Kor J Physiol (1996) 30(1), 85~96

Adenosine Agonist-induced Changes in the Transmission of Sensory Signals in the Cat Spinal Cord*

Kee Soon Kim, Hong Kee Shin and Jin Hyuk Kim

Department of Physiology, School of Medicine, Hanyang University, Seoul 133-791, Korea

=ABSTRACT=

Adenosine and its analogues are known to possess analgesic effects and to be involved in the opiate-induced antinociception as well. This study was designed to investigate the effects of three adenosine agonists, 5'-(N-cyclopropyl)-carboxamidoadenosine(CPCA), 5'-N-ethylcarboxamidoadenosine (NECA) and N^6-cyclohexyladenosine (CHA) on the signal transmission in the spinal cord and also to elucidate mechanisms of their actions in the anesthetized cat. All the tested adenosine agonists (i.v.) exerted inhibitory effects on the responsiveness of the wide dynamic range (WDR) cells, the inhibitory action of CHA, an adenosine A_1 receptor agonist, (80μg/Kg) being most weak. The intravenous CPCA, an adenosine A_2 receptor agonist, (20μg /Kg) and NECA, nonspecific adenosine receptor agonist, (20μg/Kg) inhibited the responses of WDR cells to pinch and C fiber stimulation more strongly than those to brush and A fiber stimulation. CPCA (i.v.) also suppressed the responses of WDR cells to thermal stimulus. And all the CPCA-induced inhibitions were caffeine-reversible. When CPCA was directly applied onto the spinal cord or intravenously administered into the spinal cat, on average, about three quarters of the CPCA-induced inhibitory effect was abolished. On the other hand, in the animal with spinal lesions in the ipsilateral dorsolateral area, the CPCA-induced inhibition was comparable to that oberved in the spinal cats. In conclusion, this study shows that adenosine agonists strongly suppress the responses of WDR cells to pinch, C fiber stimulation and thermal stimuli mainly through the supraspinal adenosine A_2-receptors.

Key Words: Adenosine agonists, Dorsal horn cell activity, Supraspinal mechanism, Spinal animal, Caffeine

INTRODUCTION

Since Vapaatalo et al (1975) first demonstrated antinociceptive effect of an adenosine analogue, L-N^6-(2-phenylisopropyl)adenosine in rats, antinociceptive actions of adenosine analogues have been repeatedly reported in animal studies in which effects on acute pain behaviors were assessed by tail flick (Holmgren et al, 1983; Sawynok et al, 1986), hot plate (Karlsten et al, 1991) and acetic acid writhing tests (Yarbrough & McGuffin-Clineschmidt, 1981; Herrick-Davis et al, 1989). Now it is well established that adenosine and its analogues possess analgesic effects not only on acute pain but also

* This study was supported by 1995 Grant from Korea Science & Engineering Foundation (951-0708-058-1)

tonic pain (Lee et al, 1993) in animals and human. Adenosine and the related compounds produce antinociception following systemic (Vapaatalo et al, 1975; Holmgren et al, 1983), intracerebroventricular (Herrick-Davis et al, 1989) and intrathecal (Sawynok et al, 1986; Karlsten et al, 1991) adminstration. Adenosine-induced antinociception is abolished by caffeine, theophylline and 8-phenyltheophylline (Yarbrough & McGuffin- Clineschmidt, 1981; Sawynok et al, 1986; Herrick- Davis et al, 1989), suggesting the involvement of adenosine receptors in mediating antinociceptive effects of adenosine. Adenosine receptors were classified first on the basis of opposing effects on adenylate cyclase (Van Calker et al, 1979), but later on the basis of the rank order of potency of adenosine receptor agonists (Stone, 1985). At A_1-receptor, N^6-R- phenylisopropyladenosine (R-PIA) is more potent than 5'-N-ethylcarboxamidoadenosine (NECA), while the reverse is true of A_2-receptors. Both A_1 and A_2 receptors are known to be present in the central nervous system of a variety of experimental animals (Stone, 1991).

