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Activation of the arginine-nitric oxide pathway in primary sensory neurons contributes to dipyrone-induced spinal and peripheral analgesia

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Abstract. The objective of this study was to investigate the site of action of dipyrone in rat paw prostaglandin-induced hyperalgesia. The intracerebroventricular (i.c.v.) injection of dipyrone had no effect on the hyperalgesic response to prostaglandins. In contrast, intraplantar (i.pl.) and intrathecal (i.t.) injections produced dose-dependent analgesic effects. The analgesia observed following the intraperitoneal (i.p.), i.t., i.pl. or combined i.t. and i.pl. administration of dipyrone was abolished by pretreating the paws with L-NMMA (a nitric oxide synthase inhibitor) or methylene blue (MB, an inhibitor of soluble guanylate cyclase). These results support the suggestion that dipyrone-mediated antinociception results from a combined spinal and peripheral effect in the primary peripheral sensory neuron via stimulation of the arginine/cGMP pathway.

Key words: Spinal analgesia – Intrathecal analgesia – Dipyrone – Prostaglandin E_2 – Retrograde sensitization

Introduction

Despite the numerous behavioural and electrophysiological studies that have been performed, the mode and site of action of dipyrone remain controversial. Dipyrone has been suggested to cause antinociception by stimulating the central activation of periaqueductal grey matter thus exerting an inhibitory effect on impulse transmission in the spinal chord [1, 2]. Neugebauer et al. [3], using inflamed joints, concluded that dipyrone antinociception was mainly due to a spinal effect. Unlike indomethacin, a typical cyclooxygenase inhibitor, the local application of dipyrone in the rat paw abolished the long-lasting or persistent hyperalgesia induced by a single or multiple injections of prostaglandin, respectively [4, 5]. We recently discovered that the local peripheral analgesic effect of dipyrone could be abolished by pretreating of the paws with L-NMMA, a nitric oxide synthase inhibitor, or methylene blue, an inhibitor of soluble guanylate cyclase. This observation was the basis for our suggestion that dipyrone-induced analgesia is peripheral [6].

In the present study, we have investigated the effect of pretreating rat paws with L-NMMA and methylene blue on the analgesic response to dipyrone injected by intraplantar (i.pl.), intrathecal (i.t.) or intracerebroventricular (i.c.v.) routes. We used rat paw prostaglandin-induced hyperalgesia as a model, that closely mimicks inflammatory hyperalgesia [7].

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Materials and methods

Animals

Male Wistar rats (150–200 g) with free access to food and water were used throughout the experiments.

Nociceptive test

Hyperalgesia was measured using the rat paw pressure test [7], which is a modification of the Randall-Selitto test. In this method, a constant pressure of 20 mmHg is applied to the rat's paw and discontinued (reaction time) when the animal exhibits a reaction characterized by a reduction in escape movements. The animals usually make several attempts to escape from the position imposed by the experimental situation and these are followed by an alteration in the respiratory frequency and the onset of a typical shivering reaction. The intensity of hyperalgesia was quantified as the difference in the reaction times (Δ reaction time) obtained by subtracting the value measured 1, 2 or 3 h after administration of the hyperalgesic substance from the control reaction time measured prior to the administration of this substance. The investigator performing the hyperalgesia measurements was unaware of the order of drug treatment.

Administration of drugs

The drugs were administered by the intraventricular, intrathecal or intraplantar routes. Intraventricular injections were performed by a modification of the technique described by Correa and Graeff [8]. The rat was anesthetized with ether and its head was held horizontally in a stereotaxic apparatus (David Kopf 900). The

calvarium was exposed and a trephine hole of 1 mm in diameter was drilled 1.8 mm lateral to the coronary and 1.5 mm posterior to the sagital sutures. A cannula (0.7 mm o.d.) connected to a Hamilton syringe (RN-705, 50 µl) by polyethylene tubing (PE 50) was inserted 3.2 mm into the brain by means of electrode carrier. The correct placement of the cannula was indicated by the absence of backflow when 10 µl of fluid was injected. Injections were given over a period of 10 s. The entire surgical and injection procedure lasted less than 5 min.

