Spinal adenosine A_1 and serotonin 5-HT_7 receptors mediate analgesia by systemically administered amitriptyline n the mouse formalin test

Jean Liu, Allison Reid, and Jana Sawynok
Department of Pharmacology, Dalhousie University

Dalhousie University — Inspiring Minds

Introduction

Amitriptyline is a tricyclic antidepressant used to treat chronic pain, and recruits adenosine systems in its mechanisms of action[1]. Interactions with adenosine may be of clinical importance, as **caffeine** (non-specific adenosine receptor antagonist) **reduces antinociception by amitriptyline** in acute and chronic pain models. Caffeine has an equal affinity for adenosine A_1, and A_2 receptors, but inhibition of amitriptyline antinociception is associated with adenosine A_1 receptor blockade[2] The sites of action and mechanisms involved in adenosine A_1 receptor-mediated antinociception by amitriptyline remain to be clarified.

Few studies have explored **spinal mechanisms** involved in amitriptyline actions. The 5-HT_7 receptor is positively coupled to cyclic AMP production[3] and linked to descending serotonergic projections[4]. Since 5-HT results in release of cyclic AMP and adenosine in the spinal cord[5],
5-HT_7 receptors may exert downstream analgesic effects through activation of adenosine A_1 receptors.

The **objectives of this study** were to determine:
(1) whether spinal adenosine A_1 receptors are involved in antinociception by systemic amitriptyline,
(2) whether spinal 5-HT_7 receptors contribute to antinociception by systemic amitriptyline, and
(3) whether there is a link between 5-HT_7 and A_1 receptors.

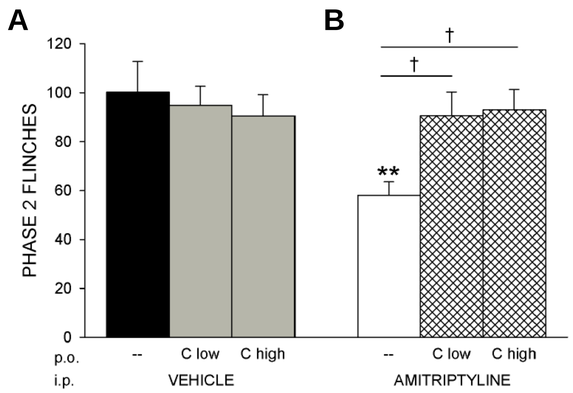
Methods

Experiments were performed in male C57BL/6 mice, as well as both sexes of adenosine A_1 receptor wild-type (+/+) and knock-out (-/-) mice. Pain responses were assessed with the formalin test, a model of persistent, inflammatory pain. Mice received an intraplantar (i.pl.) injection of formalin 2%, and the number of phase 2 (12-60 mins) flinches were counted. Data are mean number of phase 2 flinches ± SEM.

In the **chronic caffeine** experiment, caffeine was given in the drinking water for 8 days prior to testing. In other experiments, **DPCPX** (adenosine A_1 receptor antagonist), **SB269970** (5-HT_7 receptor antagonist), **AS-19** (5-HT_7 receptor agonist), or drug combinations were delivered as an **intrathecal (i.t.) pre-treatment**. Amitriptyline was given 15 minutes before formalin by intraperitoneal (i.p.) injection.

Results

FIGURE 1: Chronic Caffeine and Amitriptyline
A) Chronic caffeine administered at 0.1g/L (C low) and 0.3g/L (C high) in the drinking water did not affect flinching responses to formalin but **B)** prevented antinociception by systemic amitriptyline (3mg/kg).



Note #1: Data shown in Fig. 1A were previously published in ref. 6.
Note #2: In all figures: • p<0.05 • • p<0.01 • • • p<0.001

FIGURE 2: Block of Spinal A_1 Receptors
A) Spinal administration of the selective adenosine A_1 receptor antagonist DPCPX (10nmol) prevented antinociception by systemic amitriptyline (3mg/kg) in normal mice and **B)** antinociception by systemic amitriptyline (12mg/kg) in adenosine A_1 receptor +/+, but not -/- mice.

