Adenosine A₁ receptor agonist induces visceral antinociception via 5-HT₁A, 5-HT₂A, dopamine D₁ or cannabinoid CB₁ receptors, and the opioid system in the central nervous system

Toshikatsu Okumura a,b,⁎, Tsukasa Nozu c, Masatomo Ishio a, Sho Igarashi a, Shima Kume ib, Masumi Ohhirab

a Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical University, Japan
b Department of General Medicine, Asahikawa Medical University, Japan
c Department of Regional Medicine and Education, Asahikawa Medical University, Japan

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ABSTRACT

We have recently demonstrated that N(6)-cyclopentyladenosine (CPA), an adenosine A₁ receptor agonist, acts centrally to induce a visceral antinociception. Since serotonin (5-HT), cannabinoid (CB), dopamine or opioid signaling in the central nervous system is involved in the regulation of visceral sensation, we made a hypothesis that the signaling may play a role in the CPA-induced visceral antinociception. Visceral sensation was evaluated by colonic distension-induced abdominal withdrawal reflex (AWR) in conscious rats. Subcutaneously administered CPA significantly increased the threshold of colonic distension-induced AWR. Intracisternal injection of either 5-HT₁A or 5-HT₂A receptor antagonist blocked the CPA-induced visceral antinociception while 5-HT₁B antagonist did not block the CPA-induced visceral antinociception. Subcutaneous injection of dopamine D₁ receptor antagonist, CB₁ receptor antagonist or naloxone significantly blocked the CPA-induced visceral antinociception while neither subcutaneous injection of dopamine D₂ receptor antagonist nor CB₂ receptor antagonist blocked the CPA-induced anti-pain action. These results suggest that 5-HT₁A, 5-HT₂A, dopamine D₁, CB₁ receptors and the opioid system in the CNS may specifically mediate the CPA-induced visceral antinociception. These findings may help in understanding the physiological relevance of central adenosine with special reference to the pathophysiology of altered visceral sensation especially in irritable bowel syndrome.

1. Introduction

Adenosine is a ubiquitous endogenous neuromodulator or neurotransmitter, which plays an important role in pain modulation [1]. It is known that spinal or systemic administration of adenosine and its analogs leads to antinociception in response to somatic pain in various animal models [1-8]. Although the effects of adenosine on antinociceptive responses have been widely studied, little is known whether it is also involved in the antinociceptive action related to visceral pain sensation. We have recently demonstrated that subcutaneous or intracisternal injection of N(6)-cyclopentyladenosine (CPA), an adenosine A₁ receptor (A₁R) agonist, induced an antinociceptive action against colonic distension in a dose-dependent manner in rats, where antinociception was observed when much smaller doses of CPA were administered centrally rather than peripherally, suggesting that adenosine acts in the central nervous system (CNS) to produce an antinociceptive action against colonic distension [9]. The antinociceptive effect of intracisternal CPA was blocked completely by the subcutaneous administration of a specific A₁R antagonist, which indicates that the centrally injected CPA-induced antinociception was indeed mediated by A₁Rs.

Increasing findings have suggested that visceral sensation is regulated by some molecules other than adenosine in the CNS. For instance, we have showed that dopamine, serotonin (5-HT), cannabinoid (CB) or opioid signaling in the CNS could also induce visceral antinociception in rats [10-13]. The present study was performed to further clarify whether dopamine, 5-HT, CB or opioid signaling may be involved in the mechanisms of the visceral antinociception by CPA.
2. Experimental procedures

2.1. Ethical considerations

Approval was obtained from the Research and Development and Animal Care committees at Asahikawa Medical University (No. 13030) for all of the experiments conducted in this study.

2.2. Animals

Male Sprague-Dawley rats (Charles River Laboratory, Atsugi, Japan) weighing about 200 g were housed under controlled light/dark conditions (lights on: 07:00–19:00), and the room temperature was regulated at 23°C–25°C. Rats were allowed free access to standard rat chow (solid rat chow; Oriental Yeast Co., Tokyo, Japan) and tap water. All of the experiments were performed using conscious animals, which had been deprived of food for 24 h but with free access to water until the initiation of the experiments.

