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Effects of metabolites of the analgesic agent dipyrone (metamizol) on rostral ventromedial medulla cell activity in mice

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ABSTRACT

The molecular mechanism of action of dipyrone, a widely used antipyretic and non-opioid analgesic drug, is still not fully understood. Actions upon peripheral inflamed tissues as well as the central nervous system, especially upon the PAG-RVM axis, have been suggested. Dipyrone is a prodrug and its activity is due to its immediate conversion to its active metabolites. We tested the effect of two recently discovered metabolites of dipyrone, the arachidonoyl amides of 4-methylaminoantipyrine and 4-aminoantipyrine, on the neurons of the rostral ventromedial medulla (RVM), which are part of the descending pathway of antinociception. These compounds reduced the activity of ON-cells and increased the activity of OFF-cells. Both CB1 and TRPV1 blockade reversed these effects, suggesting that the endocannabinoid/endovanilloid system takes part in the analgesic effects of dipyrone.

1. Introduction

Dipyrone (**1**, metamizole) is one of the most commonly used analgesic and antipyretic drugs worldwide (Nikolova et al., 2012). In contrast to acidic NSAIDs, it is only weakly anti-inflammatory, but has spasmolytic effects and causes no gastrointestinal lesions.

After oral administration, dipyrone is rapidly hydrolyzed to 4-methylaminoantipyrine (**2**) (Scheme 1). Compound **2** is further metabolized to 4-aminoantipyrine (**3**) and 4-formylaminoantipyrine (**4**) (Levy et al., 1995). Two novel metabolites were recently identified by us in mice: the arachidonoyl amides of **2** and **3**, viz. **5** and **6** (Rogosch et al., 2012).

The molecular mechanism of action of dipyrone is still not fully explained. Several mechanisms were proposed, including the involvement of endogenous opioids (Vanegas and Tortorici, 2002), stimulation of the PAG-RVM-axis (Vazquez et al., 2007), COX-1/COX-2 inhibition by dipyrone and its metabolites **2** and **3** (Pierre et al., 2007), COX-2 inhibition (Hinz et al., 2007), and interference with components of the endocannabinoid system (Rogosch et al., 2012). According to recent findings, the latter mechanism is connected with a central analgesic effect (Rogosch et al., 2012, Escobar et al., 2012), although we showed that cannabinoid CB1 receptors clearly do not mediate the thermal antinociceptive actions of dipyrone (Schlosburg et al., 2012). A recent study suggested that a CB1 receptor inverse agonist and an anti-sense oligonucleotide against CB1 receptors reversed the anti-hyperalgesic effect of **3**, but not that of dipyrone or **2** (Dos Santos et al., 2014). Finally, it was reported that **2** induced hypothermia and inhibited PGE_2-dependent and –independent fever, while **3** only inhibited PGE_2-dependent fever (Malvar et al., 2014).

The periaqueductal gray-rostral ventromedial medulla (PAG-RVM) pathway plays an important role in pain processing. PAG modulates nociception via a descending pathway that relays in the RVM and terminates in the spinal cord (Vanegas and Tortorici, 2002·Urban and Gebhart, 1992). There are three classes of neurons in the RVM: ON-cells, OFF-cells and neutral cells that respond differently to pain stimuli. Activation of ON-cells results in nociception, and this makes them an interesting target for pain research (Urban and Gebhart, 1992). The activity the ON-cells was suggested

Abbreviations: CB1, cannabinoid receptor type 1; COX, cyclooxygenase; I-RTX, iodoresiniferatoxin; NSAIDs, non-steroidal antiinflammatory dugs; PAG, periaqueductal gray; RVM, rostral ventromedial medulla; TRPV1, transient receptor potential vanilloid 1

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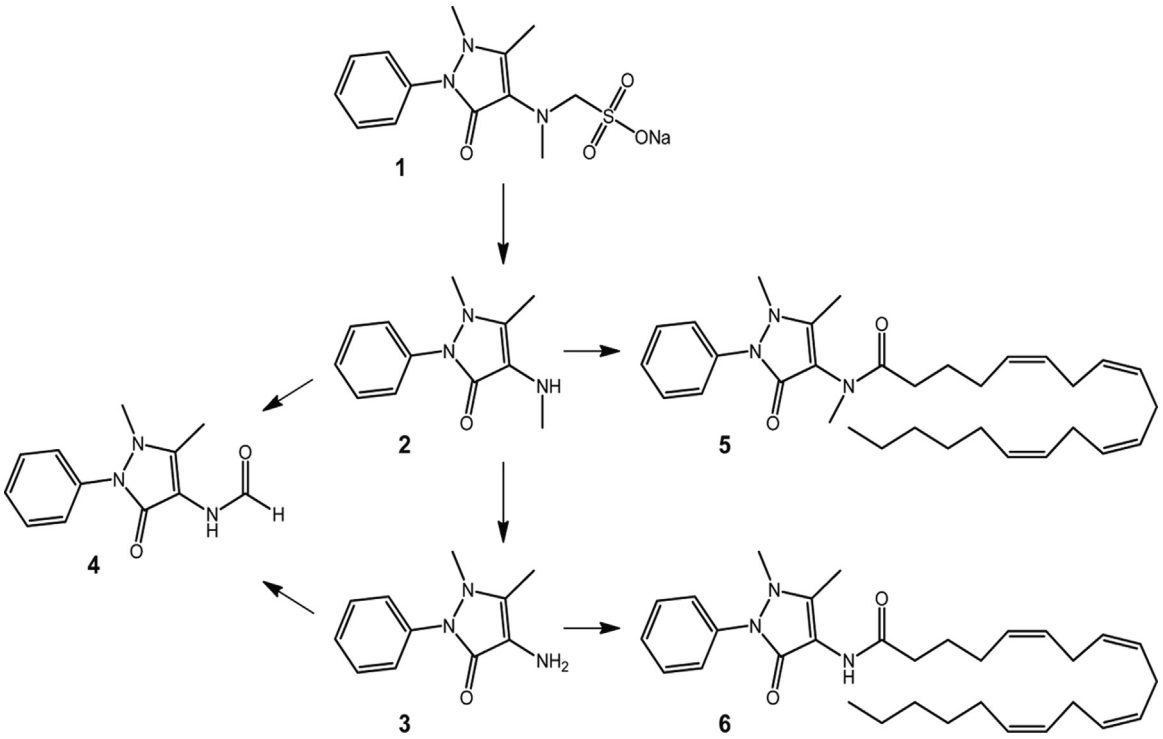

