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European Journal of Pharmacology 279 (1995) 1–5

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Possible involvement of nitric oxide in red nucleus stimulation-induced analgesia in the rat

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Received 7 July 1994; revised 6 February 1995; accepted 10 February 1995

Abstract

There is considerable evidence that nitric oxide (NO) plays a role in synaptic transmission in both central and peripheral nervous systems. Recent studies have suggested the involvement of the L-arginine-NO pathway in nociceptive transmission/modulation. Electrical stimulation of the red nucleus in the rat evokes potent analgesia. Microinjection of different concentrations of L-arginine (1 nmol–1 μmol), but not of D-arginine, produced quick and long-lasting analgesia. Pretreatment with *N*-nitro-L-arginine methyl ester (1 μmol), a nitric oxide synthase inhibitor, significantly prevented L-arginine-induced analgesia. Further, pretreatment of animals with methylene blue, a known guanylate cyclase inhibitor, also attenuated the development of analgesia. Our results suggest that L-arginine caused production of NO, which in turn activated the red nucleus analgesic system.

Keywords: Red nucleus; L-Arginine; Analgesia; Nitric oxide (NO); cGMP

1. Introduction

Nitric oxide (NO), a small relatively unstable reactive diatomic free radical, has become in the past few years one of the most studied entities in biological chemistry and has excited widespread interest. It plays a role as a biological messenger in a wide range of physiological and biochemical activities (Moncada et al., 1991; Haley et al., 1992a,b; Snyder, 1992). Several studies suggest that NO plays a role in synaptic transmission in both central as well as peripheral nervous systems (Aanonsen et al., 1990; Haley and Dickenson, 1992; Kumar et al., 1993). The recent discovery and isolation of nitric oxide synthase, an enzyme responsible for the production of NO, from neural tissue (Garthwaite et al., 1988) suggests the endogenous synthesis of NO from the amino acid, L-arginine (Palmer et al., 1988; Moncada et al., 1989). NO is known to activate soluble guanylate cyclase and to increase the intracellular content of cGMP, which may modulate various physiological functions including pain and analgesia (Moore et al., 1991; Meller et al., 1992a,b; Kawabata et al., 1993).

Electrical stimulation of the red nucleus in the rat has been shown to inhibit the tail flick reflex to noxious heat without significantly affecting motor behaviour and it is not aversive (Prado et al., 1984; Prado and Roberts, 1985). A variety of neurotransmitters, opioidic, cholinergic, adrenergic etc., have been implicated in the modulation of pain at different levels of the neuraxis. The aim of the present study was to analyse the possible role of nitric oxide in the analgesia induced by stimulation of the red nucleus.

2. Materials and methods

Male Charles Foster rats, weighing 250–300 g, of the CDRI colony were used. They were housed individually and kept in a temperature-regulated room with food and water served ad libitum. A 12-mm long 23-gauge stainless steel guide cannula was implanted in the red nucleus under sodium pentobarbitone (35 mg/kg i.p.) anaesthesia using the stereotaxic technique. The coordinates for the red nucleus were AP = −3.8 mm L = 0.8 mm, H = 7.5 mm, as described by Pellegrino and Cushman (1967). The tip of the guide cannula was kept 1 mm above the target site. It was fixed to the skull with the help of steel screws and

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Elsevier Science B.V.
SSDI 0014-2999(95)00105-0

dental cement. Each cannula was kept patent with a sterile obturator. Oxytetracycline (25 mg/kg i.m.) was administered to check infection and the animals were allowed to recover for at least a week before the experiment.

On the day of the experiment a fine needle (27 gauge) was inserted through the cannula and advanced 1 mm to reach the injection site. The drugs were dissolved in pyrogen-free normal saline and slowly injected in a volume of 0.5 μl over a 2- to 3-min period. The injection sites were later identified by histological examination of the spots produced by microinjection of pontamine sky blue at the end of each experiment.

