykinin; Nitric oxide (NO); N^G-Nitro-L-arginine; Antinociception

1. Introduction

Bradykinin is known to be a peripheral mediator of chemically induced nociception (Reeh, 1986; Martin et al., 1987; Steranka et al., 1988; Haley et al., 1989). A central antinociceptive effect of the peptide after its i.c.v. (Ribeiro et al., 1971) (Argiolas et al., 1985) or spinal (Laneuville and Couture, 1987) injection has been described. The antinociceptive effects induced by its administration to central nervous system (CNS) structures have been tentatively attributed to an excitatory presynaptic action of bradykinin on the descending noradrenergic inhibitory system (Laneuville et al., 1989). The demonstration that bradykinin stimulates the production of nitric oxide (NO) in a variety of tissues (McGhee et al., 1992; Harvey and Burgess, 1993), and the possibility that NO may modulate peptide opiate-induced antinociception (Tseng et al., 1992) suggests the involvement of this messenger in the effects of i.c.v. administration of bradykinin. Therefore, in this paper we studied the influence of L-arginine and antagonists of NO synthesis on the response to the i.c.v. administration of bradykinin.

2. Materials and methods

2.1. General

Male adult albino Swiss-Webster mice (12–15 weeks of age, weighing 26–30 g) from the vivarium of the Department of Pharmacology of the University of Concepción were used in all experiments. Mice were housed in groups of 7, maintained on a 12 h light/dark cycle at constant room temperature (22 ± 1°C) and allowed free access to food and water. Determinations of antinociceptive responses were carried out in the period between 14:30 and 18:00 h under normal room light and temperature (22 ± 2°C). Animals were used for only one experimental condition; all groups consisted of 14 mice.

2.2. Drugs

The drugs used were morphine hydrochloride (May and Baker, Dagenham, UK), naloxone, a gift of Endo Laboratories, Garden City, NY, USA, L-arginine, N^G-nitro-L-arginine, bradykinin acetate salt, and methylene blue, from Sigma Chemical Co., St Louis, MO, USA. The drugs were dissolved in saline and administered subcutaneously (s.c.)

* Corresponding author. Fax (56) (41) 245975.

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in a volume of 10 ml/kg or intracerebro-ventricularly (i.c.v.) in 5–10 μl injections. The latter route of administration was carried out according to the procedure of Haley and McCormick (1957).

2.3. Analgesic test

The hot-plate test as described by Eddy and Leimbach (1953) was used for the assessment of analgesia, but differed with respect to the temperature used (48 ± 0.5°C). The end points considered were the jumping off the plate or the kicking of the legs. Each mouse was tested twice before drug administration and the values were averaged to obtain a baseline. Reaction times were determined at 30-min intervals for a period of 90 min. In order to avoid tissue damage a cut-off time of 30 s was used. The antinociceptive responses were expressed as the area under the time-response curve calculated from values obtained every 30 min during the control period. Accordingly, the effects are expressed in terms of 'min-s,' i.e., the product of the prolongation of reaction time and the duration of such prolongation. A min-s of analgesia is defined as a delay in reaction time of 1 s, with the animal showing such delay for a period of 1 min (Winter and Flataker, 1950).

2.4. Induction of tolerance

In order to induce tolerance to opiates, a single s.c. dose of morphine (100 mg/kg) was administered 24 h prior to the assay of test doses. Control groups were injected with saline instead of the analgesics. Mice were considered tolerant to the antinociceptive effects of the opiates when the effects of a test dose of the analgesic in the primed groups differed from the effect in saline controls at a level of probability of 0.05. To induce tolerance to L-arginine, the drug was administered in doses of 600 mg/kg, twice a day at 8:00 and 19:00 h for 3 days; on the fourth day animals were given test doses of L-arginine or bradykinin. Animals were considered tolerant according to a similar criterion established for morphine-primed mice.

2.5. Statistical analysis

The significance of the differences in the mean responses of the experimental groups was determined by analysis of variance and the Newman-Keuls test. A level of probability of 0.05 was accepted as statistically significant.

3. Results

The mice injected with bradykinin, L-arginine, or NAME in the doses indicated in the present experiments did not show changes in gross behaviour over a period of 120 min when compared with saline-injected mice.

3.1. Antinociception induced by bradykinin and antagonistic effects of methylene blue and N^G-nitro-L-arginine (NAME)

The administration of bradykinin i.c.v. in doses of 4, 8 and 16 μg induced antinociceptive effects in mice. These effects were not affected by 2 and 4 mg/kg naloxone (data not shown).

