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The analgesic effect of *N*-arachidonoyl-serotonin, a FAAH inhibitor and TRPV1 receptor antagonist, associated with changes in rostral ventromedial medulla and locus coeruleus cell activity in rats

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ABSTRACT

We evaluated the effects of intra-periaqueductal grey (PAG) *N*-arachidonoyl-serotonin (AA-5-HT), a compound with a "dual" ability to inhibit the fatty acid amide hydrolase (FAAH) and to antagonize transient receptor vanilloid type 1 (TRPV1) receptors, on endocannabinoid levels, rostral ventromedial medulla (RVM) ON and OFF cell activities, thermal nociception (tail flick in anaesthetized rats) and formalin-induced nocifensive responses in awake rats. AA-5-HT increased endocannabinoid levels in the PAG and induced analgesia. Paradoxically, it also depressed the RVM OFF cell, as well as the ON cell activities. The effect of AA-5-HT was mimicked by co-injecting the selective FAAH inhibitor URB597 and the selective TRPV1 antagonist I-RTX into the PAG, which also induced analgesia and inhibition of ON and OFF cell ongoing activities. The recruitment of "alternative" pathways, such as PAG-locus coeruleus (LC)-spinal cord might be responsible for AA-5-HT effect since we found evidence that (i) intra-PAG AA-5-HT increased LC neuron firing activities, and (ii) intrathecal phentolamine or ketanserin prevented the analgesic effect of AA-5-HT. Moreover, intra-PAG AA-5-HT prevented the changes in the ON and OFF cells firing activity induced by intra-paw formalin, and it inverted the formalin-induced increase in LC adrenergic cell activity. All AA-5-HT effects were antagonized by cannabinoid CB_1 and TRPV1 receptor antagonists thus suggesting that co-localization of these receptors in the PAG can be an appropriate neural substrate for AA-5-HT-induced analgesia.

1. Introduction

The naturally produced cannabinoid receptor agonist *N*-arachidonoylethanolamide (AEA, anandamide), and its congeners or analogs *N*-arachidonoylglycine (NAGly), *N*-palmitoylethanolamide (PEA) and *N*-oleoylethanolamide (OEA), are lipid mediators with anti-nociceptive and anti-inflammatory activities in vivo exerted via several molecular targets (see Bradshaw and Walker, 2005 for review). These endogenous compounds are metabolised by enzymatic hydrolysis, which is catalysed by amidases, such as the fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996; Lichtman et al., 2004b). FAAH inhibitors may lead to the indirect (i) activation of cannabinoid CB_1 and CB_2 receptors, via accumulation of AEA and of the other endocannabinoid, 2-arachidonoylglycerol (2-AG), which is also a FAAH substrate; (ii) stimulation of peroxisome proliferation activating receptor-α, which is activated by OEA and PEA; (iii) activation/desensitisation of transient receptor potential channels of vanilloid type 1 (TRPV1) receptors, via AEA and OEA accumulation; and (iv) activation of GPR18, via NAGly accumulation (Di Marzo et al., 2002; Iversen and Chapman, 2002; Re et al., 2007; Kohno et al., 2006). Indeed, pharmacological and genetic inhibition of FAAH were shown to lead to analgesic and anti-inflammatory actions in vivo (Lichtman et al., 2004a,b; Holt et al., 2005; Jayamanne et al., 2006; Maione et al., 2006, 2007) through mechanisms that are not always mediated by cannabinoid receptors (Lichtman et al., 2004a). Also, it has been shown that *simultaneous* activation of cannabinoid CB_1 receptors and stimulation/desensitisation of TRPV1 receptors, may cause analgesic effects that are stronger than

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the targeting of each single receptor alone, due to the different respective roles and mechanisms of the two receptor types in the control of nociception (Brooks et al., 2002). TRPV1 receptors are non-selective cation channels known to be expressed mostly by peripheral sensory afferents and spinal cord neurons, and to act as polymodal pain transducers (Szallasi and Blumberg, 1999). However, recent evidence suggests that TRPV1 receptors are also expressed in the brain (Mezey et al., 2000; Cristino et al., 2006), including the periaqueductal grey (PAG) (Maione et al., 2006), where they can be activated by endogenous compounds known as "endovanilloids" (van der Stelt and Di Marzo, 2004; Starowicz et al., 2007a), including FAAH substrates such as AEA.

