Adenosine Receptor Agonists Modulate Visceral Hyperalgesia in the Rat

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Background/Aims: Adenosine is an endogenous modulator of nociception. Its role in visceral nociception, particularly in visceral hyperalgesia, has not been studied. The aim of this study was to determine the effects of adenosine receptor agonists in a model of visceral hyperalgesia.

Methods: The visceromotor response (VMR) in rats to colorectal distension (CRD; 80 mmHg, 20 seconds) was quantified by electromyographic recordings from the abdominal musculature. Three hours after the intracolonic administration of zymosan (25 mg/mL, 1 mL), VMRs to CRD were measured before and after either subcutaneous or intrathecal administration of an adenosine receptor agonist.

Results: Subcutaneous injection of 5'-N-ethylcarboxamidoadenosine (NECA; an A1 and A2 receptor agonist), R(-)-N6-(2-phenylisopropyl)-adenosine (R-PIA; a selective A1 receptor agonist), or CGS-21680 hydrochloride (a selective A2a receptor agonist) dose-dependently (10-100 mg/kg) attenuated the VMR to CRD, although hindlimb weakness occurred at the higher doses tested. Intrathecal administration of NECA or R-PIA dose-dependently (0.1-1.0 µg/kg) decreased the VMR, whereas CGS-21680 hydrochloride was ineffective over the same concentration range. Higher intrathecal doses of the A1/A2 receptor agonist NECA produced motor weakness.

Conclusions: Adenosine receptor agonists are antihyperalgesic, but also produce motor weakness at high doses. However, activation of the spinal A1 receptor significantly attenuates the VMR to CRD without producing motor weakness. (Gut and Liver 2008;2:39-46)

Key Words: Adenosine; Visceral; Hyperalgesia

INTRODUCTION

Adenosine has been known as one of the neurotransmitter and it exerts multiple influences on pain transmission. Several previous studies already proved there is an endogenous adenosine in the peripheral nerve ending and spinal cord.1,2 The endogenous compound of adenosine is present in all cells. It may be released from cells directly or via degradation of ATP and is involved in many regulatory mechanisms both in physiological and pathophysiological conditions.3,4 It affects both sensory and motor neuron. It acts as an endogenous modulator of nociception. Because of its potential as a new analgesic, interests in adenosine are increasing among physicians and pharmacologists. The various pain modulating effects of adenosine and its analogs in animal models has been known for more than 10 years. Adenosine has effects in peripheral and central nervous system, mediated through specific cell surface associated receptors. With the development of selective adenosine agonists and antagonists, its role in pain perception is being elucidated.

Adenosine receptors are found in the peripheral nerve ending and spinal cord. Adenosine has been suggested to have both pre- and postsynaptic effects within the dorsal horn indicating possible multiple sites of action of adenosine in the control of sensory events related to pain. Adenosine shows variable influences on pain transmission at peripheral and spinal sites according to its receptor.5 Adenosine A1 receptor activation in rodents, produces antinociception at peripheral nerve terminals and spinal cord level in somatic pain model. Activation of adenosine A2 receptor (A2a, A2b) exerts multiple responses accord-
ing to the concentration that was administrated and the site of action. At the peripheral nerve endings in rodents, adenosine A2 receptor activation is known to induce pro-nociceptive or pain enhancing properties. However, in the spinal cord, adenosine A2 receptor activation at lower doses reduces pain perception and at higher doses it causes motor weakness.

All those findings were observed in somatic pain models, such as acute nociceptive, inflammatory or neuropathic pain model. Its role in visceral nociception, and particularly visceral hyperalgesia, has not been studied. However, experimental and clinical data are pointing at the adenosine system as an interesting target in relation to hypersensitivity states. The aim of this study was to evaluate the effects of adenosine receptor agonists in a model of visceral hyperalgesia.

MATERIALS AND METHODS

1. Animals

Adult male Sprague-Dawley rats (400-425 g; Harlan, Indianapolis, IN) were used. Rats were housed one per cage in the animal care facility at the university of Iowa (approved by the American Association for Accreditation of Laboratory Animal Care), allowed free access to food and water, and maintained on a 12-hour light-dark cycle (lights on between 6 am and 6 pm). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Iowa.

2. Surgical Preparation

The procedure was described earlier. Rats were deeply anesthetized with pentobarbital sodium (45 mg/kg, Nembutal; Abbott Laboratories, North Chicago, IL) administered intraperitoneally. Electrodes (Teflon-coated stainless steel wire; Cooner Wire Sales, Chatworth, CA) were stitched into the external oblique musculature, just superior to the inguinal ligament, for electromyographic (EMG) recording. The electrode leads were tunneled subcutaneously and exteriorized at the nape of the neck for future access. An intrathecal catheter (polyethylene 10 tubing, 8.5 cm long) was inserted through the dura overlying the atlanto-occipital junction into the spinal subarachnoid space and guided to the lumbar enlargement. The catheter was surgically anchored to the musculature at the back of the neck and externalized with EMG leads. Animals exhibiting motor deficits after surgery due to spinal cord injury during intrathecal catheter insertion were not used. After surgery, rats were housed separately and allowed to recuperate for at least 7 days before testing. At the end of the experiments, rats were killed with an overdose of intraperitoneal pentobarbital.

3. Behavioral Testing

The stimulus used has been described previously. The descending colon and rectum were distended by pressure-controlled air inflation of a 6-7-cm-long, 2-3-cm diameter, flaccid, flexible latex balloon tied around a flexible tube (Tygon). The balloon was lubricated (Surgilube; E. Fougera & Co., Melville, NY), inserted into the colon via the anus, and anchored by taping the balloon catheter to the base of the tail. Noxious phasic CRD (80 mm Hg, 20 seconds, 5 minutes apart) were achieved by opening a solenoid gate to a constant pressure air reservoir. Intracolonic pressure was continuously monitored with the aid of a pressure control device (Bioengineering, University of Iowa, Iowa city, IA). The response quantified was the VMR, a contraction of the abdominal and hindlimb musculature. EMG activity produced by contraction of the external oblique musculature was quantified by recording the number of discharges crossing a preset voltage threshold (baseline). Each distention trial lasted 40 seconds, and EMG activity was quantified in 1-second bins for 10 seconds before distention, 20 seconds during distention, and 10 seconds after distention. The increase in total number of recorded counts during distention (above baseline) was defined as the response.

4. Drugs

Because adenosine is rapidly metabolized by endothelium within 10 seconds when administered systemically, we used adenosine analogues. Drugs used were 5'-N-ethylcarboxamidoadenosine (NECA), an A1 and A2 receptor agonist, R(-)-N6-(2-phenylisopropyl)-adenosine (R-PIA), a selective A1 receptor agonist, or CGS-21680 hydrochloride (CGS 21680), a selective A2a receptor agonist. Stock solutions were freshly prepared by dissolving the drugs in dimethyl sulfoxide solution and followed by adding sterile preservative-free saline.

5. Experimental Protocol

On the day of testing, four stable control responses to CRD (80 mm Hg, 20 seconds, 5 minutes apart) were obtained in conscious, unsedated rats before any treatment. And then, while the animals were briefly anesthetized with halothane, zymosan (25 mg/mL, 1 mL; Sigma Chemical Co., St. Louis, MO), was instilled into the colon through a gavage needle inserted into the colon to a depth of about 7-8 cm. Three hours after intracolonic treatment, four control responses to CRD were obtained to establish whether rats were hyperalgesic (i.e., gave significantly increased responses to 80 mm Hg CRD). Two