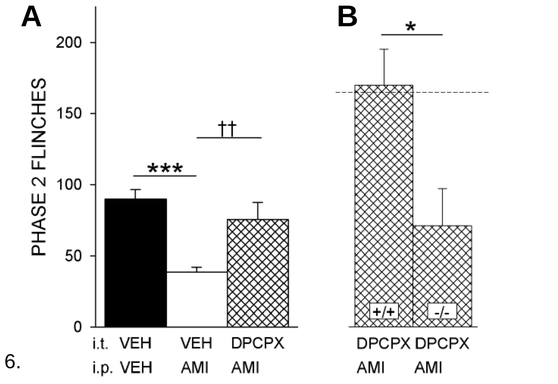


FIGURE 3: Block of 5-HT_7 Receptors
A) Spinal administration of the selective serotonin 5-HT_7 receptor antagonist SB269970 prevented antinociception by systemic amitriptyline (3mg/kg) in normal mice and **B)** antinociception by systemic amitriptyline (12mg/kg) in adenosine A_1 receptor +/+, but not -/- mice.

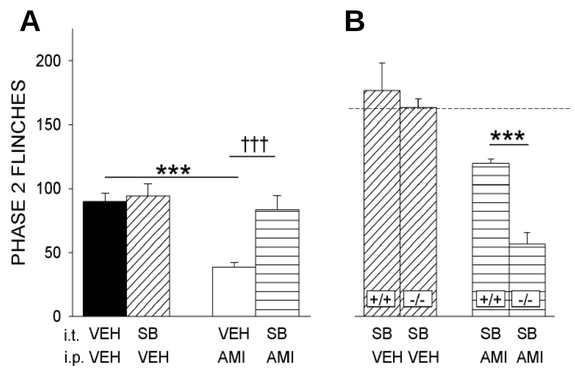
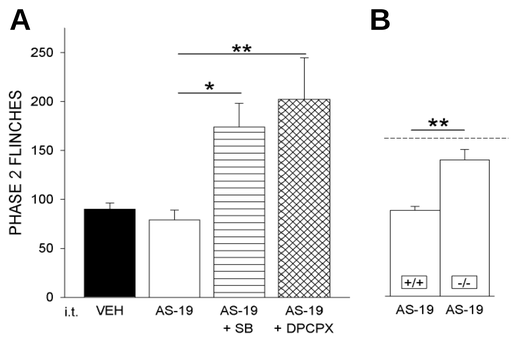


FIGURE 4: 5-HT_7 and A_1 Receptor Interactions
A) Spinal co-administration of the selective serotonin 5-HT_7 receptor agonist AS-19 (20µg) with SB269970 (3µg) or AS-19 (20µg) with DPCPX (10nmol) produced pronociception. **B)** Spinal administration of AS-19 (20µg) was analgesic in adenosine A_1 receptor +/+, but not -/- mice.

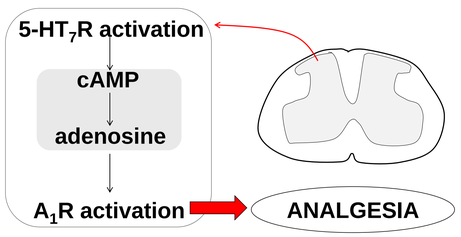


Note #3: Dotted lines represent averaged phase 2 formalin 2% (control) flinches in A_1R +/+ and -/- mice, previously published in ref. 6. Values are 170.9 ± 12.5 (A_1R +/+) and 153.1 ±13.1 (A_1R -/-), with a mean of 162 flinches.

Summary and Conclusions

1. Chronic oral caffeine prevents antinociception by systemic amitriptyline, likely via blockade of adenosine A_1 receptors.
2. Spinal adenosine A_1 receptors are involved in, but not mandatory for, antinociception by systemic amitriptyline.
3. Spinal 5-HT_7 receptor blockade prevents antinociception by systemic amitriptyline.
4. In the presence of a spinal 5-HT_7 receptor agonist, pronociception results from:
   a) block of 5-HT_7 receptors and
   b) block or deletion of adenosine A_1 receptors.

Activation of spinal 5-HT_7 and adenosine A_1 receptors appear to mediate one component of antinociception by amitriptyline, as long as adenosine A_1 receptors are present.



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