Methylene blue was administered in a concentration of 1 μg i.c.v. concomitant with the injection of bradykinin. NAME was given in a dose of 10 mg/kg, s.c., 30 min before the injection of the peptide. Although NAME induced a low hypoalgesic response, both NAME and methylene blue significantly antagonized the antinociceptive effects of bradykinin (Table 1).

3.2. Effects of L-arginine on bradykinin-induced antinociception

The administration of L-arginine (300 and 450 mg /kg s.c.) induced antinociception, but when administered 30 min before bradykinin, L-arginine significantly reduced the effects of the peptide. These results are shown in Table 2.

3.3. Effects of bradykinin in mice tolerant to morphine or L- arginine

Results obtained with morphine-pretreated mice are shown in Table 3. Morphine was administered in doses of 100 mg/kg s.c. 24 h before the injection of bradykinin. Opiate administration resulted in tolerance as demonstrated by a reduction in the effects of a test dose. Morphine

Table 1
Effects of methylene blue (MB) and N^G-nitro-L-arginine (NAME) on the antinociceptive effects of bradykinin in mice

Dose μg, mice	Antinociception (area under the time-response curve, in min-s units).		
	Bradykinin and saline	Bradykinin and MB, 1 μg i.c.v.	Bradykinin and NAME, 10 mg/kg, s.c.
Saline	7 ± 4	8 ± 4	20 ± 6 [a]
Bradykinin, 4	116 ± 16	73 ± 9 [b]	71 ± 10 [b]
Bradykinin, 8	216 ± 19	91 ± 13 [b]	96 ± 16 [b]
Bradykinin, 16	228 ± 24	144 ± 12 [b]	120 ± 19 [b]

MB was administered in conjuction with bradykinin (i.c.v.). NAME was administered i.p., 30 min before bradykinin (i.c.v.). $n = 14$ mice per experimental group. [a] Statistically different from saline-injected mice, $P < 0.05$; [b] statistically significant from the effects induced by bradykinin in saline-injected mice, $P < 0.05$.

Table 2
Effects of L-arginine on the antinociceptive effects of bradykinin in mice

Dose μg, mice	Antinociception (area under the time-response curve, in min-s units).		
	Saline	Bradykinin and L-arginine 300 mg/kg	Bradykinin and L-arginine 450 mg/kg
Saline	9 ± 4	94 ± 22 [a]	112 ± 12 [a]
Bradykinin, 4	119 ± 19	80 ± 16 [a]	91 ± 18 [a]
Bradykinin, 8	207 ± 25	110 ± 15 [a]	126 ± 20 [a]
Bradykinin, 16	230 ± 22	118 ± 12 [a]	134 ± 23 [a]

L-Arginine was administered i.p., 30 min before bradykinin (i.c.v.). $n = 14$ mice per group. [a] Indicates statistically different from the effects induced by bradykinin in saline-injected mice $P < 0.05$.

administered in a test dose of 5 mg/kg s.c. in saline-treated mice gave a mean area value of 198 ± 24 min-s. The responses to the same dose of mice previously treated with the priming dose of the opiate were significantly lower: 37 ± 7.3. As shown in the table, the effects of bradykinin in morphine-tolerant mice did not differ from those observed in saline-treated animals.

The effects of bradykinin in mice tolerant to L-arginine are shown in Table 4. L-Arginine was administered for three days in doses of 600 mg/kg twice a day. This treatment reduced the effect of the amino acid: the area under the curve was reduced from 218 ± 26 to 48 ± 12 min-s. As shown in Table 4, the response induced by bradykinin was also significantly reduced when bradykinin was administered to mice tolerant to the precursor of NO.

4. Discussion

The present study indicates that i.c.v. administration of bradykinin produces dose-related antinociceptive responses in mice as assessed by the hot-plate procedure. These results confirm previous reports of various authors who administered the drug i.c.v. (Ribeiro et al., 1971; Argiolas et al., 1985) or in the spinal cord (Laneuville and Couture, 1987). More recently, Bauer et al. (1993) demonstrated that bradykinin stimulates cGMP formation in cultured dorsal root ganglion neurons. Since NO is a physiological stimulus for cGMP synthesis, it seems admissible to postulate a role for NO in the bradykinin-induced responses. However, the involvement of other neurotransmitters in the antinociceptive action of i.c.v. bradykinin cannot be ruled out. In this regard, the antagonism of intrathecal bradykinin-antinociception induced by α_2-adrenoceptor inhibitors has been reported by Laneuville et al. (1989).