2.3. Chemicals

CPA (Abcam, Tokyo, Japan) was dissolved in dimethyl sulfoxide (DMSO). WAY100635, a 5-HT1A receptor antagonist (Abcam, Tokyo, Japan), isamoltane, a 5-HT1B receptor antagonist (Tocris Bioscience, Ellisville, MO, USA), ketanserin, a 5-HT2A receptor antagonist (Tokyo Kasei Co., Tokyo, Japan) or naloxone hydrochloride (Cayman Chemical, Ann Arbor, MI, USA) was dissolved in saline. SCH-23390, D1 dopamine receptor antagonist, sulpiride, D2 dopamine receptor antagonist, AM251, CB1 receptor antagonist or AM630, CB2 receptor antagonist (Wako Chemical, Osaka, Japan) were dissolved in DMSO.

2.4. Implantation of electrodes and placement of colorectal balloon

The electrodes used to obtain electrophysiological measurements of abdominal muscle contractions were implanted acutely on the day of the experiment, as described previously [14, 15]. Briefly, a skin incision measuring 5 mm was created while the rats were under isoflurane anesthesia. The electrodes (Teflon-coated stainless steel, 0.05 mm diameter; MT Giken, Tokyo, Japan) were inserted approximately 2 mm into the left side of the external oblique musculature through the incision and fixed to the incised skin with cyanoacrylate instant adhesive (Aron Alpha, Toagosei, Tokyo, Japan). The electrode leads were externalized through this closed incision and threaded through a urethane tube. Immediately after implanting the electrodes, a distension balloon was inserted intraanally where the distal end was positioned 2 cm proximal to the anus. A 6-Fr (2 mm external diameter) disposable silicone balloon-urethral catheter for pediatric use (JU-SB0601; Terumo, Tokyo, Japan) was employed. The maximal inflation volume of the balloon was 1.5 ml, and the length of the maximally inflated balloon was 1.2 cm. The balloon was secured in place by taping the catheter to the tail.

2.5. Detection of visceral sensitivity

The abdominal withdrawal reflex (AWR) test was performed as described previously to detect the pain threshold, which was defined as the intensity of colorectal distension that elicited AWR [16]. Briefly, colorectal distension was performed as described previously [14, 15], that is, an ascending method for limited phasic distension was applied by inflating the balloon manually with water using a syringe until the AWR was detected by the EMG. After completing the surgery for electrode implantation and balloon placement, the sedated rats were placed in Bollman cages where they were allowed to recover and adjust for 20 min before testing. Next, the electrode leads were connected to a custom-made EMG amplifier. The EMG signals were amplified, filtered (3,000 Hz) and digitized using a PowerLab system and recorded using computer software (LabChart 7). The pain threshold was assessed two times (2-min interval), and the mean of the threshold was calculated as the results for the animals. In the majority of animals, the pain threshold in the first test was consistent with that in the second test.