For intrathecal injections, a slightly modified version of the technique described by Papir-Kricheli et al. [9], was used. Each animal was anesthetized with ether, the dorsal fur was shaved and a small incision in the skin was made in the lumbar region to facilitate the injections. With the spinal column arched, a 27-gauge hypodermic needle was inserted into the subarachnoid space at the level of the lower sacral spinal chord-cauda equine by puncturing on the midline at an angle of approx. 45° between the L4 – L5 (±1) vertebrae. A 20-µl bolus of drug or sterile saline was injected intrathecally using a Hamilton 710 Lt (100 µl) microsyringe. The needle was subsequently withdrawn and the skin sutured. In skilled hands, the injection procedure from the beginning of ether administration until withdrawal of the hypodermic needle takes about 3 min. The animals regained consciousness within 2 min of injection were fully awake and after 30–60 min, behavioral testing began.

In the paws, saline and all agents were administered as bolus injections of 100 µl into the plantar region. The dose, route and time of administration and the statistical analysis employed are indicated in the legend. Standard errors of the mean (SEM) smaller than the symbols used are not shown. A one-way analysis of variance (ANOVA) was used to compare the changes between different doses or treatments.

Drugs

The following drugs were used: PGE_2 (Sigma, St. Louis, USA), L-NMMA (Alexis Corporation, Läufelfinger, Switzerland), methylene blue (Reagen, São Paulo, Brazil), and dipyrone (Hoechst, Brazil).

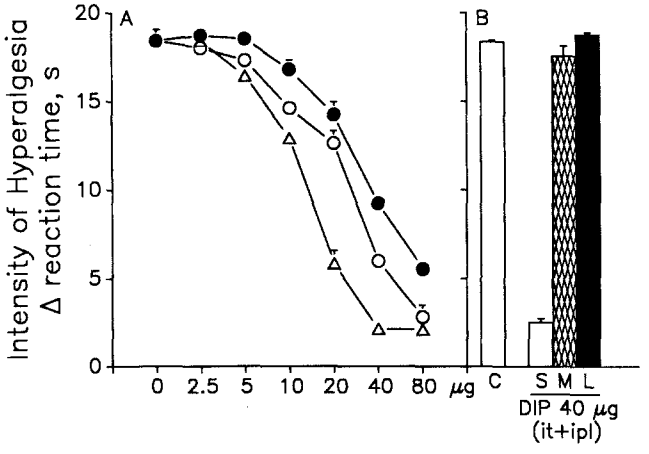

Fig. 1. Comparison of the responses to single and combined (i.t. and i.pl.) injections of dipyrone. The hyperalgesic measurements were taken three hours after the injection of prostaglandin E_2 (100 ng/paw). In panel A, the abscissa indicates the doses injected i.t. (●) and i.pl. (○). For combined injections (△), the doses indicated on the abscissa were equally between i.t. and i.pl. injections. Saline was administered either i.t. or i.pl. as a control in the i.pl. or i.t. alone groups, respectively. In panel B, C shows the control of PGE_2 hyperalgesia and S is the control of dipyrone analgesia by i.t. and i.pl. administration. Dipyrone was administered 2 h after PGE_2 challenge. The contralateral paws were pretreated (30 min before of dipyrone) with MB (M, 500 µg/paw) and L-NMMA (L, 50 µg/paw). The data are the mean ± SEM of five animals per group.

Results

Figure 1 shows that dipyrone induced a dose-dependent antinociceptive effect on the hyperalgesia induced by the i.pl. injection of PGE_2 when administered i.p., i.pl. and i.t. Pretreating the contralateral paw with either methylene blue or L-NMMA abolished the response to dipyrone injected by any of the three routes.