In order to elucidate whether endogenous opioid systems influence bradykinin-induced antinociception, the drug was given to mice rendered tolerant to morphine by pretreatment with a high dose of the opiate; generally, tolerance to a drug involves a decreased potency of pharmacologically related agents. Antinociception was unaffected by this pretreatment or by naloxone, indicating that opiopeptidergic mechanisms may not be involved in bradykinin antinociception.

The involvement of nitric oxide in pain perception is currently a matter of investigation; thus, Moore et al. (1991) and Haley et al. (1992) have described antinociception when NO formation is antagonized by NO synthase inhibitors. However, in contrast to what might be expected, L-arginine and other precursors of NO synthesis (Duarte and Ferreira, 1992) reduced pain perception. A possible involvement of central opiopeptidergic systems has been suggested, since L-arginine antinociception is antagonized by naloxone (Duarte and Ferreira, 1992; Kawabata et al., 1992). In regard to the antinociception induced by NAME Hirsch et al. (1993) have suggested that inhibitors of NO synthase decrease neurotransmitter release in brain synaptosomes induced by excitatory amino acids, including those responsible for pain perception. Our results showing antagonistic effects on bradykinin-induced antinociception produced by concomitant administration of methylene blue or NAME, two inhibitors of NO formation (Rees et al., 1989; Mayer et al., 1993), do not support the hypothesis that NO is a messenger for nociceptive stimulation.

Table 3
Effects of bradykinin in mice tolerant to morphine

	Antinociception (area under the time-response curve, in min-s units)	
	Saline	Pretreated with morphine
Saline	9 ± 4	40 ± 12 [a]
Bradykinin, 4 μg	122 ± 20	93 ± 15
Bradykinin, 8 μg	201 ± 23	190 ± 23
Bradykinin,16 μg	222 ± 20	203 ± 15

Mice pretreated with morphine received one dose of 100 mg/kg s.c., 24 h before bradykinin administration. $n = 14$ mice per group. [a] Statistically different from saline-injected mice. $P < 0.05$.

Table 4
Effects of bradykinin in mice tolerant to L-arginine

	Antinociception (area under the time-response curve, in min-s units)	
	Saline	Pretreated with L-arginine
Saline	9 ± 4	48 ± 12 [a]
Bradykinin, 4 μg	115 ± 14	34 ± 8 [b]
Bradykinin, 8 μg	196 ± 26	75 ± 22 [b]
Bradykinin, 16 μg	219 ± 23	94 ± 14 [b]

L-Arginine was administered i.p. in doses of 600 mg/kg, twice a day, for 3 days. $n = 14$ mice per group. [a] Statistically different from saline-injected mice, $P < 0.05$. [b] Statistically significant from the effects induced by bradykinin in saline-injected mice, $P < 0.05$. The effect of L-arginine in non-pretreated mice (acute administration) was 218 ± 26.

The present findings show that bradykinin and L-arginine share common mechanisms: for both agents, antinociception is antagonized by NO synthase inhibitors and tolerance to the antinociceptive effects of L-arginine also reduces the responses to bradykinin. Furthermore, the concomitant administration of bradykinin and L-arginine to mice decreases antinociception, which suggests the interference of these drugs with common pathways. It is possible that L-arginine antagonism of bradykinin antinociception might be due to the stimulation of a hyperalgesic pathway. This pathway may be 'switched off' when the antinociceptive pathway is activated by the sole presence of L-arginine. However, the two drugs differ with respect to the possible involvement of opiopeptidergic mechanisms in their hypoalgesic effects since L-arginine, in contrast to bradykinin, exhibits a naloxone-sensitive response.

For a better understanding of some of the controversies of neurophysiological and behavioural studies regarding antinociception, it is worthwhile to consider that pain perception is an expression of the complex functional organization of the CNS. Thus, the effects of systemically administered drugs, even those applied in the intracerebral ventricular system, may be induced simultaneously at various sites of the CNS and the response seen after their application in a discrete area may be quite different from the integrated behavioural response. This rationale should be kept in mind in neurophysiological studies which conclude that NO behaves only as a pronociceptive agent when inhibitors of NO synthase are applied to restricted areas of the CNS. It is now becoming clear that NO formed after L-arginine administration may produce antinociception or hyperalgesia according to its action on different neuronal pathways (Kawabata and Takagi, 1994).

Our present results do not allow us to infer the precise site of action of bradykinin; however, it has been reported that microinjections in the periaqueductal grey matter antagonize nociceptive responses (Ribeiro et al., 1971), whereas injections in other brain loci are ineffective.

In conclusion, if it is accepted that bradykinin antinociception is induced through an increase in NO synthesis and the consequent formation of cGMP, our results do not favour the hypothesis that NO has a pro-nociceptive role in the CNS.

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