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Synergistic antinociceptive effects of anandamide, an endocannabinoid, and nonsteroidal anti-inflammatory drugs in peripheral tissue: A role for endogenous fatty-acid ethanolamides?

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit fatty-acid amide hydrolase (FAAH), the enzyme responsible for the metabolism of anandamide, an endocannabinoid. It has been suggested that the mechanisms of action of NSAIDs could be due to inhibition of cyclooxygenase (COX) and also to an increase in endocannabinoid concentrations. In a previous study we have demonstrated that the local analgesic interaction between anandamide and ibuprofen (a non-specific COX inhibitor) was synergistic for the acute and inflammatory phases of the formalin test. To test this hypothesis further, we repeated similar experiments with rofecoxib (a selective COX-2 inhibitor) and also measured the local concentrations of anandamide, and of two fatty-acid amides, oleoylethanolamide and palmitoylethanolamide. We established the ED_{50} for anandamide (34.52 pmol ± 17.26) and rofecoxib (381.72 pmol ± 190.86) and showed that the analgesic effect of the combination was synergistic. We also found that paw tissue levels of anandamide, oleoylethanolamide and palmitoylethanolamide were significantly higher when anandamide was combined with NSAIDs and that this effect was greater with rofecoxib. In conclusion, local injection of anandamide or rofecoxib was antinociceptive in a test of acute and inflammatory pain and the combination of anandamide with rofecoxib was synergistic. Finally, locally injected anandamide with either NSAID (ibuprofen or rofecoxib) generates higher amount of fatty-acid ethanolamides. The exact comprehension of the mechanisms involved needs further investigation.

Keywords: Anandamide; Endocannabinoids; Fatty-acid ethanolamides; Oleoylethanolamide; Palmitoylethanolamide; Cannabinoid receptors; Rofecoxib; Ibuprofen; Fatty-acid amide hydrolase; Nonsteroidal anti-inflammatory drugs; Capsazepine; Formalin test; Inflammatory pain

1. Introduction

The principal active ingredient of Cannabis, Δ^9 — tetrahydrocannabinol, produces its effect by binding to G protein-coupled receptors, identified as the cannabinoid CB_1 receptor (Matsuda et al., 1990) localised primarily in the central nervous system (including the spinal cord and dorsal root ganglia) and in the periphery (Rice et al., 2002 for review), and the cannabinoid CB_2 receptor (Munro et al., 1993) mainly expressed in immune tissues (Galiègue et al., 1995). Endogenous cannabinoids (or endocannabinoids) such as anandamide (arachidonylethanolamide), a cannabinoid CB_1 receptor agonist, were also identified (Devane et al., 1992). The therapeutic utility of using compounds that would modulate the endocannabinoid system is beginning to attract more interest (Piomelli et al., 2000). Anandamide can act in the periphery to attenuate pain behaviour. Indeed, when injected into the ipsilateral hind paw of the rat, anandamide reduced hyperalgesia induced by carrageenan (Richardson et al., 1998) or pain induced by formalin injection (Calignano et al., 1998). Those

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studies showed that pain relief was produced by the activation of peripheral cannabinoid receptors. Other authors have demonstrated the peripheral mechanisms involved against persistent somatic inflammatory pain (Malan et al., 2001; Nackley et al., 2003).

Anandamide is hydrolysed into arachidonic acid and ethanolamine by a membrane-bound enzyme named fatty-acid amide hydrolase (FAAH) (Cravatt et al., 1996). Anandamide can also be oxygenated by cyclooxygenase-2 (COX-2) to form prostamides, a new class of prostaglandin analogs (Weber et al., 2004; Yang et al., 2005). Other endogenous lipid compounds called fatty-acid ethanolamides also exist such as palmitoylethanolamide and oleoylethanolamide. The former has both anti-inflammatory and antinociceptive properties (Calignano, 1998); it does not bind to cannabinoid receptors (Showalter et al., 1996) although its effects are antagonised by cannabinoid CB_2 antagonists (Calignano et al., 1998). The latter is a naturally occurring lipid that regulates feeding and body weight (Fu et al., 2003; Lo Verme et al., 2005b).