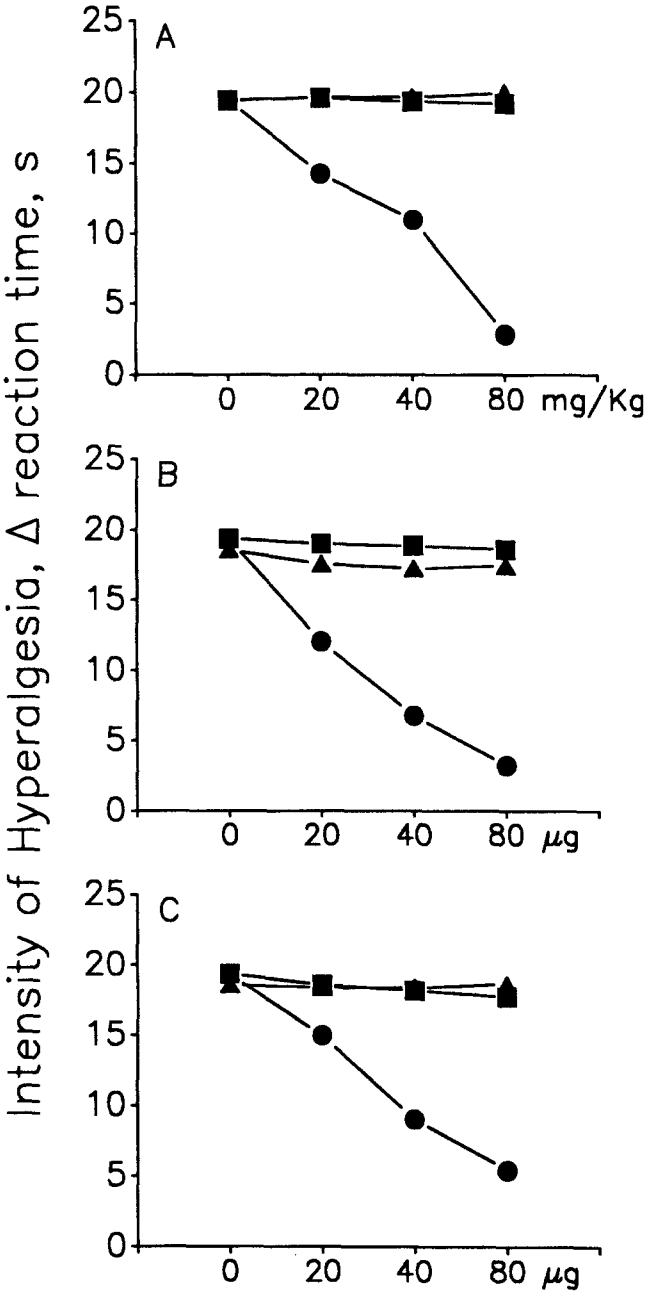

Fig. 2. Blockade of the intraperitoneal (A), intraplantar (B) and intrathecal (C) analgesic effect of dipyrone by pretreatment of the paws with L-NMMA or MB. The hyperalgesic measurements were taken three hours after the injection of prostaglandin E_2 in both hind paws (100 ng/paw). Dipyrone was injected 30 min before the measurements. In the intraplantar experiments, dipyrone was injected in both paws. The paw contralateral to the control one (●) was pretreated with L-NMMA (■, 50 µg/paw, five animals) or MB (▲, 500 µg/paw, five animals) 30 min before the injection of dipyrone. The data are the mean ± SEM.

Figure 2 compares the combined antinociceptive effect of i.t. and i.pl. administration of dipyrone with that observed after a single injection by each of these routes separately. There was no difference (ANOVA) between the i.t. and i.pl. effects, although the combination was more effective when compared with the single administration (ANOVA, $p < 0.01$). Panel B shows that methylene blue and L-NMMA abolished the antinociception produced by combined i.t. (20 µg) and i.pl. (20 µg) injections. When dipyrone was injected i.c.v. in doses up to 80 µg, no antinociceptive response was observed. As a positive control for the i.c.v. injection we used morphine which, at a dose of 60 µg, produced more than 95% antinociception (not shown).