2.6. Experimental procedures

Initially, to clarify whether the 5-HT signaling is involved in the CPA-induced antinoceptive action against colonic distension, we examined the effect of intracisternal injection of either WAY100635, 5-HT1A receptor antagonist, isamoltane, 5-HT1B receptor antagonist or ketanserin, 5-HT2A receptor antagonist at a dose of 1 μg/10 μl on the subcutaneously administered CPA (0.2 mg/rat)-induced visceral antinociception. Intracisternal injection was performed under brief isoﬂurane anesthesia using a 10-μl Hamilton microsyringe after the rats were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA), as reported previously [17]. Next, to clarify whether the dopamine, cannabinoid or opioid signaling is involved in the CPA-induced antinociceptive action against colonic distension, we examined the effect of the subcutaneous injection of SCH23390 (0.2 mg/rat), sulpiride (40 mg/rat), naloxone hydrochloride (0.5 mg/rat), AM251 (0.5 mg/rat) or AM630 (0.5 mg/rat) on the CPA-induced antinociceptive action against colonic distension. We selected the doses of WAY100635, isamoltane or ketanserin [12, 18-20], and AM251, AM630, SCH23390, sulpiride or naloxone hydrochloride according to previous studies [10, 11, 13, 15]. In the previous studies, we have clearly demonstrated that the same dose of WAY100635, isamoltane, ketanserin, AM251, AM630, SCH23390, sulpiride or naloxone hydrochloride alone failed to change the AWR threshold in the same experimental setting [10-12]. Following subcutaneous and/or intracisternal injection, the rats were implanted with electrodes, and the balloons were inserted, after which the rats were moved into Ballman cages to evaluate the AWR threshold volume as described above. In rats that received subcutaneous and intracisternal injections, the procedures on each rat were performed within 5 min.

2.7. Statistical analysis

The data were expressed as means ± standard error (SE). The data were compared with the one-way analysis of variance followed by Dunnett’s multiple comparisons test. P < 0.05 was considered statistically significant.

3. Results

We examined the effect of intracisternal injection of 5-HT receptor antagonists on the subcutaneously administered CPA-induced visceral antinociception. As shown in Fig. 1, subcutaneously administered CPA at a dose of 0.2 mg significantly increased the AWR threshold volume. Either intracisternal WAY 100635, 5-HT1A receptor antagonist or ketanserin, 5-HT2A receptor antagonist significantly blocked the CPA-induced visceral antinociception while intracisternal isamoltane, 5-HT1B receptor antagonist failed to change the AWR threshold volume (Fig. 1), suggesting that 5-HT1A and 5-HT2A receptor signaling may specifically mediate the CPA-induced visceral antinociception.

To identify whether CB signaling may be involved in the CPA-induced visceral antinociception, we examined the effect of subcutaneous injection of either AM251, CB1 receptor antagonist or AM630, CB2 receptor antagonist on the subcutaneously administered CPA-induced visceral antinociception. As shown in Fig. 2, AM251 but not AM630 potently blocked the CPA-induced visceral antinociception, suggesting that CB1 receptor signaling may be implicated in the CPA-induced visceral antinociception while CB2 receptor could not mediate the CPA-induced visceral antinociception.

The effect of subcutaneous injection of either SCH23390, dopamine D1 receptor antagonist, or sulpiride, dopamine D2 receptor antagonist...
on the subcutaneously administered CPA-induced visceral antinociception was examined to identify whether dopamine signaling may be involved in the CPA-induced visceral antinociception, we examined SCH23390 but not sulpiride potently blocked the CPA-induced visceral antinociception (Fig. 3), suggesting that dopamine D1 receptor signaling may be implicated in the CPA-induced visceral antinociception while dopamine D2 receptor signaling could not mediate the CPA-induced visceral antinociception.

To clarify whether the opioid system is involved in the CPA-induced antinociceptive action during colonic distension, the effect of naloxone was evaluated. As shown in Fig. 4, naloxone by itself did not change the threshold of colonic distension-induced AWR as shown in our recent

Fig. 1. Effect of intracisternal (ic) injection of WAY100635, a 5-HT_{1A} antagonist, isamoltane, a 5-HT_{1B} antagonist or ketanserin, a 5-HT_{2A} antagonist on the subcutaneously (sc) administered CPA-induced visceral antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± SE. Number of rats was 5-8 in each group. *, **, *** P < 0.01.

Fig. 2. Effect of subcutaneous (sc) injection of dopamine D_{1} antagonist, or dopamine D_{2} antagonist on the subcutaneously administered CPA-induced visceral antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± SE. Number of rats was 5-7 in each group. *, **, *** P < 0.01.
Fig. 3. Effect of subcutaneous (sc) injection of cannabinoid 1 receptor antagonist, or cannabinoid 2 receptor antagonist on the subcutaneously administered CPA-induced visceral antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± SE. Number of rats was 5-6 in each group. *, **, *** P < 0.01.

