Adenosine $A_1$ but Not $A_{2a}$ Receptor Agonist Reduces Hyperalgesia Caused by a Surgical Incision in Rats

A Pertussis Toxin-sensitive G Protein-dependent Process

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Background: Activation of $A_1$ adenosine receptors ($A_1$Rs) causes antinociception after nerve injury and inflammation. However, the role of $A_{2a}$ adenosine receptors ($A_{2a}$Rs) for pain processing is less clear. In the current study, the authors investigated the role of spinal adenosine $A_1$Rs and $A_{2a}$Rs for the maintenance of mechanical hyperalgesia in an animal model for postoperative pain.

Methods: Rats with intrathecal catheters were anesthetized and underwent plantar incision. Spontaneous pain behavior and withdrawal threshold to punctuate stimulation were measured before and after administration of intrathecal R-phenylisopropyl-adenosine (R-PIA; $A_1$R agonist), 2-w-2-carbonyl-ethyl-phenethylaminox-5X-N-ethylcarboxamido-adenosine (CGS21680; $A_{2a}$R agonist), or vehicle. In separate groups of animals, the effects of pertussis toxin, forskolin, glibenclamide, 4-aminopyridine, tetraethylammonium, apamin, charybdotoxin, or margatoxin on R-PIA-induced antinociception were examined.

Results: Intrathecal administration of 5 nmol R-PIA but not 10 nmol CGS21680 decreased nonevoked spontaneous pain behavior. Furthermore, intrathecal administration of R-PIA but not of CGS21680 increased withdrawal thresholds after incision. Pretreatment with pertussis toxin and administration of forskolin, glibenclamide, 4-aminopyridine, and tetraethylammonium inhibited R-PIA-induced antinociception. In addition, intrathecal administration of apamin, charybdotoxin, or margatoxin did not modify mechanical hypoalgesia mediated by R-PIA.

Conclusions: Spinal $A_1$Rs but not $A_{2a}$Rs play an important role in the maintenance of nonevoked and evoked pain behaviors after an incision. Furthermore, $A_1$R-induced spinal antinociception is mediated by interactions with pertussis toxin-sensitive G proteins. In addition, the opening of adenosine triphosphate-sensitive K channels but not of calcium-activated potassium channels and voltage-gated Kv1.3 or Kv1.6 channels contribute to the antinociceptive effect of $A_1$R agonists.

ADENOSINE, an important endogenous modulator of neurotransmission, inhibits synaptic transmission in the central nervous system and is involved in the regulation of several biologic functions, including anxiety, cognition, and memory, mediating its actions by stimulation of adenosine $A_1$, $A_{2a}$, $A_{2b}$, and $A_3$ G protein-coupled receptors. There is now ample evidence that activation of spinal $A_1$ adenosine receptors ($A_1$Rs), present in superficial layers of the dorsal spinal cord and on afferent terminals of nociceptors, causes antinociception after nerve injury or inflammation and decreases C fiber-driven responses in dorsal horn neurons. Mice lacking $A_1$Rs exhibited increased nociceptive responses. However, the exact mechanisms by which $A_1$Rs agonists cause antinociception remain to be defined. Patel et al. demonstrated that activation of $A_1$Rs hyperpolarizes spinal dorsal horn neurons by increasing potassium conductance, resulting in postsynaptic inhibition of excitatory transmission. Furthermore, the inhibitory action of adenosine in the spinal cord may be caused by activation of presynaptic $A_1$Rs present on sensory afferent terminals and dorsal root ganglion neurons, leading to a decrease in cyclic adenosine monophosphate (cAMP) production and an inhibition of excitatory neurotransmitter release.