Sawynok et al (1986) and Karlsten et al (1991) reported that tail flick and hot plate latencies were significantly prolonged after intrathecal administration of adenosine A_1 receptor agonists but not by adenosine A_2 receptor agonists. However, DeLander and Hopkins (1987) strongly suggested the involvement of adenosine A_2 receptor in spinal mechanism of antinociception. Recently, Fastbom et al (1990) indicated that there is no compelling evidence to ascribe the antinociceptive effect of adenosine analogues to A_2 rather than A_1 receptors. Both presynaptic and postsynaptic mechanisms have been implicated in the adenosine-induced antinociception. Adenosine and its analogues dose-dependently suppress Ca^{++} uptake and ω-conotoxin-sensitive Ca^{++} current measured in hippocampal neurons (Scholz & Miller, 1991; Wu & Saggau, 1994), sympathetic ganglionic neurons (Zhu & Ikeda, 1993), dorsal root ganglionic neurons (Gross et al, 1989; Kasai & Aosaki, 1989) and brain and dorsal horn synaptosomes (Goncalves et al, 1991). This inhibitory actions on inward Ca^{++} current was potentiated by dipyridamole (Wu et al, 1982) whereas antagonized by pertussis toxin (Gross et al, 1989), theophylline (Wu et al, 1982) and other A_1 receptor antagonists (Scholz & Miller, 1991; Wu & Saggau, 1994). Bath application of adenosine and CADO reduced excitatory synaptic potential recorded in presynaptic hippocampal neurons (Thompson et al, 1992; de Mendonca & Ribeiro, 1993). All these presynaptic mechanisms may lead to the suppression of neurotransmitter release. In fact, adenosine agonists have been reported to inhibit the releases of acetylcholine (Ach) (Kirkpatrick & Richardson, 1993), noradrenaline (NA) (Fredholm et al, 1983), 5-hydroxytryptamine (5-HT) (Feuerstein et al, 1985) and excitatory amino acids (Poli et al, 1991) from various brain synaptosomes and also to reduce the amount of SP-like and calcitonin gene-related peptide-like (CGRP) immunoreactivities released from capsaicin-sensitive primary afferent fibers (Santicioli et al, 1992; 1993).

It has been suggested that ATP-sensitive K^+ channels (K_{ATP} channels) play an important role in adenosine-induced inhibition. In hippocampal and striatal neurons, adenosine induced long-lasting hyperpolarization accompanied by steady-state outward current in association with an increase in conductance, which was 4-aminopyridine- and theophylline-reversible (Trussell & Jackson, 1985; Gerber et al, 1989) and insensitive to tetrodotoxin (TTX). This outward current was suggested to be K^+ current and identified to be K_{ATP} channels in ventricular myocytes (Kirsh et al, 1990). Hyperpolarization induced by opening the K^+ channels leads presynaptically to the suppression of neurotransmitter release by preventing the opening of voltage-dependent Ca^{++} channels (Quast & Cook, 1989) and postsynaptically to the reduction of excitatory postsynaptic potential (EPSP) (de Mendonca & Ribeiro, 1993). K^+ channel opener has been

known to have antinociceptive action and to potentiate adenosine-induced analgesic effect (Ocana & Baeyens, 1994). Few studies have focused on the effect of adenosine agonist on the responses of wide dynamic range (WDR) cells to nociceptive stimuli, which have been known to play important role in nociceptive transmission in the spinal cord.

In the present study, we investigated the effects of adenosine agonists on the responses of WDR cells to mechanical, electrical and thermal stimuli and also examined whether adenosine agonists-induced inhibition was mediated through spinal and/or supraspinal mechanisms. A preliminary report of this work has been published in an abstract form (Choi et al, 1993; Kim et al, 1994).