The rats were tested for analgesia using the tail flick method (D'Amour and Smith, 1941). They were placed in a transparent plastic rat holder with the tail protruding out and laid gently across a wire coil. Initially, sufficient current was passed through the coil to cause tail flick latencies of 4–5 s duration. A cut-off time of 10 s was employed to avoid damage to tail skin. Two baseline tail flick latencies (TFL) were recorded at 5 min intervals, before drug administration and thereafter up to 60 min. The tail flick latency was expressed as index of analgesia (IA), calculated by the following formula:

$$IA = \frac{\text{Observed TFL} - \text{Control TFL}}{10 - \text{Control TFL}}$$

L-Arginine, a nitric oxide precursor, D-arginine, *N*-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor, and methylene blue, a guanylate cyclase inhibitor, were dissolved in pyrogen-free normal saline. L-Arginine or D-arginine was microinjected in doses of 1 nmol to 1 μmol to groups of 5–8 animals each. Methylene blue (1 μmol) and *N*-nitro-L-arginine methyl ester (1 μmol) were given 10 min prior to the injection of L-arginine. The two-tailed Mann-Whitney *U*-test was used for statistical analysis of results and the *U* values were converted into the more familiar *P* values.

3. Results

3.1. Effects of L-arginine microinjection on tail flick latency

L-Arginine, when microinjected into the red nucleus of rats, produced a concentration-dependent antinociceptive effect (Fig. 1). A low dose of L-arginine (1 nmol) had little effect on tail flick latency. Microinjection of a higher dose of L-arginine (10 nmol) produced an analgesic response which lasted for 10–15 min. The inhibition of tail flick latency produced by 100 nmol of L-arginine lasted for about 40 min. Further, a higher dose of L-arginine (1000 nmol) had potent analgesic effects. The highest dose of L-arginine (1000 nmol) produced analgesia with a rapid onset and which lasted for nearly an hour.

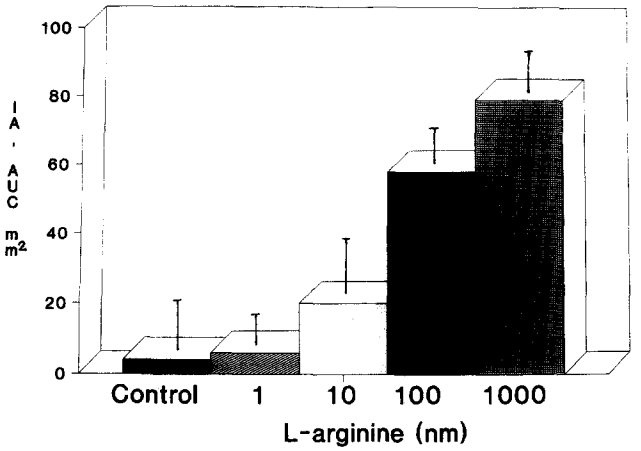

Fig. 1. Dose-dependent facilitation of the antinociceptive tail flick reflex produced by L-arginine (1–1000 nmol). Ordinate represents the index of analgesia (IA) measured as area under the curve (AUC, mm^2). The values are means ± SE. Each group consisted of five animals.

3.2. Antagonism of L-arginine-induced analgesia by N-nitro-L-arginine methyl ester

The analgesia induced by microinjection of L-arginine (1 μmol) into the red nucleus was completely antagonised by pretreatment with the nitric oxide synthase inhibitor, *N*-nitro-L-arginine methyl ester (1 μmol) (Fig. 2). It did not affect the basal tail flick latency per se.

3.3. Effects of methylene blue on L-arginine-induced analgesia

Methylene blue (1 μmol), a guanylate cyclase inhibitor, when administered 10 min prior to L-arginine

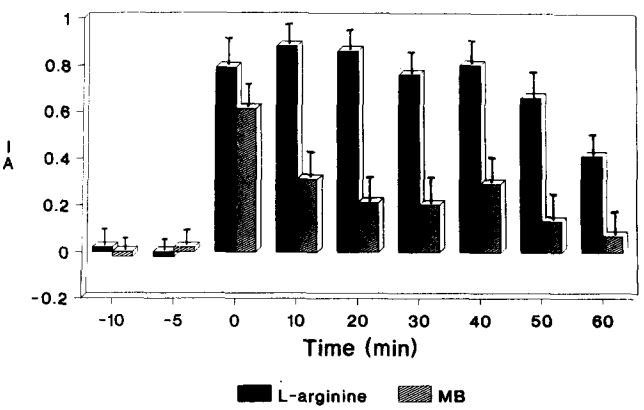

Fig. 2. The time course of the analgesic effects of microinjection of L-arginine (1 μmol) into the red nucleus of rats ($n = 6$) and antagonistic effect of methylene blue (MB, 1 μmol), microinjected 10 min prior to administration of L-arginine. The readings at −10 and −5 min indicate the level of analgesia before drug injection.