In contrast, the role of the spinal $A_{2a}$ adenosine receptor ($A_{2a}$R), most likely present on spinal presynaptic terminals of sensory afferents, for pain transmission is uncertain. In a recent study, Yoon et al. demonstrated that intrathecal administration of the $A_{2a}$R agonist 2-w-2-carbonyl-ethyl-phenethylaminox-5X-N-ethylcarboxamido-adenosine (CGS21680) inhibited both phases of formalin-induced pain behaviors. Only a modest antinociceptive effect of spinally administered CGS21680 was observed for inflammation-induced hyperalgesia. In contrast, mice lacking the adenosine $A_{2a}$R were hypoalgesic, and $A_{2a}$R antagonists produced antinociception, indicating that $A_{2a}$Rs mediate proncephalic effects. Other investigators did not observe any role of spinal $A_{2a}$Rs for nociceptive modulation.

A common form of acute pain and hyperalgesia in humans is postoperative pain. To reduce pain in the perioperative period and improve patients outcome, new treatment strategies must be developed. There is now plenty of evidence that postoperative pain is based on distinct pathophysiologic and pharmacologic mechanisms compared with other pain models.

We hypothesize that $A_1$R but not $A_{2a}$R activation modifies pain behaviors caused by an incision. Furthermore, we assumed that $A_1$R-induced antinociception may be mediated by different mechanisms, including the activation of pertussis toxin (PTX)-sensitive G proteins or different potassium channels. Therefore, the aim of the current study was to investigate the role of spinal adenosine $A_1$R and $A_{2a}$R for the maintenance of spontaneous nonevoked pain behavior and evoked mechanical hyper-
algesia after a surgical incision by using a rat model for postoperative pain.

Materials and Methods

General

These experiments were reviewed and approved by the Institution’s Animal Care and Use Committee in Muenster, Germany. The animals were treated in accordance with the Ethical Guidelines for Investigations of Experimental Pain in Conscious Animals as issued by the International Association for the Study of Pain.

Experiments were performed in 135 adult (weight, 280–350 g) male Sprague-Dawley rats (Harlan, Indianapolis, IN) housed in pairs before surgery. All persons performing experiments were blinded to the drug administered. Food and water were available ad libitum. Postoperatively, the animals were housed individually. Eight animals were excluded for wound dehiscence or clotted catheters; at the end of the protocol, all animals were anesthetized and killed with an overdose of potassium chloride administered intracardially.

Surgery

For subarachnoid drug administration, a lumbar intrathecal catheter was placed using a technique modified from one described previously. Briefly, after isoflurane anesthesia, the lumbar skin was cleansed and incised. The intervertebral space between L5 and L6 was punctured with a hypodermic needle, and a 32-gauge polyurethane catheter (length 10 cm, 32-PU, OD 0.010 inches, ID 0.005 inches; Micor, Allison Park, PA) was advanced through the needle in the lumbar area of the spinal cord. The distal end was secured, inserted into PE-10 tubing, and tunneled to the cervical region. The catheter (8 μl dead space) was flushed with saline and sealed. One day after catheter placement, 20 μl lidocaine, 2%, was administered, and only rats with a brief bilateral hind limb paresis were studied. Experiments were begun not less than 3 days after intrathecal catheter placement. At the end of the experiment, the localization of the catheter was verified by injecting 30 μl methylene blue, killing the rat, and dissecting the lumbar spinal cord. In rats with a functional intrathecal catheter, segments of the lumbar spinal cord were dyed with methylene blue.

For paw incisions, all rats were anesthetized with 1.5–2% isoflurane delivered via a nose cone. As described previously, a 1-cm longitudinal incision was made through skin and fascia of the plantar aspect of the right hind paw including the plantaris muscle. The skin was apposed with two mattress sutures of 5-0 nylon. After surgery, the animals were allowed to recover in their cages. Sutures were removed approximately 30 h later at the end of postoperative day 1, and the wounds healed well within 5–6 days. The animals did not receive additional analgesics after surgical treatments such as catheter placement or paw incision.

Pain Behaviors

On the day of the experiment, the rats were placed individually on an elevated plastic mesh floor. After adaptation to testing conditions, baseline nonevoked or evoked pain behaviors before paw incision were measured as described.