Fig. 4. Effect of subcutaneous (sc) injection of naloxon on the subcutaneously administered CPA-induced visceral antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± SE. Number of rats was 5-7 in each group. *, ** P < 0.01.
The antinociceptive effects of subcutaneous CPA was significantly reversed by subcutaneously injected naloxone, indicating that the opioid system is involved in the CPA-induced visceral antinociception.

4. Discussion

We have recently showed that intracisternal injection of ketanserin, a 5-HT2A antagonist, but not WAY-100635, 5-HT1A receptor antagonist or isomaltane, 5-HT1B receptor antagonist blocked the levodopa-induced visceral antinociception [12], suggesting that 5-HT2A receptors in the CNS, especially the spinal cord specifically plays a role in the visceral antinociception by levodopa. The present findings also showed that 5-HT2A receptor antagonist potently blocked the CPA-induced visceral antinociception. These results indicate that 5-HT2A receptors in the CNS are involved in the visceral antinociception by not only levodopa but also activation of A1R signaling. With regard to the 5-HT1A receptors in the CNS, Barriere et al. [21] have demonstrated that intrathecal injection of 5-HT1A receptor antagonist, WAY-100635, prevented the antinociceptive action of 4-aminophenol, suggesting that 5-HT1A receptors in the spinal cord plays a role in the pain control. The 5-HT1A receptor antagonist significantly attenuated the CPA-induced visceral antinociception as shown in the present study. We would therefore speculate that not only 5-HT2A but 5-HT1A receptors in the spinal cord may be implicated in the CPA-induced visceral antinociception. A couple of papers have demonstrated a tight relationship between 5-HT2A or 5-HT1A receptor, and adenosine. For instance, the involvement of 5-HT1A receptors in the antidepressant-like effect of adenosine was reported in the mouse forced swimming test [22]. Stutzmann et al. [23] have demonstrated adenosine preferentially suppresses 5-HT2A receptor-enhanced excitatory postsynaptic currents in layer V neurons of the rat medial prefrontal cortex. These findings may support the functional relation between adenosine and 5-HT1A and 5-HT2A receptors in the CPA-induced visceral antinociception.

As shown in this study, CPA-induced visceral antinociception was blocked by dopamine D1 receptor antagonist but not dopamine D2 receptor antagonist, suggesting that dopamine D1 receptors may be involved in the A1R-mediated visceral antinociception. We have recently showed that either central injection of A1R agonist or dopamine D1 receptor agonist increased the AWR threshold volume against colonic distension in rats, suggesting that adenosinergic or dopamine D1 receptor signaling is capable of inducing visceral antinociceptive action. With regard to the relationship between adenosine and dopamine D1 receptor signaling, adenosine-dopamine interaction in the pathophysiology of CNS disorders has been established [24]. Yabuuchi et al. [25] have demonstrated a role of A1Rs in the modulation of dopamine D1 receptor signaling in the neostriatum. According to Hobson et al. [26], A1R and dopamine D1 receptor regulate cocaine-seeking behavior in mice. Thus, there is a functional relationship between A1Rs and dopamine D1 receptors in the CNS. Based on the findings, the present results might support an existence of functional relationship between A1R and dopamine D1 receptors to regulate visceral sensation.

