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Short communication

Synergistic antinociception by the cannabinoid receptor agonist anandamide and the PPAR-α receptor agonist GW7647

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Abstract

The analgesic properties of cannabinoid receptor agonists are well characterized. However, numerous side effects limit the therapeutic potential of these agents. Here we report a synergistic antinociceptive interaction between the endogenous cannabinoid receptor agonist anandamide and the synthetic peroxisome proliferator-activated receptor-α (PPAR-α) agonist 2-(4-(2-(1-Cyclohexanebutyl)-3-cyclohexylureido)ethyl)phenylthio)-2-methylpropionic acid (GW7647) in a model of acute chemical-induced pain. Moreover, we show that anandamide synergistically interacts with the large-conductance potassium channel ($K_{Ca}1.1$, BK) activator isopimaric acid. These findings reveal a synergistic interaction between the endocannabinoid and PPAR-α systems that might be exploited clinically and identify a new pharmacological effect of the BK channel activator isopimaric acid.

Keywords: PPAR-α; Cannabinoid; Pain; Palmitoylethanolamide; Isopimaric acid; Formalin

1. Introduction

Cannabinoid receptor agonists, which include the endogenous lipid mediators anandamide and 2-arachidonoyl glycerol, as well as exogenous substances such as Δ^9-tetrahydrocannabinol (THC), are well characterized for their broad-spectrum analgesic effects (Mackie, 2006). These drugs produce, however, a variety of psychotropic side effects that severely limit their therapeutic utility (Piomelli, 2005). Alternative cannabinoid-based therapeutic strategies are being explored, therefore, which include activating peripheral cannabinoid receptors with brain impermeant receptor agonists, prolonging the half-life time of endocannabinoids by inhibiting their degradation (Bari et al., 2006; Piomelli et al., 2006) or using low-dose combinations of cannabinoid receptor agonists in conjunction with other analgesic agents to produce synergistic reductions in pain (Calignano et al., 2001; Cichewicz, 2004; Guindon et al., 2006). An example of the combination approach is when anandamide is administered together with the naturally occurring analgesic and anti-inflammatory factor palmitoylethanolamide (PEA) (Calignano et al., 1998; Jaggar et al., 1998; Mazzari et al., 1996) — the two agents exert a synergistic antinociceptive effect in mice without eliciting overt psychotropic responses (Calignano et al., 2001).

PEA was once used in the clinic to treat respiratory infections (Masek et al., 1974) and is currently utilized as a topical treatment for pruritus (Szepietowski et al., 2005). It exerts its anti-inflammatory and analgesic effects by activating the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPAR-α) (LoVerme et al., 2005, 2006). Indeed, synthetic PPAR-α receptor agonists produce broad spectrum analgesia in a dose-dependent manner (LoVerme et al., 2006). In the present study, we asked whether normally ineffective doses of an endocannabinoid such as anandamide (Calignano et al., 1998), might synergistically interact with low and ineffective doses of a synthetic PPAR-α receptor agonist such as 2-(4-(2-(1-Cyclohexanebutyl)-3-cyclohexylureido)ethyl)phenylthio)-2-methylpropionic acid (GW7647) (Brown et al., 2001). To investigate this possibility, in the present study we used the

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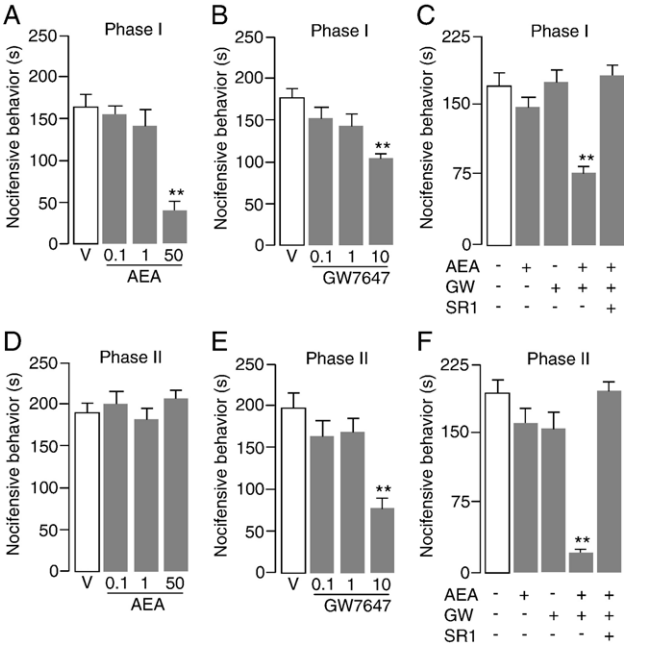