Nonevoked (spontaneous) pain behaviors were assessed by a cumulative pain score as described previously. Unrestrained rats were placed on a plastic mesh floor, and the incised foot of each animal was closely observed during a 2.5-min period for 30 min. Depending on the position in which the foot was found during the majority of the 2.5-min scoring period, a 0, 1, or 2 was given. Full weight bearing of the foot (score = 0) was present if the wound was blanched or distorted by the mesh. If the foot was completely off the mesh, a score of 2 was recorded. If the area of the wound touched the mesh without blanching or distorting, a 1 was given. The sum of the 12 scores (0–24) obtained during the 30-min session for the incised foot was obtained.

Withdrawal responses to punctate mechanical stimulation (evoked pain behavior) were determined using calibrated nylon von Frey monoofilaments applied from underneath the cage through openings (12 × 12 mm) in the plastic mesh floor to an area adjacent to the wound. Each von Frey filament was applied once starting with 15 mN and continuing until a withdrawal response occurred or 247 mN (the cutoff value) was reached. The median of the lowest force from the three tests producing a response was considered the withdrawal threshold. For this study, 563 mN was recorded as the withdrawal threshold if there was no withdrawal response to the next lowest filament (247 mN). However, using standard von Frey filaments to assess mechanical pain thresholds has several limitations. Most importantly, shape and size of the filaments are critical factors for mechanical pain perception. Von Frey filaments with flat ends bend on the skin at their sharp corners, and different probe angles may cause variable mechanical thresholds. Greenspan et al. reported that a change in probe angle of 15° produced variable pain thresholds with a stimulus of the same tip size. Furthermore, different von Frey filament strengths vary in their tip diameters up to 100-fold, indicating that there is a trade-off between the force applied and the probe circumference for pain threshold. In addition, Hogan et al. further indicate that decreased withdrawal thresholds caused by a peripheral nerve injury assessed by von Frey filaments are not valid measures for neuropathic pain. However, we speculate
that this is different to acute postoperative pain. In contrast to neuropathic pain, a surgical incision causes robust mechanical hyperalgesia characterized by decreased withdrawal thresholds to von Frey filaments; mechanical hyperalgesia was blocked by morphine or other analgesics. Therefore, we suggest that besides the limitations of standard von Frey filaments, mechanical hyperalgesia is a robust and important feature of postoperative pain.

**Experimental Protocols**

**Experiment 1: Effects of A1R and A2aR Agonists on Nonevoked Pain Behavior.** Twenty-one rats were pretreated for nonevoked pain behaviors as described in the first two paragraphs of the Pain Behaviors section. The incision was made in the plantar aspect of the foot, and after a recovery time of 2 h, spontaneous pain behavior was assessed.

Subsequently, 5 nmol R-phenylisopropyl-adenosine (R-PIA; adenosine A1 receptor agonist), 10 nmol CGS21680, or vehicle was administered intrathecally (n = 6–8/group). Nonevoked pain behavior was measured every 30 min after drug injection for the next 3 h on the day of surgery and postoperative day 1.

**Experiment 2: Evaluating Effects of A1R and A2aR Agonists on Evoked Mechanical Hyperalgesia after Incision.** Twenty-eight rats were pretreated for mechanical withdrawal threshold as described above. The incision was made in the plantar aspect of the foot, and after a recovery time of 2 h, responses to mechanical stimuli were tested.

Subsequently, 0.3, 1, or 5 nmol R-PIA (adenosine A1 receptor agonist) or vehicle was administered intrathecally (n = 6–8/group). Mechanical hyperalgesia was measured at 30 and 60 min and then every hour after drug injection for the next 5 h. In separate groups of animals, administration of an A1R antagonist (CPT, 242 nmol) or an A2aR antagonist (DMPX, 50 nmol) was followed by 5 nmol R-PIA (n = 4).