The standard hypothesis for the mechanism of action of nonsteroidal anti-inflammatory drugs (NSAIDs) is by inhibiting COX enzymes responsible for the production of prostaglandins. However, other mechanisms have been proposed, such as interactions with endocannabinoids (Fowler et al., 1997). Indeed, FAAH activity, responsible for the degradation of anandamide, is inhibited by NSAIDs such as ibuprofen, ketorolac and flurbiprofen (Fowler et al., 1997, 1999). However, very little information is available in the literature on the antinociceptive effects of the combination of a cannabinoid with an NSAID. It has been shown that intrathecal indomethacin (Gühring et al., 2002) or flurbiprofen (Ates et al., 2003) is antinociceptive in the formalin test in the rat. This effect was antagonised by a cannabinoid CB_1 antagonist and was absent in cannabinoid CB_1 knockout mice. Furthermore, orally administered palmitoylethanolamide, an endogenous peroxisome proliferator-activated receptor (PPAR)-α agonist with anti-inflammatory properties (Lo Verme et al., 2005a) and indomethacin have demonstrated anti-oedema and anti-inflammatory properties in a rat model of acute inflammation (Conti et al., 2002). Finally in a previous study, we have shown that locally injected anandamide, ibuprofen (a non-specific COX inhibitor) or combination thereof, decreased pain behaviour in the formalin test (Guindon et al., 2006). The combination of anandamide with ibuprofen produced synergistic antinociceptive effects involving both cannabinoid CB_1 and CB_2 receptors. However, the mechanism of this interaction was not explained and the interaction of anandamide with a specific COX-2 inhibitor has not been studied yet. Furthermore, there is evidence of cellular colocalisation of FAAH and transient receptor potential vanilloid 1 (TRPV1) receptors in primary sensory neurons (Price et al., 2005). Therefore, the role of TRPV1 receptors in the context of anandamide/rofecoxib (a selective COX-2 inhibitor) interactions was also evaluated by using a specific antagonist of TRPV1 receptors.

The present study was thus designed to further investigate the antinociceptive interactions between endocannabinoids and NSAIDs. Anandamide and rofecoxib were studied in a test of acute and inflammatory pain. Finally, paw tissue concentrations of fatty-acid ethanolamides were also determined.

2. Methods

2.1. Animals

This research protocol was approved by the Animal Ethics Committee of the Université de Montréal and all procedures conformed to the guidelines of the Canadian Council for Animal Care. Male Wistar rats (Charles River, St-Constant, Québec, Canada), 180–220 g at the time of testing were housed in standard plastic cages with sawdust bedding in a climate-controlled room on a 12-h light/dark cycle. Animals were allowed free access to food pellets and water.

2.2. Drug administration

Anandamide, an endogenous cannabinoid, is a receptor agonist with a four-fold selectivity for the cannabinoid CB_1 receptor (Ki = 89 nM) over the cannabinoid CB_2 receptor (Ki = 371 nM) and was purchased already in a liquid form in water-dispersible emulsion and further dissolved in 0.9% NaCl (Pertwee, 1999). Capsazepine, a TRPV1 receptor antagonist, was dissolved in 0.9% NaCl solution containing 50% dimethyl sulfoxide (DMSO) (Kwak et al., 1998). Anandamide and capsazepine were purchased from Tocris (Ellisville, MO, USA). Ibuprofen (Sigma, St-Louis, USA), a non-specific COX inhibitor, and rofecoxib, a specific COX-2 inhibitor, were dissolved in 0.9% NaCl solution. For rofecoxib, the compressed tablet from commercial preparations (VIOXX®, 25 mg) was weighed and crushed (using a mortar) into a fine suspension with physiological saline (Francischi et al., 2002). Finally, NS-398 (another specific COX-2 inhibitor) was dissolved in 0.9% NaCl solution containing 4% DMSO and was purchased from Cayman Chemicals (MI, USA).

2.3. Formalin test

The formalin test is a well-established model of inflammatory pain (Tjölsen et al., 1992). Rats were acclimatised to the testing environment (clear Plexiglass box 29 × 29 × 25 cm) during 15 min or until cessation of explorative behaviour. Anandamide (2.88, 8.63, 28.77, 86.31, 288, 2 877 or 14 385 pmol in 50 μl), rofecoxib (31.81, 95.43, 318, 954, 6 362 or 31 810 pmol in 50 μl), NS-398 (159 nmol in 50 μl; Choi et al., 2003), ibuprofen (438 pmol in 50 μl) and capsazepine (2.65 μmol in 50 μl; Kwak et al., 1998) were injected subcutaneously (s.c.) on the dorsal surface of the right hind paw 15 min before the injection (28G needle) of 2.5% formalin (50 μl) next to the previous injection. Following each injection, the rat was immediately put back in the observation chamber. Nociceptive behaviour was observed with the help of a mirror angled at 45° below the observation chamber. Observation of the animal's behaviour was made in consecutive 5-min periods for 60 min following formalin administration. In each 5-min period, the total time the animal spent in three different behavioural categories was recorded: (1) the injected paw has little or no weight placed on it; (2) the injected paw is raised; (3) the injected paw is licked, shaken or bitten. Nociceptive behaviour was quantified using the