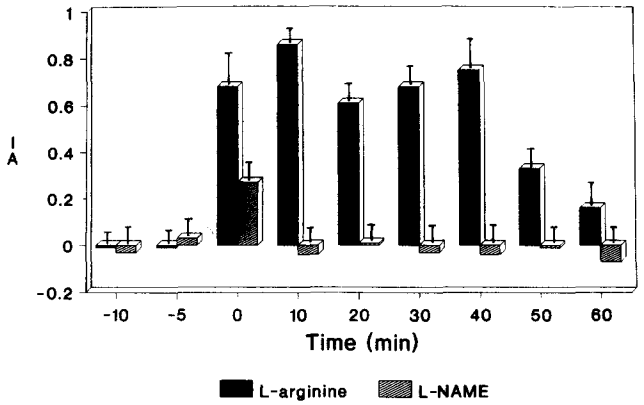

Fig. 3. The attenuation of analgesia induced by microinjection of L-arginine (1 μmol, $n = 8$) by *N*-nitro-L-arginine methyl ester (L-NAME, 1 μmol), microinjected 10 min prior to L-arginine administration.

(1 μmol) microinjection into the red nucleus, significantly prevented the development of analgesia (Fig. 3). Methylene blue also had no effects on basal tail flick latency per se.

3.4. Effect of D-arginine

Microinjection of D-arginine (1 μmol) into the red nucleus failed to produce an analgesic response as measured by the tail flick test (Fig. 4).

The placement of the injection site within the red nucleus was consistent in all the groups (Fig. 5). The slight variation in the site did not have any effect on the analgesic response to L-arginine or its modification by the pretreatments.

4. Discussion

The results of the present study demonstrate that microinjection of L-arginine into the red nucleus produced analgesia of long duration. It has been previ-

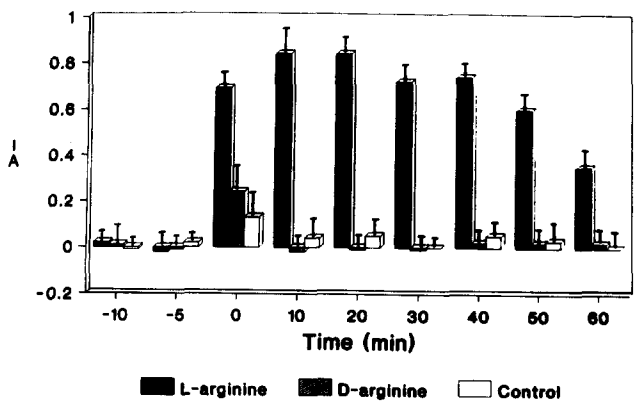

Fig. 4. The effects of microinjection of D-arginine (1 μmol, $n = 5$) into the red nucleus and comparison with the effect of L-arginine (1 μmol) and saline (5 μl).