In another group of rats, 2, 10, or 20 nmol CGS21680 (adenosine A2a receptor agonist) or vehicle was administered intrathecally (n = 6–8/group). Mechanical hyperalgesia was measured at 30 and 60 min and then every hour after drug injection for the next 5 h. In separate groups of animals, administration of an A1R (CPT, 242 nmol) or A2aR antagonist (DMPX, 50 nmol) was followed by 20 nmol CGS21680 (n = 4).

**Experiment 3: Evaluating Mechanisms of A1R-Induced Antinociception.** In a separate experiment, rats were pretreated with 0.5 µg PTX 7 days before incision. A greater effect of the toxin at 6 days compared with 2 days to disrupt the inhibitory secondary messenger pathway has been observed. In the current study, PTX-pretreated rats were tested before and 2 h after incision, with observation of similar mechanical thresholds compared with untreated animals, indicating that 0.5 µg PTX did not affect pain behaviors on its own. In agreement, other investigators demonstrated that low PTX doses (0.5–1 µg) did not produce pain behavior in uninjured animals.

In another group of animals, 5 nmol R-PIA was administered after pretreatment with vehicle or 20, 50 or 100 nmol of the adenosine triphosphate-sensitive potassium (KATP) channel blocker glibenclamide (n = 6–8/group) or after pretreatment with the nonselective potassium channel blockers 4-aminopyridine (4-AP; n = 5; 5 nmol) and tetraethylammonium (n = 5; 100 nmol); mechanical hyperalgesia was assessed for 5 h as described above. Doses for tetraethylammonium and 4-AP were evaluated by a dose finding experiment. Greater doses of tetraethylammonium and 4-AP cause significant adverse effects, including intense pain behaviors or motor impairment.

In a separate group of animals, R-PIA was administered 10–15 min after intrathecal administration of apamin (n = 4; 3 ng), charybdoxin (n = 4; 1 ng), or margatoxin (n = 6; 10 ng), and pain behaviors were assessed. None of these selective potassium channel blockers alone produced pain behaviors.

**Drugs**

All drugs were purchased from Sigma (Hamburg, Germany) or Tocris (Bristol, United Kingdom). To enhance their solubility, R-PIA (0.3–10 nmol, A1R agonist; Ki A1/A2a: 2/860 nm),32 was dissolved in a pH-adjusted HCl solution, and CGS21680 (2–20 nmol, A2aR agonist; Ki A1/A2a: 2,600/15 nm),13 DMPX (A2a antagonist, 50 nmol; Ki A1/A2a: 2,600/15 nm),33 and glibenclamide (KATP channel inhibitor, Kd: 1.7–5 nmol; 3 ng),34 charybdoxin (large- and intermediate-conductance calcium-activated potassium channel inhibitor, Kd: 60 ps–100 nm; 3 ng),34 margatoxin (voltage-gated potassium channel blocker, 100 nmol),34 tetraethylammonium (nonselective potassium channel blocker, 100 nmol),34 4-AP (nonselective potassium channel blocker, 5 nmol), PTX (0.5 µg),34 apamin (small-conductance calcium-activated potassium channel inhibitor, Kd: 60 ps–100 nm; 3 ng),34 forskolin (cAMP activator, 10 µg),34 were dissolved in 10% ethanol, and glibenclamide (KATP channel inhibitor, 10–100 nmol)35 was dissolved in 50% dimethyl sulfoxide. Peng et al. demonstrated that up to 50% of dimethyl sulfoxide did not change the activity of dorsal horn neurons.
and greater doses of dimethyl sulfoxide were administered intrathecally in in vivo experiments by others. Intrathecal injection volumes for all substances were 10 μl followed by a 10-μl flush of preservative-free saline. All drugs were prepared on the day of the experiment.

Statistical Analysis

The results are presented as median for ordinal data, and all data were compared using nonparametric analyses. The Friedman test for within-group comparisons and the Kruskal-Wallis test and Wilcoxon–Mann–Whitney test for between-groups comparisons were used. Multiple comparisons after the Friedman test and Kruskal-Wallis test were performed using a Dunnett test or Dunn test for nonparametric analysis. *P* < 0.05 was considered significant.

