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Research report

Antinociception induced by intravenous dipyrone (metamizol) upon dorsal horn neurons: Involvement of endogenous opioids at the periaqueductal gray matter, the nucleus raphe magnus, and the spinal cord in rats

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Abstract

Microinjection of dipyrone (metamizol) into the periaqueductal gray matter (PAG) in rats causes antinociception. This is mediated by endogenous opioidergic circuits located in the PAG itself, in the nucleus raphe magnus and adjacent structures, and in the spinal cord. The clinical relevance of these findings, however, is unclear. Therefore, in the present study, dipyrone was administered intravenously, and the involvement of endogenous opioidergic circuits in the so-induced antinociception was investigated. In rats, responses of dorsal spinal wide-dynamic range neurons to mechanical noxious stimulation of a hindpaw were strongly inhibited by intravenous dipyrone (200 mg/kg). This effect was abolished by microinjection of naloxone (0.5 μg/0.5 μl) into the ventrolateral and lateral PAG or into the nucleus raphe magnus or by direct application of naloxone (50 μg/50 μl) onto the spinal cord surface above the recorded neuron. These results show that dipyrone, a non-opioid analgesic with widespread use in Europe and Latin America, when administered in a clinically relevant fashion causes antinociception by activating endogenous opioidergic circuits along the descending pain control system.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Descending pain control; Endogenous opioids; Nucleus raphe magnus; NSAIDs; Periaqueductal gray; Spinal nociceptive neurons

1. Introduction

Non-opioid analgesics exert their effects by acting upon peripheral tissues as well as upon central nervous system structures. Central targets of non-opioid analgesics include the periaqueductal gray matter (PAG) [3,27,28,31], the rostral ventromedial medulla (RVM), i.e., the nucleus raphe magnus (NRM) and adjacent structures [16], and the spinal cord (see [30] for review). Dipyrone (metamizol) is an antipyretic and non-opioid analgesic with widespread clinical use in Europe and Latin America [4,14,15]. This pyrazolone derivative readily forms neutral solutions in water and has inhibitory activity upon cyclooxygenases 1, 2, and 3 [2,5,7,14,22]. The biologically active metabolites of dipyrone quickly enter the cerebrospinal fluid and reach a concentration in brain tissue of about 50% plasma concentration [9]. We have shown [27] that dipyrone microinjection into PAG induces changes in the activity of spinally projecting neurons located in the RVM, specifically, the so-called on- and off-cells (see [10] for review). These changes are in the expected direction for the proposed role of on- and off-cells as pain-modulating intermediaries between the PAG and spinal nociceptive circuits [27]. PAG-microinjected dipyrone consequently induces inhibition of spinal neuronal responses to peripheral noxious stimulation [31–33] and inhibition of the tail flick reflex [3,27]. The antinociceptive effects of PAG-microinjected dipyrone thus mimic the effects of PAG-microinjected opioids [6,11,35] and are abolished by naloxone admin-

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istration to the same PAG site [29], to the RVM [32], or to the spinal cord [13]. Therefore, the antinociceptive effect of PAG-microinjected dipyrone is mediated by endogenous opioids at the PAG, the RVM, and the spinal cord, i.e., along the descending pain control system. The clinical relevance of these findings remains unknown, however, because dipyrone is not normally administered by microinjection into the PAG. In the present study, dipyrone was therefore administered intravenously, as often done to induce analgesia in humans [4,14,15], and naloxone was subsequently applied at various levels of the descending pain control system in order to investigate whether the antinociceptive effect of systemically administered dipyrone is also mediated by endogenous opioids acting at such levels. Some of the results have been presented in preliminary form [12].

2. Methods

2.1. General

Recommendations of the Society for Neuroscience and the International Association for the Study of Pain regarding experiments in animals were followed throughout. Male Sprague–Dawley rats (260–320 g), bred at the *Instituto Venezolano de Investigaciones Cientificas*, were deeply anesthetized with thiopental (60 mg/kg i.p. initial dose and 3.5–5 mg/kg/h i.v. continuously for maintenance). After insertion of a tracheal cannula, a carotid catheter, and a jugular catheter, a lumbar laminectomy was performed. Carotid pressure remained within normal range, and rectal temperature was kept around 37 °C. The animals were neither paralyzed nor artificially ventilated.

