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Antinociception by systemically-administered acetaminophen (paracetamol) involves spinal serotonin 5-HT$_7$ and adenosine A$_1$ receptors, as well as peripheral adenosine A$_1$ receptors

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HIGHLIGHTS

- Spinal 5-HT and adenosine receptors are involved in antinociception by acetaminophen.
- Peripheral adenosine A$_1$ receptors are involved in peripheral actions of acetaminophen.
- Peripheral adenosine A$_1$ receptors also contribute to systemic actions of acetaminophen.

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ABSTRACT

Acetaminophen (paracetamol) is a widely used analgesic, but its sites and mechanisms of action remain incompletely understood. Recent studies have separately implicated spinal adenosine A$_1$ receptors (A$_1$Rs) and serotonin 5-HT$_7$ receptors (5-HT$_7$Rs) in the antinociceptive effects of systemically administered acetaminophen. In the present study, we determined whether these two actions are linked by delivering a selective 5-HT$_7$R antagonist to the spinal cord of mice and examining nociception using the formalin 2% model. In normal and A$_1$R wild type mice, antinociception by systemic (i.p.) acetaminophen 300 mg/kg was reduced by intrathecal (i.t.) delivery of the selective 5-HT$_7$R antagonist SB269970 3 μg. In mice lacking A$_1$Rs, i.t. SB269970 did not reverse antinociception by systemic acetaminophen, indicating a link between spinal 5-HT$_7$R and A$_1$R mechanisms. We also explored potential roles of peripheral A$_1$Rs in antinociception by acetaminophen administered both locally and systemically. In normal mice, intraplantar (i.pl.) acetaminophen 200 μg produced antinociception in the formalin test, and this was blocked by co-administration of the selective A$_1$R antagonist DPCPX 4.5 μg. Acetaminophen administered into the contralateral hindpaw had no effect, indicating a local peripheral action. When acetaminophen was administered systemically, its antinociceptive effect was reversed by i.pl. DPCPX in normal mice; this was also observed in A$_1$R wild type mice, but not in those lacking A$_1$Rs. In summary, we demonstrate a link between spinal 5-HT$_7$Rs and A$_1$Rs in the spinal cord relevant to antinociception by systemic acetaminophen. Furthermore, we implicate peripheral A$_1$Rs in the antinociceptive effects of locally- and systemically-administered acetaminophen.

1. Introduction

Acetaminophen (paracetamol) is an over-the-counter drug that has been widely used alone, or in combination formulations, for the treatment of mild-to-moderate pain since the 1960s. Acetaminophen acts at supraspinal and spinal sites, and simultaneous application to the two sites produces synergy ("self-synergy") [17]. While its mechanisms of antinociceptive action are incompletely understood, cyclooxygenase inhibition, as well as serotonin (5-HT), endocannabinoid and vanilloid systems are all implicated in its action [15,16,22,25]. Recently, we demonstrated that spinal adenosine A$_1$ receptors (A$_1$Rs) are involved in the antinociceptive actions of systemically administered acetaminophen [21]. Shortly thereafter, spinal serotonin 5-HT$_7$ receptors (5-HT$_7$Rs) also were implicated in antinociception by acetaminophen [8]. Spinal 5-HT$_7$Rs are present in the superficial layers of the dorsal spinal cord [7], and are positively coupled to adenylyl cyclase and increased cyclic AMP formation [18]. 5-HT releases cyclic AMP and adenosine from the spinal cord [24], and spinal 5-HT$_7$ and A$_1$Rs are linked in contributing to antinociception by amitriptyline [13]. In the present study, we determined whether spinal 5-HT$_7$Rs and A$_1$Rs also are linked in contributing to systemic

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antinociception by acetaminophen by administering a selective 5-HT_7R antagonist (SB269970, (2R)-1-[(3-hydroxyphenyl) sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride) intrathecally in normal mice, wild type mice and mice lacking A_1Rs, and assessing nociception using the formalin test.

A further consideration in our study concerned which sites of action, central or peripheral, were involved in the antinociceptive effect of acetaminophen. Following systemic administration, acetaminophen is widely distributed throughout the body [2]. Since acetaminophen crosses the blood-brain-barrier and produces self-synergy in supraspinal and spinal compartments, it is generally regarded to act centrally to reduce nociception [22,25]. However, some preclinical reports indicate that acetaminophen also can produce peripheral antinociception [5,9]. Given that peripheral A_1Rs lead to antinociception [1,6,12], we determined whether such receptors also contribute to the actions of acetaminophen in the formalin model by delivering a selective A_1R antagonist (DPCPX, 8-cyclopentyl-1,3-dipropylxanthine) locally by intraplantar (i.pl.) injection along with acetaminophen. Furthermore, we determined whether peripheral A_1Rs contributed to systemic antinociception by acetaminophen in normal mice, wild-type mice and those lacking A_1Rs by delivering DPCPX locally by i.pl. injection.