Stimulation of TRPV1 receptors in the dorsal PAG generates analgesia or hyperalgesia followed by analgesia by activating or desensitizing specific neurons involved in nociception in this region and terminating onto ON and OFF cells of the rostral ventromedial medulla (RVM) (Palazzo et al., 2002; McGaraughty et al., 2003). NEUTRAL, ON and OFF cells are different pain responding/modulating neuron types found in the RVM. Pain stimuli increase ON, inhibit OFF and do not affect NEUTRAL cell activity (Fields et al., 1991). We have shown more recently that functional TRPV1 receptors in the ventrolateral PAG (VL-PAG) form part of this descending modulatory system, since their stimulation produces analgesic effects by acting on the same RVM-projecting neurons that mediate specific functional changes in RVM ON and OFF neurons (Maione et al., 2006; Starowicz et al., 2007b). Based on recent evidence that *N*-arachidonoyl-serotonin (AA-5-HT, the first ever compound to be developed with the "dual" ability to inhibit FAAH and to antagonize TRPV1 receptor), shows strong analgesic activity after systemic administration in acute or chronic pain models in rodents (Maione et al., 2007), the effect of intra-VL-PAG microinjections of AA-5-HT on (i) the PAG levels of anandamide (AEA), 2-arachidonoylglycerol (2-AG), *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA); (ii) acute thermal nociception (tail flick test) in anaesthetized rats; (iii) ongoing and tail flick-related changes in RVM ON and OFF cell activities in healthy and formalin-treated rats; (iv) changes in locus coeruleus (LC) cell activities in healthy and formalin-treated rats and (v) formalin-induced nocifensive responses in awake rats, has been investigated in this study. Additional experiments with intrathecal adrenergic/serotonergic blockers were also performed in order to clarify the involvement of "alternative" descending pathway circuitries, such as those in the LC, in the analgesic mechanisms of intra-VL-PAG AA-5-HT administration.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were housed under controlled conditions (12 h light/12 h dark cycle; temperature 20–22 °C; humidity 55–60%) with chow and tap water available ad libitum. All surgery and experimental procedures were done during the light cycle and were approved by the Animal Ethics Committee of the Second University of Naples. Animal care was in compliance with the European regulations on the protection of laboratory animals (O.J. of E.C. L358/1 18/12/86). In agreement with the Ethical Guidelines of the IASP (Zimmermann, 1983), all efforts were made to reduce both animal numbers and suffering during the experiments.

2.2. Surgical preparation

For electrophysiology experiments combined to tail flick tests, rats were anaesthetised with pentobarbital (50 mg/kg, i.p.) and a 31-gauge, 12 mm long stainless steel guide cannula was stereotaxically lowered until its tip was 1.5 mm above the left VL-PAG by applying coordinates from the Atlas of Paxinos and Watson (1986) (*A*: −7.8 mm from bregma, *L*: 0.5 mm, *V*: 4.5 mm below the dura) on the day of the experiment (Fig. 1). VL-PAG was considered in this study because previous data from this laboratory have focused on this area (Maione et al., 2006; Starowicz et al., 2007b) and because neurons from this area have been shown to project to the RVM (Sandkuhler and Gebhart, 1984; Moreau and Fields, 1986). The cannulae were anchored with dental cement to a stainless steel screw in the skull. We used a David Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA) with the animal positioned on a homeothermic temperature control blanket (Harvard Apparatus Limited, Edenbridge, Kent).