Fig. 1. The PPAR-α receptor agonist GW7647 and the endocannabinoid anandamide synergistically inhibit pain behavior. A,B and D,E: dose dependent inhibition of (A,D) phase I (0–15 min) or (B,E) phase II (15–45 min) formalin-evoked pain behavior in Swiss mice by intraplantarly administered (A,D) anandamide (0.1–10 μg/paw), (B,E) GW7647 (0.1–10 μg/paw) or vehicle (V). C and F: effects of anandamide (AEA, 0.1 μg/paw) alone or together with GW7647 (GW, 0.1 μg/paw) in the presence or absence of SR141716A (SR1, 1 mg/kg, i.v.) on (C) phase I or (F) phase II formalin-evoked pain behavior in Swiss mice (n=6–10) ** $P<0.01$ vs. V.

formalin model of acute pain — a model in which anandamide, PEA and a number of synthetic PPAR-α receptor agonists are active (Calignano et al., 1998; LoVerme et al., 2006).

2. Materials and methods

Anandamide was synthesized as described (Astarita et al., 2006). (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4,-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxyamide hydrochloride) (Rimonabant, SR141716) was provided by RBI (Natick, Massachusetts) as part of the Chemical Synthesis Program of the National Institutes of Health (NIH). GW7647 was obtained from Sigma-Aldrich (St. Louis, Missouri) and isopimaric acid from Tocris (Avonmouth, United Kingdom). Fresh drug solutions were prepared immediately before use. All procedures met the NIH guidelines for the care and use of laboratory animals, and those of the Italian Ministry of Health (D.L. 116/92). Male Swiss mice (20–25 g) were obtained from Charles River (Wilmington, Massachusetts). All animals were maintained on a 12-h/12-h light/dark cycle with free access to water and chow (RMH 2500, Prolab, Framingham, Massachusetts).

Tonic nociception was assessed by injecting formalin (5% formaldehyde in sterile saline, 10 μl) into the plantar surface of mice using a 27-gauge needle fitted to a microsyringe. Vehicle (0.9% sterile saline/5% PEG-400/ 5% Tween-80) or drugs were dissolved in the formalin solution. For synergism experiments, drugs were dissolved together in the formalin solution. Following injections, animals were immediately transferred to a transparent observation chamber where formalin-evoked pain behavior (time spent licking and biting the injected paw) was continuously monitored for 45 min (phase-I: 0–15 min; phase-II: 15–45 min). Rimonabant was dissolved in a vehicle of 0.9% sterile saline/5% PEG-400/5% Tween-80 and injected intravenously (i.v.) 30 min prior to formalin treatments at a dose of 1 mg/kg.

Experimental results are expressed as the mean±s.e.m. of n experiments. Data analyses were conducted using the GraphPad Prism software (GraphPad Software, San Diego, California). The significance of differences between groups was determined by one-way ANOVAs followed by the Dunnett's test for multiple comparisons. Isobolographic analyses were not conducted, as the doses (0.1 μg/paw) used for our synergism experiments did not cause any observable pain behavior.