2. Methods

2.1. Animals

All experiments were approved by the Dalhousie University Committee on Laboratory Animals (Halifax, Nova Scotia, Canada) and conducted in compliance with the ethical guidelines of the Canadian Council on Animal Care. Adult male C57BL/6 (normal) mice (Charles River Laboratories, Saint-Constant, Québec, Canada) or both sexes of A_1R +/+ (wild type) and −/− (knock-out) colony mice, all weighing 20–30 g, were used for all experiments. Mice were kept on a 12-h light/12-h dark cycle and housed at a temperature of 21 ± 1 °C in groups of 2–5, with free access to food and water. A_1R colony mice were raised on a C57BL/6 genetic background, and were initially derived from heterozygotes supplied by Dr. Bertil Fredholm (Karolinska Institutet, Stockholm, Sweden). Genotypes of the A_1R colony mice were confirmed by polymerase chain reaction performed on DNA isolated from tail-clips (Karolinska Institutet, Stockholm, Sweden).

2.2. Nociceptive test system and analysis

The formalin test, a model of ongoing and inflammatory pain, was used to assess pain behaviors. Briefly, each mouse received a subcutaneous i.pl. injection of formalin 2% (20 μl, in saline) into the hindpaw [13,21]. Instances of flinching (elevation of the hindpaw and/or rapid shaking) were counted over 60 min, and 2 mice were monitored in alternating 2 min bins in separate adjacent plexiglass observation chambers. Each mouse was euthanized at the conclusion of the test. The biphasic pattern of formalin-evoked pain behaviors was analyzed as the cumulative number of flinches during phase 1 (0–8 min) and phase 2 (12–60 min). Phase 1 flinching behaviors were generally unaffected by acetaminophen, and values depict mean cumulative number of phase 2 flinches ± standard error of the mean (SEM). Statistical tests were performed using the Student's *t*-test or an analysis of variance (ANOVA), followed by the Student–Neuman–Keuls (SNK) post hoc test. Results were taken to be statistically significant at $P < 0.05$.

2.3. Drug treatments

Acetaminophen was delivered 20 min before i.pl. formalin 2% either by intraperitoneal (i.p.) injection (300 mg/kg; 5 ml/kg) or by subcutaneous i.pl. injection (200 μg/10 μl) to the ipsilateral paw. On one occasion, i.pl. acetaminophen was delivered to the contralateral hindpaw. The i.p. dose was selected on the basis of a dose–response analysis [21], and the i.pl. dose on the basis of preliminary experiments (with 50, 200 and 500 μg doses). Selective receptor antagonists were administered 5 min before formalin by acute lumbar puncture (intrathecal or i.t. injection), or 15–20 min i.pl. before formalin injection. The selective serotonin 5-HT_7R antagonist SB 269970 ((2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride) [14] was given under isoflurane anesthesia by i.t. injection (5 μl) via acute lumbar puncture. The A_1R antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) [4] was given as an i.pl. injection (10 μl). These experiments involved complex injection regimens, and in order to minimize the number of control groups used, all drugs were given in 20% dimethylsulfoxide (DMSO)/saline; all experiments use appropriately matched vehicle controls.

3. Results

3.1. Systemic acetaminophen, role of spinal 5-HT_7 and adenosine A_1 receptors

In normal mice, i.t. administration of the 5-HT_7R antagonist SB269970 3 μg did not produce an intrinsic effect on phase 2 flinching responses to formalin 2% (Fig. 1A). Systemic (i.p.) administration of acetaminophen 300 mg/kg reduced phase 2 formalin responses by over 50%; this effect was significantly reversed by i.t. SB269970 3 μg (Fig. 1A), indicating that spinal 5-HT_7Rs are involved in systemic antinociception by acetaminophen. SB269970 reversal of antinociception by acetaminophen 300 mg/kg also was observed in A_1R +/+ mice, but not in A_1R −/− mice (Fig. 1B). We have previously shown that A_1R colony mice exhibit stable responses to i.pl. formalin 2% alone, with similar control responses in +/+ and −/− genotypes, as well as similar and significant antinociceptive responses to i.p. acetaminophen [21].

3.2. Peripheral acetaminophen, role of adenosine A_1 receptors

We next determined whether acetaminophen can act peripherally in the formalin 2% model. In normal mice, administration of acetaminophen 200 μg into the hindpaw ipsilateral to formalin produced significant phase 2 antinociception, and this was reversed by i.pl. administration of the A_1R antagonist DPCPX 4.5 μg (Fig. 2, middle columns). This dose of DPCPX had no intrinsic effect on flinching responses (Fig. 2, left columns). The action of acetaminophen occurred locally, as i.pl. administration of acetaminophen 200 μg into the contralateral hindpaw failed to produce antinociception (Fig. 2, right column). This i.pl. dose corresponds to a systemic dose of 8 mg/kg in a 25 g mouse.

3.3. Systemic acetaminophen, peripheral adenosine A_1 receptors

The final experiment determined whether drug actions in the peripheral compartment contribute to systemic antinociception by acetaminophen. Intraplantar DPCPX 4.5 μg reversed antinociception by acetaminophen 300 mg/kg in normal mice (Fig. 3A and B). Furthermore, it reversed the effect of acetaminophen 200 mg/kg in A_1R +/+ mice, but not A_1R −/− mice (Fig. 3C). A lower dose of acetaminophen was used in this experiment because of the higher sensitivity of colony mice to acetaminophen [21]. These experiments confirm that peripheral A_1Rs are involved in the blockade of systemic acetaminophen actions.