Discussion

Dipyrone produced a well-defined dose-dependent peripheral [4] and spinal [3] antinociceptive effect in the rat paw prostaglandin-induced hyperalgesia test and acute inflammation in the knee joint in cats. Our method has an advantage over other hyperalgesia tests in that it eliminates the possibility that the peripheral effect of the analgesic results from a blockade of the release or action of mediators produced during the inflammatory process. Thus, indomethacin, a typical cyclooxygenase inhibitor, has a significant antinociceptive effect on carrageenin- but not on prostaglandin E_2-induced hyperalgesia [7].

We previously described the ability of L-NMMA and methylene blue to block the antinociceptive effect of analgesics such as dipyrone and diclofenac on ongoing prostaglandin E_2-induced hyperalgesia in the rat paw [4, 6]. We used the antinociceptive pretreatment of paws in the present series of experiments to define the participation of primary sensory neurons in the peripheral, spinal and systemic (i.p.) action of dipyrone (Fig. 1). As shown, pretreating the paws with L-NMMA or methylene blue, abolished the analgesia induced by dipyrone inject via any of the three routes (i.p., i.pl. and i.t.). This observation suggests that the systemic effect results from an action on the primary sensory neuron and that dipyrone acts at a receptor present at peripheral and presynaptic sites of this neuron.

Our results further suggest that systemic dipyrone-mediated antinociception may result from a synergistic effect at spinal and peripheral sites since the responses to combined i.t. and i.pl. injections were significantly greater than those observed with a two-fold higher dose injected by a single route (Fig. 2). The ability of intraplantar pretreatment with L-NMMA and methylene blue to abolish the systemic response to dipyrone points to an involvement of the primary sensory neuron in this antinociceptive effect. If secondary neuron sensitization were involved, one would expect only a partial inhibition by L-NMMA and methylene blue. Based on an inflammatory sensitization test in the cat knee joints, had been suggested a spinal action to explain dipyrone antinociception [3]. In agreement with their observations, we were also unable to implicate a central action for dipyrone, since i.c.v. injections were ineffective. Our conclusions are in apparent conflict with those which reported [1, 2] that dipyrone analgesia has a central origin, and is mediated by spinal inhibition via the activation of periaqueductal grey matter. Their explanation, however, is not straightforward since they also described that spinalization of rats, did not block antinociception by systemic (i.p.) administration of dipyrone [1]. The main reason for these discrepancies may lie in the fact that their conclusions were based on the tail flick test, the results of which do not generally correlate with those of tests for inflammation-induced nociception.

An intriguing finding in the present experiments, was that the intraplantar administration of methylene blue or L-NMMA affected intrathecally-induced hyperalgesia or analgesia. This observation was surprising and may indicate an unexpected rapid distribution of NO, methylene blue and L-NMMA in the cytosol of the primary afferent neuron. Thus, the axonal transport of substances between the periphery and spinal chord (and vice versa) may be much quicker than is currently thought [10]. Alternatively, the primary sensory neurons associated with inflammation may respond as an integrated unit in spite of the functional specializations at their peripheral and central presynaptic terminations. This raises the possibility that biochemical membrane events triggered at one neuronal termination may be rapidly transmitted to another termination by an as yet undefined mechanism.

In conclusion, our results support the suggestion that the major antihyperalgesic site of action of dipyrone is at the primary peripheral sensory neuron and that the systemic response may result from a combined spinal and peripheral effect. The present observations further support our earlier conclusion that dipyrone-induced analgesia is associated with the stimulation of the arginine/cGMP pathway in sensory neurons [6].

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