3. Results

As previously shown, the endocannabinoid anandamide and the PPAR-α receptor agonist GW7647 dose-dependently inhibited phase I pain behavior (Fig. 1A,B) in the mouse formalin model (Fig. 1A,B). Moreover, GW7647, but not anandamide, effectively reduced phase II pain behavior (Fig. 1D,E) (Calignano et al., 1998; LoVerme et al., 2006). The lack of efficacy displayed by anandamide during phase II-pain is in agreement with published reports (Calignano et al., 1998, 2000) and is likely due to the compounds short half-life *in vivo*. To determine whether cannabinoid and PPAR-α receptor agonists cooperatively reduce formalin-induced pain behavior, we

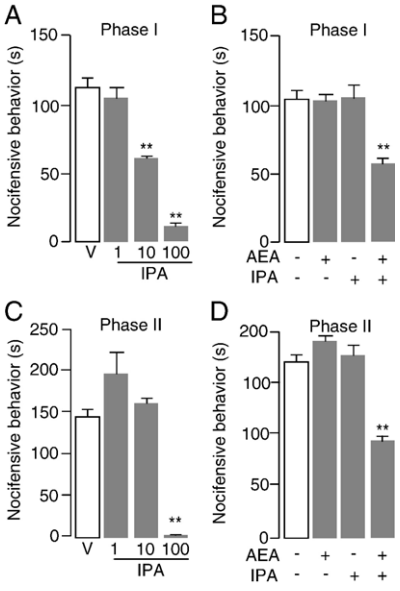

Fig. 2. The BK channel opener isopimaric acid and the endocannabinoid anandamide synergistically inhibit pain behavior. A and C: Dose dependent inhibition of (A) phase I (0–15 min.) or (C) phase II (15–45 min) formalin-evoked pain behavior in Swiss mice by intraplantarly administered isopimaric acid (IPA, 1–100 μg/paw) or vehicle (V). B and D: Effects of anandamide (AEA, 0.1 μg/paw) alone or together with isopimaric acid (IPA, 0.1 μg/paw) on (B) phase I or (D) phase II formalin-evoked pain behavior in Swiss mice (n=6) ** $P<0.01$ vs. V.

administered a single ineffective dose of anandamide along with an equal and ineffective dose of GW7647 into the mouse paws. At a dose of 0.1 μg/paw, anandamide and GW7647 failed to reduce either phase I (Fig. 1A) or phase II (Fig. 1D) of formalin-evoked nociception. However, when the same doses of the two drugs were injected together, the combination reduced both phase I (Fig. 1C) and phase II (Fig. 1F) pain. Confirming the contribution of cannabinoid receptors to this response, the synergistic effects of anandamide and GW7647 were abrogated by the selective cannabinoid type-1 (CB_1) receptor antagonist rimonabant (1 mg/kg, i.v.) (Fig. 1C and F). These findings suggest that the CB_1 and PPAR-α signaling pathways converge to reduce peripheral pain synergistically.

The antinociceptive effects of PPAR-α receptor agonists are blocked by the large conductance potassium channel ($K_{Ca}1.1$, BK, slo) inhibitors charybdotoxin and iberiotoxin (LoVerme et al., 2006), suggesting that activation of PPAR-α leads to a downstream opening of such channels, which are known to regulate neuronal excitability (Scholz et al., 1998). We hypothesized, therefore, that (i) pharmacological agents that open BK channels, such as isopimaric acid (Imaizumi et al., 2002), might produce antinociception, and (ii) cannabinoids may also interact synergistically with such agents to reduce pain. As predicted, isopimaric acid (1–100 μg/paw) dose-dependently reduced phase I (Fig. 2A) and phase II (Fig. 2C) formalin-evoked pain behavior. In line with the hypothesis that BK channel opening events lie downstream of PPAR-α activation, a combination of ineffective doses of anandamide (0.1 μg/paw) and isopimaric acid (0.1 μg/paw) (Fig. 2B,D) synergistically reduced phase I (Fig. 2B) and phase II (Fig. 2D) formalin pain.

4. Discussion

The present study suggests that the endocannabinoid anandamide, and presumably other cannabinoid receptor agonists, productively interacts with PPAR-α receptor agonists to reduce acute pain behaviors in a synergistic manner. Moreover, the results indicate that direct pharmacological activation of BK channels is sufficient to produce antinociception in an acute pain model and that BK channels, the putative downstream targets of PPAR-α (LoVerme et al., 2006), can also synergistically interact with cannabinoids to reduce nocifensive behaviors.

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