2.2. Preparation for naloxone administration

When naloxone was to be microinjected, a stainless-steel guide cannula (22-gauge) was stereotaxically [23] driven through a small craniotomy to reach 2 mm above the intended target in the PAG, the NRM, or their vicinities. When naloxone was to be applied to the spinal cord, the dura mater over the lumbar enlargement was opened, and a thin-walled plastic ring was sealed with grease onto the dorsal spinal cord surface over the intended recording site. This ring was filled with 50 μl normal saline, and 2% agar was poured around it to cover the surgical area as far as the stretched skin flaps. When naloxone was not to be applied to the spinal cord, no ring was installed, and the whole area was covered with agar except for a small saline-filled window above the spinal cord recording site (see below).

2.3. Recording and stimulation

Tungsten microelectrodes were introduced into the spinal cord through the saline solution in the plastic ring or in the agar window in order to record action potentials from dorsal horn neurons with receptive fields in the ipsilateral hindpaw. The neurons chosen for study had no or negligible spontaneous activity and were differentially excited by the dorsoventral application to the hindpaw of a weak clamp (innocuous when applied to the experimenter's fifth finger) or a strong clamp (noxious to the experimenter). Both the innocuous and the noxious clamp were spring-loaded so that the pressure applied in each case was maintained during the 10 s stimulation period (see below). The neurons chosen for study were also excited by noxious heat and pinch applied to their receptive field skin and can thus be classed as wide-dynamic range (WDR) or multireceptive neurons. When two or (seldom) three neurons were simultaneously recorded, their spikes were discriminated by means of the *BrainWave*® software. The number of neurons reported may thus be larger than the number of animals.

2.4. Experimental protocol

The following recording protocol was carried out every 5 min: (a) 1 min on-going activity, (b) 10 s application of the innocuous clamp, (c) 1 min on-going activity, and (d) 10 s application of the noxious clamp. After three or more cycles with stable responses were obtained (baseline), dipyrone (*Novalcina*®, formerly Hoechst–Marion–Rousell) was injected (200 mg in 0.8 ml saline per kilogram of body weight) through the jugular catheter in 10 s. Sixteen minutes after the dipyrone injection, naloxone was administered. For administration to the PAG or the NRM, a stainless-steel microinjection cannula (29-gauge), connected by polyethylene tubing to a 1 μl Hamilton syringe, was introduced through the guide cannula to reach the desired target, and 0.5 μg naloxone in 0.5 μl saline was microinjected in 10 s. For application to the spinal cord, the saline in the ring was replaced with 50 μg naloxone in 50 μl saline. Only one protocol was performed per animal.

We have previously shown that 200 mg/kg and 400 mg/kg i.v. dipyrone dose-dependently inhibit the tail flick reflex in rats [27]. According to the equation of Pong et al. [24], an oral dose of 200 mg/kg of dipyrone in mice would be equivalent to the recommended analgesic oral dose of 500 mg for adult humans (see [26], p. 538, and [15], p. 13). If the Pong equation also holds for rats, the i.v. dose of dipyrone chosen for the present study (200 mg/kg) is equivalent to the recommended single oral or i.v. dose for humans. On the other hand, the doses of naloxone used in the present study have been effective in the RVM (0.5 μg/0.5 μl) [32] and on the spinal cord (50 μg/50 μl) [13] for blocking the antinociceptive action of PAG-microinjected dipyrone.

At the end of the experiment, the microinjection site was marked by microinjecting 0.5 μl cresyl violet, and the microelectrode recording site was marked electrolytically (20 μA, 20 s). The animal was killed with an overdose of thiopental, and the brain and lumbar spinal cord were fixed