Results

**Experiment 1: Effects of A₁R and A₂aR Agonists on Nonevoked Pain Behavior**

In all groups of animals, similar nonevoked pain behavior 2 h after surgery was observed (figs. 1A–C). Intrathecal administration of R-PIA (5 nmol) but not of CGS21680 (10 nmol) produced a decrease of the median pain score from 18.5 (0 h) to 7 during the first hour after drug administration (*P* < 0.05 vs. 0 min; figs. 1B and C). Decreased nonevoked pain behaviors lasted for more than 2 h. The next day, baseline pain scores were assessed, and similar results were obtained (*P* < 0.05 vs. 0 min; fig. 1D).

**Fig. 1. Effect of intrathecally administered A₁ and A₂a adenosine receptor agonists on cumulative pain scores caused by an incision. (A–C) Nonevoked pain behaviors after incision in rats treated with vehicle, R-PIA (5 nmol), or CGS21680 (10 nmol) on the day of surgery. (D) Summary of nonevoked pain behaviors 60 min after administration of vehicle, R-PIA (5 nmol), or CGS21680 (10 nmol) on postoperative day 1. *P* < 0.05 versus 0 min by Friedman and Dunnett test. † versus vehicle by Kruskal-Wallis and Dunn test.

**Experiment 2: Evaluating Effects of A₁R and A₂aR Agonists on Evoked Mechanical Hyperalgesia after Incision**

In vehicle-treated rats, the median withdrawal threshold to von Frey filaments decreased from 563 mN (pre) before surgery to 36.9 mN 2 h (0 min before intrathecal drug administration) after incision. Hyperalgesia was persistent; median withdrawal thresholds were 36.9 mN or less throughout the day of surgery (fig. 2A). Intrathecal administration of 0.3, 1, and 5 nmol R-PIA produced a dose-dependent increase of the reduced withdrawal thresholds after incision for 30 and 60 min, respectively (*P* < 0.05 vs. 0 min; figs. 2B–D), indicating an A₁R-induced reduction of hyperalgesia. Intrathecal pretreatment with CPT (242 nmol), a specific A₁R antagonist, but not with DMPX (50 nmol), a specific A₂aR antagonist, blocked R-PIA (5 nmol)-induced antinociception (figs. 2E and F). Similar results were observed on postoperative day 1 (fig. 2G).

In separate groups of animals, intrathecal administration of a vehicle or 2 or 10 nmol CGS21680 did not modify pain behaviors (figs. 3A–C), but intrathecal injection of 20 nmol CGS21680 caused a brief increase in withdrawal thresholds 30 min after injection (*P* < 0.05 vs. 0 min; fig. 3D).
This short-lasting antihyperalgesic effect was blocked by intrathecal pretreatment with CPT (A1R antagonist) but not with the corresponding A2aR antagonist DMPX, indicating that the CGS21680-induced increase of withdrawal thresholds was due to an unspecific activation of the A1R (figs. 3E and F). Similar results were observed on postoperative day 1 (fig. 3G).

**Experiment 3: Evaluating Mechanisms of A1R-induced Antinociception**

Intrathecal pretreatment with 0.5 μg PTX 7 days before administration of 5 nmol R-PIA blocked R-PIA-induced increase of withdrawal thresholds (fig. 4A). Similarly, in rats pretreated with forskolin, the subsequent intrathecal administration of R-PIA did not modify mechanical withdrawal thresholds after incision (fig. 4B). In addition, pretreatment of PTX or forskolin followed by a vehicle injection (data not shown) did not increase hyperalgesia in rats after an incision, indicating that the inhibition of R-PIA-induced antinocepcion by PTX or forskolin pretreatment was not caused by a baseline shift of withdrawal thresholds.