The CB system plays a role in the regulation of visceral pain sensation. We have very recently demonstrated that intraperitoneal injection of either CB1 or CB2 receptor agonist, WIN 55212 or O-Arachidonoyl ethanolamine increased the threshold volume of colonic distension-induced AWR in rats, suggesting CB could induce visceral antinociception [10]. Pretreatment with either the CB1 or CB2 receptor antagonist potently blocked the centrally injected orexin-A-induced antinociceptive action against colonic distension in rats, suggesting that endogenous CB may be involved in the central orexin-induced antinociceptive action through the CB1 or CB2 receptors. Thus, the CB system plays a role in the regulation of visceral sensation through the specific receptors, CB1 or CB2. We have examined the roles of CB receptors in the CPA-induced visceral antinociception and showed that CB1 but not CB2 receptor antagonist significantly blocked the CPA-induced visceral antinociception, suggesting that CB1 receptors are involved in the visceral antinociceptive action by CPA. Interaction between A1R and CB1 receptors has been reported in vivo studies, where an A1R-mediated enhancement of cannabinoid CB1 receptor-induced impairment of short-term spatial memory and motor incoordination were observed [27, 28].

In order to assess the possible involvement of the opioid system in the CPA-induced antinociceptive effect during colonic distension, the opioid antagonist, naloxone hydrochloride was administered. The antinociceptive action during colonic distension of CPA was reversed by subcutaneous injection of naloxone hydrochloride, indicating that the CPA-induced visceral antinociceptive action is mediated by the opioid system. A couple of reports have shown the close relation between adenosine and opioid pathways. Demirkapu et al. [30] have demonstrated that administration of the A1R agonist significantly decreased opioid-withdrawal behaviors. Liao et al. [31] have shown electroacupuncture attenuates induction of inflammatory pain by regulating opioid and adenosine pathways in mice. Such tight relationship between adenosine and opioid pathways might play a role in the CPA-induced visceral antinociception.

Whether adenosine A1 signaling is involved in related receptors directly or indirectly to induce visceral antinociception is not clarified by the present limited pharmacological experimental data. Although the present study could not identify the interactive sites of action of molecules between adenosine A1 signaling and receptors such as 5-HT, dopamine, cannabinoid and opioid, we might be allowed to speculate a possible mechanism by which adenosine A1 signaling induces visceral antinociception (Fig. 5). Earlier investigators have demonstrated that spinal 5-HT1A [32, 33] or 5-HT2A receptors [34, 35] play an antinociceptive role. We would therefore suggest that 5-HT1A or 5-HT2A receptors in the spinal cord may be involved in the visceral antinociception by adenosinergic signaling. Immunohistochemical [36] and electrophysiological [37] studies suggested that adenosine A1 receptors localize in the dopamine-containing neurons in the ventral tegmental area (VTA). Since dopamine D1 receptor in the VTA is involved in the descending pain control system [38], dopamine D1 receptor in the VTA may mediate the visceral antinociception by adenosine A1 receptor activation. A well-characterised descending pathway originates within the periaqueductal grey (PAG) and projects to the spinal dorsal horn via the rostral ventromedial medulla (RVM) [39]. Activation of this descending system, either from within the PAG, RVM or higher centers, elicits analgesia by inhibiting ascending nociceptive transmission at the spinal cord level. Opioids and cannabinoid agonists influences PAG and RVM neurons via presynaptic μ-opioid and CB receptors, respectively [40-43]. Considering the findings, we would suggest that opioid and CB1 receptor in the PAG-RVM-spinal pathway may play a role in regulation of visceral sensation by adenosinergic signaling. A possibility of interaction of receptors such as relation between 5-HT receptors and dopamine D1 receptor to induce antinociception could not be excluded. Further studies should be needed to clarify the issues.

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal tract [44, 45]. Brain-gut interaction plays an important role in the pathophysiology of IBS. Visceral hypersensitivity to mechanical distension of the gut is related to symptom severity and it is considered to play a role in the pathophysiology of IBS [33]. However, a therapeutic strategy to improve visceral hypersensitivity in IBS has not been established. In other words, we are able to provide a novel therapeutic option if we could clarify mechanisms in the CNS of visceral hypersensitivity and control it. Based on the present study, we would therefore suggest that activation of adenosine A1 signaling through 5-
HT2A, 5-HT1A, dopamine D1 or CB1 receptors, or opioid signaling in the CNS should be considered to be a novel therapeutic target for IBS patients. These findings help us understand the pharmacological property of CPA and the pathophysiology of visceral hypersensitivity especially in IBS.