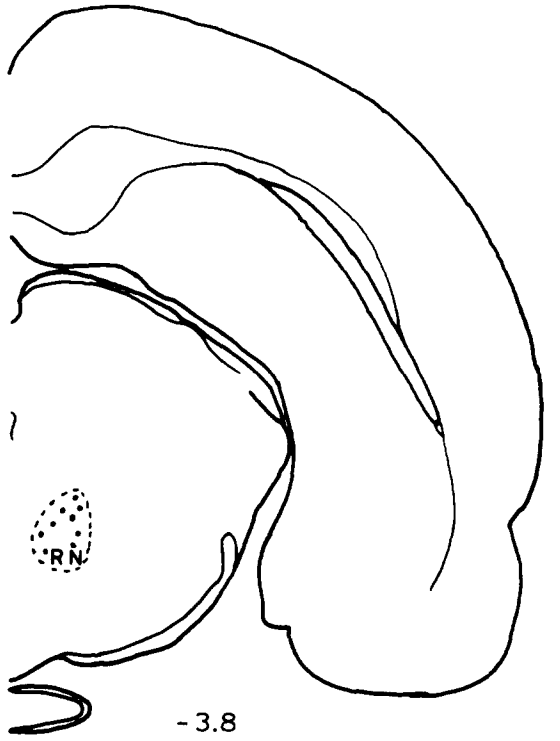

Fig. 5. A coronal section of the rat brain stem (derived from the atlas of Pellegrino and Cushman, 1967). Dotted areas indicate the region in which injection sites were located. The number indicates the distance in mm posterior to the bregma.

ously reported that L-arginine when administered either subcutaneously or intracerebroventricularly produces antinociception in mice (Kamei et al., 1994; Kawabata et al., 1993). There are no nitric oxide synthase-containing neurons or nitric oxide synthase terminals in the red nucleus, but nitric oxide synthase-containing neurons are present in the pretectal nucleus (Vincent and Kimura, 1992), which gives dense projections to the red nucleus (Prado, 1989). The present study suggests that NO may be involved in the antinociceptive mechanisms mediated by the red nucleus.

The failure of D-arginine to induce analgesia suggests that L-arginine increased the NO concentration in the red nucleus, which in turn produced analgesia. This is further supported by blocking of the analgesic effect of L-arginine by *N*-nitro-L-arginine methyl ester, which prevents its conversion into NO. The attenuation of analgesia by methylene blue, a guanylate cyclase inhibitor, suggests the possible involvement of cGMP in the red nucleus analgesic mechanism(s). Several groups of workers have recently shown that L-arginine is converted into NO in the brain by the stereoseletive action of nitric oxide synthase, which stimulates guanylate cyclase activity to generate cGMP (Moncada et al., 1991; Southam and Garthwaite, 1993). There is growing evidence which suggests the possible involvement of the L-arginine-NO-cGMP pathway in central anal-

gesic mechanisms (Duarte et al., 1990, 1992; Meller et al., 1992). It seems that a similar mechanism may operate in red nucleus stimulation induced analgesia, as this was significantly attenuated by *N*-nitro-L-arginine methyl ester and methylene blue. The increasing concentrations of L-arginine should result in a proportionate increase in endogenous NO levels and cGMP formation to produce a concentration-dependent analgesic effect. This is suggestive of a physiological function of NO, and therefore it appears that L-arginine-NO-cGMP pathways may serve as important central non-opioid analgesic mechanisms involving the red nucleus.

It has already been reported that kyotorphin, an endogenous neuropeptide isolated from bovine brain, produces naloxone-sensitive antinociception by enhancing [Met]enkephalin release in the brain and spinal cord (Takagi et al., 1979a,b; Ueda et al., 1982, 1987). Kyotorphin is formed from L-tyrosine and L-arginine by the enzyme kyotorphin synthase (Ueda et al., 1987). It is also suggested that L-arginine may be a rate-limiting factor for this enzyme; L-arginine is considered to be a precursor of kyotorphin (Ueda et al., 1987). In this regard it is also reported that L-arginine administered s.c. or i.c.v. produces naloxone-sensitive antinociception in mice and rats with carrageenin-induced hyperalgesia (Kawabata et al., 1992a,b). It is possible that microinjection of L-arginine into the red nucleus produces analgesia via the kyotorphin-[Met]enkephalin pathway in this specific brain region.

In conclusion, our results suggest a neuromodulatory or neurotransmitter role of NO in red nucleus analgesia. However, the precise underlying physiological and molecular mechanisms remain to be elucidated. This may be clinically relevant if activation of this pathway leads to analgesia devoid of tolerance as seen with opioids.

Acknowledgements

The financial support by the Council of Scientific and Industrial Research, New Delhi to A.K. is gratefully acknowledged.

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