Intrathecal pretreatment with the nonselective potassium blocker tetroethylammonium (100 nmol) or 4-AP (5 nmol) reduced R-PIA-induced antinocepcion, indicating that potassium channels were involved in this process (P < 0.05 vs. 0 min; figs. 5A and B). Intrathecal pretreatment with glibenclamide produced a dose-dependent inhibition of R-PIA-induced antinocepcion (P < 0.05 vs. 0 min; figs. 5C–F). Intrathecal administration of glibenclamide (100 nmol) and subsequent vehicle did not modify persistent hyperalgesia after incision (data not shown). Intrathecal administration of either apamin nor charybdotoxin modified hypoalgesia mediated by R-PIA (P < 0.05 vs. 0 min; figs. 6A and B); a small but not statistically significant inhibition of R-PIA-induced hypoalgesia was observed with a pretreatment of margatoxin (P < 0.05 vs. 0 min; fig. 6C). Higher intrathecal doses of apamin, charybdotoxin, or margatoxin produced severe pain behaviors and were not used in this study. Therefore, KATP channels but not calcium-activated potassium channels and the voltage-gated potassium channels Kv1.3 and Kv1.6 are partially involved in A1R-induced antinocicepcion.

**Discussion**

In the current study, we demonstrated that the intrathecal administration of an A1R agonist (R-PIA) but not of an A2aR agonist (CGS21680) decreased nonevoked pain behaviors and evoked mechanical hyperalgesia after a surgical incision. Furthermore, A1R-induced spinal antinocicepcion is mediated by interactions with PTX-sensitive G proteins and due to the inhibition of adenylyl
cyclase. In addition, the opening of KATP channels but not of calcium-activated potassium channels or Kv1.3 or 1.6 channels contribute to the antinociceptive effect of adenosine receptor agonists.

Effect of Spinal A1 and A2a Adenosine Receptor Agonists on Hyperalgesia in Animal Models

Several investigators have examined the effects of spinally administered A1R agonists in models of persistent pain and hyperalgesia. In vitro and in vivo electrophysiologic experiments demonstrated that activation of A1Rs hyperpolarize dorsal horn neurons in lamina II and inhibit the C fiber–induced windup phenomenon.\(^5,8,11,38\) Intrathecal administration of selective A1R agonists reduced pain behaviors after chemical irritation,\(^12\) nerve injury,\(^32,39\) spinal cord injury,\(^40\) and inflammation.\(^13\) Furthermore, mice lacking the A1R exhibited increased nociceptive responses under normal conditions and enhanced heat hyperalgesia after inflammation and nerve injury.\(^6,7\) In agreement, results from the current study revealed that spinal administration of an A1R agonist decreased dose dependently mechanical hyperalgesia after an incision, indicating that activation of the A1R affects the maintenance of exaggerated pain behaviors in this model for postoperative pain. Accordingly, previous experiments demonstrated a dose-dependent antihyper-

Fig. 3. Effect of intrathecal A1R adenosine receptor agonist CGS21680 on punctuate mechanical hyperalgesia caused by incision. (A–D) Withdrawal threshold after incision in rats treated with vehicle or 2, 10, or 20 nmol of the A1R adenosine receptor agonist CGS21680. (E and F) Blockade of the antihyperalgesic effect of 20 nmol CGS21680 by intrathecal administration of the noncorresponding A1 adenosine receptor antagonist CPT but not by the corresponding A2a adenosine receptor antagonist DMPX, indicating an unspecific A1 adenosine receptor antinociceptive effect. (G) Summary of withdrawal thresholds 30 min after administration of vehicle or different doses of CGS21680. \(* P < 0.05\) versus 0 min by Friedman and Dunnett test. \(†\) versus vehicle by Kruskal-Wallis and Dunn test.