Scheme 1. Metabolites of dipyrone (**1**).

to serve as an electrophysiological marker of nociception (De Novellis et al., 2012). OFF-cells are inhibited by pain stimuli, and neutral cells show no response.

We have reported that the arachidonic acid amide **5** inhibits TRPV1 (Sinning et al., 2008a), and that some fatty acid amides exert their antinociceptive effect partly by acting on the spinal cord and the PAG (De Novellis et al., 2012), and hence we have hypothesized that the dipyrone metabolites **5** and **6** also act upon the PAG-RVM axis, contributing to the strong analgesic effect of dipyrone. The central analgesic effects of this compound were suggested to be effected through PAG neurons and the descending antinociceptive pathway already in 1986 (Carlsson et al., 1986). We monitored the activity of ON- and OFF-cells after injection of **2**, **5** and **6** into the PAG and the influence of coinjection of TRPV1 and CB1 antagonists. The aim of this study was to evaluate the interaction of the arachidonic acid conjugates of **5** and **6** with the PAG-RVM axis and thus further evaluate how and how much the analgesic activities of dipyrone are mediated through this axis.

2. Materials and methods

2.1. Animals

Adult male CD1 mice (20–25 g) were purchased from Harlan Laboratories (Milan, Italy) and were housed under controlled illumination (12:12 h light/dark cycle; light on 06.00 h) and environmental conditions (room temperature 20–22 °C, humidity 55–60%) for at least 1 week before the beginning of experiments. Chow and tap water were available ad libitum. All experimental procedures were conducted in conformity with protocols approved by the Animal Ethics Committee of the Second University of Naples. Animal care was in compliance with the IASP and European Community (E.C. L358/1 18/12/86) guidelines on the use and protection of animals in experimental research. All efforts were made to minimize animal suffering and to reduce the number of animals used.

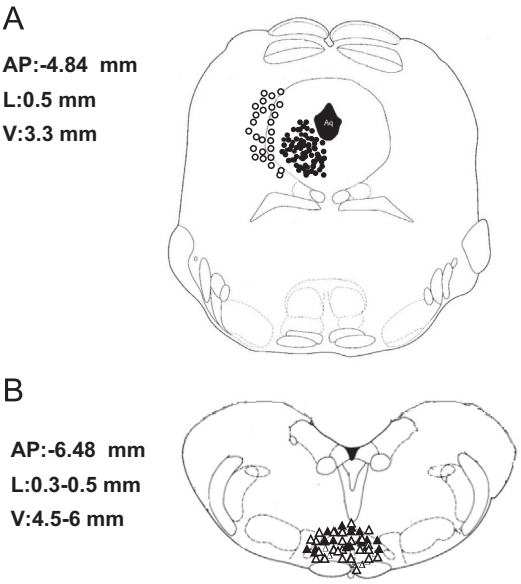

Fig. 1. Schematic illustration of the location of ventrolateral periaqueductal gray (VL PAG) microinjection sites (A) and RVM ON or OFF cell recording sites (B). Vehicle or drug microinjections were performed in the VL PAG (filled circles). The open circles indicate microinjections accidentally or intentionally performed outside of VL PAG, the effects of which have been considered in the study for location specificity (A). Cell recordings were performed by lowering a tungsten electrode into the RVM. ON cells (filled triangles) or OFF cells (open triangles) recording sites are shown in B.

2.2. Surgical preparation for intra-PAG microinjection

(Fig. 1) For electrophysiological experiments, mice were anesthetized with pentobarbital (50 mg/kg, i.p.) and a 26-gauge, 10 mm long stainless steel guide cannula was stereotaxically lowered until its tip was 1 mm above the left VL-PAG by applying coordinates (AP: −4,84 mm from bregma, L: 0.5 mm from midline, V: 3,3 mm below