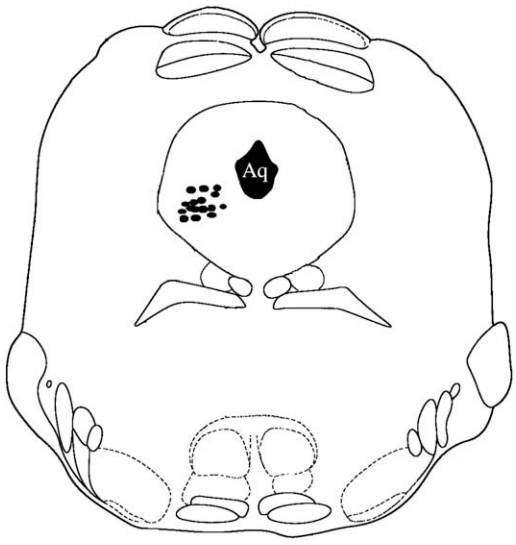

Fig. 1. Schematic illustration of the location of the microinjection sites. Vehicle or drug microinjections were performed into the left ventrolateral PAG matter (filled circles). Many sites are not shown because of the overlaping the of symbols.

2.3. RVM or LC extracellular recordings

After implantation of the guide cannula into the VL PAG, a tungsten microelectrode was stereotaxically lowered, through a small craniotomy, into the RVM (2.8–3.3 mm caudal to lambda, 0.4–0.9 mm lateral to it and 8.9–10.7 mm depth from the surface of the brain) or into the LC (3.7 mm caudal to the lambda, 1.1–1.4 mm lateral and 5.7–6.8 mm depth from the surface of the brain) (Paxinos and Watson, 1986). The jugular vein was cannulated to allow intravenous anaesthetic administration (propofol, 8–10 mg/kg/h, i.v.). The RVM ON cells and OFF cells were identified by the characteristic OFF cell pause, and ON cell burst of activity just before tail flick responses (Fields et al., 1983a,b; Meng and Johansen, 2004). Basal values were obtained by averaging the activities recorded 30–50 s before the application of 3–4 thermal stimulation. Anaesthesia was adjusted so that tail flicks were elicited with a constant latency of 4–5 s. From 35 °C, the temperature increased linearly to 53 °C. The thermal stimulus was elicited by a radiant heat source of a tail flick unit (Ugo Basile, Varese, Italy) focused on the rat tail approximately 4–5 cm from the tip of the tail. Tail flicks were elicited every 3–4 min for at least 15 min prior to microinjecting drugs, or the respective vehicle (20% DMSO in ACSF), with or without formalin injection (50 μl, 5%), into the right hind paw, into the VL-PAG.

The LC cells were identified by criteria described by Cedarbaum and Aghajanian (1976). We identified as adrenergic neurons those single units showing spontaneous firing activity of 0.5–5 Hz, a long-lasting positive–negative spike and a biphasic excitation–inhibition response to contralateral hind paw application of different nociceptive stimuli (paw pinch or pressure). Moreover, an additional clue to the correct positioning of the electrode in the LC was the electrical silence immediately dorsal to LC, due to the IVth cerebral ventricle (Ugedo et al., 1998).

The recorded signals were amplified and displayed on analog and digital storage oscilloscope to ensure that the unit under study was unambiguously discriminated throughout the experiment. Signals were also fed into a window discriminator, whose output was processed by an interface (CED 1401) (Cambridge Electronic Design Ltd., UK) connected to a Pentium III PC. Spike2 software (CED, version 4) was used to create peristimulus rate histograms on-line and to store and analyze digital records of single-unit activity off-line. Configuration, shape, and height of the recorded action potentials were monitored and recorded continuously, using a window discriminator and Spike2 software for on-line and off-line analyses. Once an RVM or LC cell was identified from its background activity, we optimised spike size before all treatments. This study only included neurons whose spike configuration remained constant and could clearly be discriminated from activity in the background throughout the experiment, indicating that the activity from one neuron only and from the same one neuron was measured. The recording site was marked with a 20 μA DC current for 20 s. After fixation by immersion in a 10% formalin, the recording sites were identified. In each rat, only one neuron was recorded. The neuron responses, before and after intra-VL-PAG drug microinjections, with or without the peripheral formalin injection, were measured and expressed as spikes/s (Hz).