Fig. 4. Intrathecal administration of pertussis toxin (PTX) and forskolin modulates R-PIA–induced antihyperalgesia. (A and B) Intrathecal administration of PTX or forskolin reduced the antihyperalgesic action of R-PIA, suggesting that the antinociceptive responses to R-PIA are transduced via PTX-sensitive G protein–coupled A1Rs and due to the inhibition of cyclic adenosine monophosphate production.
sensitivity effect of the positive allosteric adenosine receptor modulator T62 after intrathecal administration in the incisional model for postoperative pain. In contrast, the role of the spinal A2aR for pain processing is uncertain. Recently, Yoon et al. demonstrated that intrathecal administration of the A2aR agonist DPMA inhibited both phases of formalin-induced pain behaviors. Poon et al. revealed only a modest antinociceptive effect of the spinally administered A2aR agonist CGS21680 on inflammation-induced thermal hyperalgesia, arguing that this might be due to an unspecific activation of A1Rs. However, other investigators did not observe antinociceptive effects after spinal A2aR activation. In electrophysiologic studies of spinal cord slices, application of CGS21680 (A2aR agonist) was without effects on neuronal transmission or exhibited a mix of excitatory and inhibitory effects. In the current study, intrathecal administration of CGS21680 did not decrease mechanical hyperalgesia after incision. Moderate effects on incision-induced hyperalgesia after application of higher doses of CGS21680 were due to an unspecific activation of A1Rs and therefore blocked by an A1R antagonist but not by an A2aR antagonist.

Possible Mechanisms of A1R-induced Antinociception: Role of PTX-sensitive Inhibitory G Proteins

Although there is ample evidence that adenosine-induced spinal antinociception is largely mediated by ac-
tivation of A1Rs, little is known about the underlying mechanisms. A1Rs are coupled to PTX-sensitive Gi proteins reducing cAMP levels by inhibiting adenylyl cyclase. Because increased cAMP levels facilitate the release of neurotransmitters, enhance the excitability of dorsal horn neurons or spinothalamic tract cells, and result in hyperalgesia, it has been suggested that modifying the cAMP transduction cascade represents one downstream mechanism by which activation of A1Rs produces antinociception. In agreement, presynaptic A1Rs located on sensory afferent terminals and dorsal root ganglion neurons decreased cAMP production and inhibited the release of neurotransmitters such as CGRP and glutamate. Furthermore, a decrease of spinal glutamate release caused by presynaptic A1R activation was observed after capsaicin injection and nerve injury, indicating that characteristic mechanisms of A1R activation occur after different types of tissue injuries. Although not investigated in the current study, we speculate that activation of presynaptic A1Rs is crucial for A1R-induced antinociception.

Pertussis toxin ribosylates the /H9251 subunit of inhibitory G proteins linked to adenylate cyclase and disrupt this inhibitory secondary messenger pathway, leading to increased levels of cAMP. Previous studies demonstrated that PTX prevented A1R-mediated inhibition of adenylate cyclase and reduced effects of A1R agonists on normal nociception in uninjured animals. In the current study, pretreatment with PTX inhibited the hypoalgesic effect of intrathecal R-PIA, implicating that A1R-induced spinal antinociception is mediated by PTX-sensitive G proteins. If this pathway occurs in the spinal cord, we would expect that the intrathecal administration of an A1R agonist in PTX-pretreated animals causes pain behaviors due to inhibition of adenylate cyclase and subsequently an accumulation of cAMP. In contrast, in the current study, the R-PIA–induced antinociceptive effect was largely blocked by PTX pretreatment without producing increased pain behaviors, suggesting an interaction between A1R and PTX-sensitive Gi proteins but not PTX-insensitive Gs or Gq proteins. However, a pronociceptive effect mediated by PTX-resistant Gs or Gq proteins using higher doses of R-PIA, other A1R agonists, or a different animal pain model cannot be discarded.