Conflicts of interest
We have no conflicts of interest to declare.

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References
[1] J. Sawynok, Adenosine receptor activation and nociception, Eur. J. Pharmacol. 347 (1998) 1–11.
[2] P.M. Lavand’homme, J.C. Eisenach, Exogenous and endogenous adenosine enhance the spinal antiallodynic effects of morphine in a rat model of neuropathic pain, Pain 80 (1999) 31–36.
[3] Y.W. Lee, T.L. Yaksh, Pharmacology of the spinal adenosine receptor which mediates the antiallodynic action of intrathecal adenosine agonists, J. Pharmacol. Exp. Ther. 277 (1996) 1642–1648.
[4] J.A. Gomes, X. Li, H.L. Pan, J.C. Eisenach, Intrathecal adenosine interacts with a spinal noradrenergic system to produce antinociception in nerve-injured rats, Anesthesiology 91 (1999) 1276–1285.
[5] T. Okumura, Y. Ohba, O. Kemmotsu, Characterization of adenosine receptors mediating spinal sensory transmission related to nociceptive information in the rat, Anesthesiology 87 (1997) 577–584.
[6] A. Poon, J. Sawynok, Antinociception by adenosine analogs and inhibitors of adenosine metabolism in an inflammatory thermal hyperalgesia model in the rat, Pain 74 (1998) 235–245.
[7] B. Johansson, L. Hallldner, T.V. Dunwiddie, S.A. Masino, W. Poelchen, L. Giménez-Lloret, et al., Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor, Proc. Natl. Acad. Sci. USA 98 (2001) 9407–9412.
[8] W.P. Wu, J.X. Hao, L. Halldner, C. Lövdahl, G.E. DeLander, Z. Wiesenfeld-Hallin, et al., Increased nociceptive response in mice lacking the adenosine A1 receptor, Pain 113 (2005) 395–404.
[9] T. Okumura, T. Nozu, S. Kumei, K. Takakusaki, S. Miyagishi, M. Ohhira, Adenosine A1 receptors mediate the intracisternal injection of orexin-induced antinociceptive action against colonic distension in conscious rats, J. Neurol. Sci. 362 (2016) 106–110.
[10] T. Okumura, T. Nozu, S. Kumei, M. Ohhira, Role of the cannabinoid signaling in the brain orexin- and ghrelin-induced visceral antinociception in conscious rats, J. Pharmacol. Sci. 137 (2018) 250–252.
[11] T. Okumura, T. Nozu, S. Kumei, K. Takakusaki, S. Miyagishi, M. Ohhira, Involvement of the dopaminergic system in the central orexin-induced antinociceptive action against colonic distension in conscious rats, Neurosci. Lett. 605 (2015) 34–38.
[12] Okumura T., Nozu T., Ishioh M., Igarshi S., Kumei., Ohhira M., 5-HT2A receptors in the central nervous system mediate the levodopa-induced visceral antinociception in conscious rats, Naunyn-Schmiedeberg's Arch. Pharmacol. in press.
[13] T. Okumura, T. Nozu, S. Kumei, M. Ohhira, Central oxytocin signaling mediates the central orexin-induced visceral antinociception through the opioid system in conscious rats, Physiol. Behav. 198 (2019) 96–101.
[14] T. Okumura, T. Nozu, S. Kumei, K. Takakusaki, S. Miyagishi, M. Ohhira, Antinociceptive action against colonic distension by brain orexin in conscious rats, Brain Res. 1598 (2015) 12–17.
[15] T. Okumura, T. Nozu, S. Kumei, K. Takakusaki, M. Ohhira, Ghrelin acts centrally to induce an antinociceptive action during colonic distension through the orexigenic, dopaminergic and opioid systems in conscious rats, Brain Res. 1686 (2018) 48–54.
[16] E.D. Al-Chaer, M. Kawasak, P.J. Pasricha, A new model of chronic visceral hyperalgesia in adult rats induced by colon irritation during postnatal development, Gastroenterology 119 (2000) 1276–1285.
[17] T. Okumura, K. Fukagawa, P. To, L.L. Taylor, T.N. Pappas, Intracisternal injection of apolipoprotein A-I-V inhibits gastric secretion in pylorus-ligated conscious rats, Gastroenterology 107 (1994) 1861–1864.
[18] A. González-Hernández, G. Martínez-Lorenzana, J. Rodríguez-Jiménez, G. Rojas-Pilón, G. M. Cendes-Lara, Intracisternal injection of palmitoylethanolamide inhibits the peripheral nociceptive evoked responses of dorsal horn wide dynamic range neurons, J. Neuroal. Transm. (Vienna) 122 (2015) 369–374.
[19] S.H. Avila-Rojas, I. Velázquez-Lagunas, A.B. Salinas-Abarca, P. Barragán-Iglesias, J.B. Pineda-Farías, V. Granados-Soto, Role of spinal 5-HT1A, and 5-HT1A/1B/1D receptors in neuropathic pain induced by spinal nerve ligation in rats, Brain Res. 1622 (2015) 377–385.
[20] Z. Song, B. A. Meyerson, B. Linderoth, Spinal 5-HT receptors that contribute to the pain-relieving effects of spinal cord stimulation in a rat model of neuropathy, Pain 152 (2011) 1666–1673.
dependent generation of antinociceptive drug metabolites acting on TRPV1 in the brain, PLoS One 5 (2013) e70690.

[22] M.P. Kaster, A.R.S. Santos, A.L.S. Rodrigus, Involvement of 5-HT1A receptors in the antidepressant-like effect of adenosine in the mouse forced swimming test, Brain Res. Bull. 67 (2005) 53-61.

[23] D.E. Stuttmann, G.J. Marek, G.K. Agbajanjan, Adenosine preferentially suppresses serotonin2A receptor-enhanced excitatory postsynaptic currents in layer V neurons of the rat medial prefrontal cortex, Neuroscience 105 (2001) 55-69.

[24] K. Fuzi, D. Marcellino, D.O. Borroto-Escuela, M. Guescini, V. Fernández-Dueltas, S. Tanganelli, A. Rivera, F. Ciruela, L.F. Agnati, Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders, CNS Neurosci. Ther. 16 (2010) e18-e42.

[25] K. Yabunishi, M. Kuroiwa, T. Shuto, N. Sotogaku, H. Higashi, K. Fuxe, D. Marcellino, D.O. Borroto-Escuela, M. Guescini, V. Fernández-Dueltas, S. Tanganelli, A. Rivera, F. Ciruela, L.F. Agnati, Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders, CNS Neurosci. Ther. 16 (2010) e18-e42.

[26] V.C. Sousa, N. Assaife-Lopes, J.A. Ribeiro, J.A. Pratt, R.R. Brett, A.M. Sebastião, B.D. Hobson, C.E. O’Neill, S.C. Levis, L.M. Monteggia, R.L. Neve, D.W. Self, K. Yabuuchi, M. Kuroiwa, T. Shuto, N. Sotogaku, G.L. Snyder, H. Higashi, K. Fuxe, D. Marcellino, D.O. Borroto-Escuela, M. Guescini, V. Fernández-Dueltas, S. Tanganelli, A. Rivera, F. Ciruela, L.F. Agnati, Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders, CNS Neurosci. Ther. 16 (2010) e18-e42.

[27] M.P.Kaster,A.R.S. Santos,A.L.S.Rodrigus,Involvementof 5-HT1Areceptors inthe

[28] J.J. Azukue, Subtype-specific changes in 5-HT receptor-mediated modulation of C

[29] R.K. Bachtell, Adenosine A1 and dopamine d1 receptor regulation of AMPA receptor phosphorylation and cocaine-seeking behavior, Neuropsychopharmacology 38 (2013) 1974–1983.