Among various Gi proteins, Gz is the only member of this subfamily with a PTX-resistant /H9251 subunit. The PTX-resistant Gz protein is located predominantly in the central nervous system and retina and interacts with several Gi-coupled receptors, including A1R and opioid receptors. To date, the only known signaling function of Gz is inhibition of adenyl cyclase. Although a role of Gz proteins for A1R-induced antinociception cannot be discarded, we demonstrated that a blockade of PTX-sensitive G protein receptors prevented completely the antinociceptive effect of R-PIA, speculating that the PTX-resistant G protein receptor Gz will be of limited importance for A1R-mediated antihyperalgesia.
Although similar results were obtained for normal nociception in uninjured animals by using the tail flick and hot plate tests, results of the current study demonstrate for the first time that A1R-induced spinal antinociception after an incision is mediated by interactions with PTX-sensitive G proteins and due to the inhibition of adenylate cyclase.

**Possible Mechanisms of A1R-induced Antinociception: Interaction with Potassium Channels**

There is ample evidence that potassium channels are involved in nociception and that the opening of specific potassium channels is critical for G protein-coupled receptor-mediated antinociception including opioid receptors, γ-aminobutyric acid type B receptors, α2 receptors, and A2Rs. Ocana and Baeyens revealed in uninjured animals that different intracerebroventriculally administered K<sub>ATP</sub> channel blockers antagonized A1R-induced supraspinal antinociception. In the current study, we assessed for the first time the importance of different potassium channels for A1R-mediated antinociception in animals after a tissue injury (incision). In agreement with Ocana et al., we demonstrated that R-PIA-induced hypoalgesia was attenuated by intrathecal administration of a K<sub>ATP</sub> channel blocker. Similarly, intrathecal administration of the nonselective potassium channel blockers tetraethylammonium and 4-AP decreased the antinociceptive effect of R-PIA on incisional pain, indicating a possible role of other potassium channels for A1R-induced hypoalgesia.

Calcium-activated potassium channels are subdivided in small (SK), intermediate (IK), or large (BK) conductance channels and are activated by an increased intracellular calcium concentration. The bee venom toxin apamin (high-affinity SK channel inhibitor; Ki: 60 pm-100 nm) and the scorpion toxin charybdotoxin (IK, BK inhibitor; Ki: 1.7–5 nm) have been used to demonstrate that spinal SK channels seem to be involved in antinociception mediated by cannabinoid and δ-opioid receptors. In the current study, the intrathecal application of neither apamin nor charybdotoxin modified R-PIA-induced hypoalgesia, indicating that calcium-activated potassium channels are not involved in A1R-mediated antinociception.

Besides its effects on BK and IK conductance channels, charybdotoxin inhibits also the voltage-gated potassium channel Kv1.3 (Ki: 0.17 nm). Margatoxin blocks Kv1.3 and Kv1.6 (Ki: 0.03 and 5 nm) but not calcium-activated potassium channels. Both scorpion toxins have been used to demonstrate that Kv1 channels are important for α<sub>2</sub>adrenoceptor-, μ-opioid receptor-, and nonsteroidal antiinflammatory drug-induced antinociception. In the current study, neither charybdotoxin nor margatoxin decreased R-PIA-induced hypoalgesia, indicating that Kv1.3 and Kv1.6 channels are not involved in A1R-mediated antinociception.

Therefore, the opening of K<sub>ATP</sub> but not of calcium-activated potassium channels and of Kv1.3 and 1.6 channels is important for antinociception mediated by A1R agonists. The role of other potassium channels for A1R-induced antinociception must be clarified.

**Conclusion**

In the current study, we demonstrated that the intrathecal administration of an adenosine A1R agonist (R-PIA) but not of an adenosine A2aR agonist (CGS21680) decreased mechanical hyperalgesia after a surgical incision. Furthermore, A1R-induced spinal antinociception is mediated by interactions with PTX-sensitive G proteins and due to the inhibition of adenylate cyclase. The opening of K<sub>ATP</sub> channels but not of calcium-activated potassium channels or the voltage-gated channels Kv1.3 and Kv1.6 may contribute to the antinociceptive effect of A1R agonists.

**References**