[30] C.W. Vaughan, M. Connor, E.E. Bagley, M.J. Christie, Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro, Mol. Pharmacol. 57 (2000) 288–295.

[31] C.W. Vaughan, I.S. McGregor, M.J. Christie, Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons in vitro, Br. J. Pharmacol. 127 (1999) 935–940.

[32] Z. Airea, I. Buesa, M. Salguerino, J. Bilbao, L. Aquílera, M. Zimmermann, J.J. Azukue, Subtype-specific changes in 5-HT receptor-mediated modulation of C

[33] F.C. Colpaert, W.P. Wu, J.X. Hao I. Royer, F. Sautel, Z. Wiesenfeld-Hallin, X.J. Xu, High-efficacy 5-HT1A receptor activation causes a curative-like action on allodynia in rats with spinal cord injury, Eur. J. Pharmacol. 497 (2004) 29–33.

[34] X. Pichon, A.S. Wattiez, C. Becamel, et al., Disrupting 5-HT(2A) receptor/PDZ protein interactions reduces hyperalgesia and enhances SSRI efficacy in neuropathic pain, Mol. Ther. 18 (2010) 1462–1470.

[35] J.S.M. Chia, A.A Omar Farouk, A.S. Mohamad, M.R. Sulaiman, E.K. Perimal, Zerumbone alleviates chronic constriction injury-induced allodynia and hyperalgesia through serotonin 5-HT receptors, Biomed. Pharmacother. 83 (2016) 1303-1310.

[36] S.A. Rivkees, S.L. Price, F.C. Zhou, Immunohistochemical detection of A1 adenosine receptors in rat brain with emphasis on localization in the hippocampal formation, cerebral cortex, cerebellum, and basal ganglia, Brain Res 677 (1995) 193–203.

[37] Y.N. Wu, N.B. Mercuri, S.W. Johnson, Presynaptic inhibition of gamma-aminobutyric acidB-mediated synaptic current by adenosine recorded in vitro in midbrain dopamine neurons, J. Pharmacol. Exp. Ther. 273 (1995) 576–581.

[38] P.B. Wood, Role of central dopamine in pain and analgesia, Expert. Rev. Neurother. 8 (2008) 781–797.

[39] B. Lau, C.W. Vaughan, Descending modulation of pain: the GABA disinhibition hypothesis of analgesia, Curr. Opin. Neurobiol. 29 (2014) 159–164.

[40] C.W. Vaughan, M. Connor, E.E. Bagley, M.J. Christie, Actions of cannabinoids on membrane properties and synaptic transmission in rat periaqueductal gray neurons in vitro, Mol. Pharmacol. 57 (2000) 288–295.

[41] C.W. Vaughan, I.S. McGregor, M.J. Christie, Cannabinoid receptor activation inhibits GABAergic neurotransmission in rostral ventromedial medulla neurons in vitro, Br. J. Pharmacol. 127 (1999) 935–940.

[42] B. Chieng, M.J. Christie, Inhibition by opioids acting on mu-receptors of GABAergic and glutamatergic postsynaptic potentials in single rat periaqueductal gray neurones in vitro, Br. J. Pharmacol. 113 (1994) 303–309.

[43] C.W. Vaughan, M.J. Christie, Presynaptic inhibitory action of opioids on synaptic transmission in the rat periaqueductal grey in vitro, J. Physiol. 498 (1997) 463–472.

[44] G.P. Longstreth, W.G. Thompson, W.D. Chey, L.A. Houghton, F. Mearin, R.C. Spiller, Functional bowel disorders, Gastroenterology 130 (2006) 1480–1491.

[45] E.A. Mayer, G.F. Gebhart, Basic and clinical aspects of visceral hyperalgesia, Gastroenterology 107 (1994) 271–293.