MATERIALS AND METHODS

Forty two cats (2.5～3.5 kg) of either sex were used in this experiment. After pretreating with ketamine HCl (10 mg/kg, i.m), the animals were anesthetized by the intravenous injection of α-chloralose (60 mg/kg). A tracheostomy was made and the animals were artificially ventilated with a respirator (Model 665, Harvard, U.S.A.). Throughout the entire period of experiment, pancuronium bromide (0.3 mg/kg/hr) was intravenously infused to paralyze the musculature. End tidal CO_2 was monitored and maintained between 3.5 and 4.5% (Ohmeda 5200, U.S.A.). The rectal temperature was kept near 37°C with a homeothermic blanket system (Harvard, Cat. NO.50-7129, U.S.A.). Also monitored was the arterial blood pressure. The lumbosacral spinal cord was exposed by laminectomy at the L4-S1 levels. In some animals, spinal cord between T13-L1 was additionally exposed for spinalization and graded lesions. Seven aniamals were spinalized at the T13-L1 levels and a lesion was made in the ipsilateral (N=6) or contralateral (N=5) dorsolateral area of the spinal cord (T13-L1). At the end of experiments, the segment of spinal cord containing lesions was removed and fixed in 5% formalin for more than 1 week. Spinal cord lesions were confirmed by histological examination. The common peroneal and tibial nerves were dissected from the surrounding tissues at popliteal fossa and the paraffin pools were made over the exposed tissues. After finishing all these surgical procedures, animals were placed in a stereotaxic apparatus.

The search stimulus for dorsal horn cells was an electrical stimulation of the afferent nerves. The single unit activity of the spinal neuron was recorded with the carbon filament microelectrode. Once the single activity of the spinal neuron was recorded, the type of neuron was determined according to the response pattern to mechanical stimulation of the receptive field (RF). Only wide dynamic range (WDR) cells that responded both to A and C fiber inputs were used in this experiment. The recorded activities were amplified (WPI, DAM-80, U.S.A.), displayed on oscillosope and fed into a window discriminator, whose output was used by a computer to compile the poststimulus time histogram. The A and C fiber responses of WDR cells were compiled from 15 consecutive stimulations of the afferent nerves with a single pulse (0.1msec duration) or a train of three pulses (internal frequency: 30～50Hz, 0.5msec duration), respectively. The stimulus strength was expressed as times the threshold (T) of the largest A fibers. The intensity of stimuli was adjusted to activate only A fibers (5～10T) or all A and C fibers (150～200T). Mechanical stimulus was applied to RF for 10 sec., brushing the skin with a hair brush or pinching a fold of skin with forceps. The intensities of thermal stimulus were 44, 48 and 52°C and each thermal stimulus was applied to RF for 20 sec.

In the animals with an intact spinal cord, the control responses of WDR cells to mechanical stimulation of RF or electrical stimulation of the afferent nerves were compared with those obtained after intravenous administration of N^6-cyclohexyladenosine (80 μg/kg, CHA), 5'-(N-cyclopropyl)-carboxamidoadenosine (20 μg/kg, CPCA) and 5'-N-

ethylcarboxamidoadenosine (20 μg/kg, NECA). The thermal responses of WDR cells were recorded before and after administration of CPCA (20 μg/kg, i.v). In a few animals (N=6) with intact spinal cord, CPCA solution (1 μg/ml) was applied directly onto the spinal cord around the recording electrode after carefully removing liquid paraffin. The changes in the responses of WDR cells evoked by direct spinal application of CPCA were compared with those produced by intravenous infusion of CPCA. And also studied were the effects of caffeine (25 mg/kg. i.v) on the CPCA-induced changes in the responses of WDR cells.

In the spinal animals or animals with lesions in the spinal cord, the responses of WDR cells to mechanical or electrical stimulation were recorded before and after intravenous injection of CPCA (20 μg/kg). These changes in the responses induced by CPCA were compared with those obtained in the animals with an intact spinal cord. The location of dorsal horn cells was established by the depth of microelectrode tip below the surface of the spinal cord. Because the size of evoked responses varied from one unit to another, data were expressed as percentage of discharges in the control state.

RESULTS

About three quarters of all the WDR cells recorded in this experiment were found to be located in the depth of 1400~1800 μm below the surface of spinal cord and remaining one quarter was located in the deeper layers (1900~2500 μm). The maximal effects of adenosine agonists were observed about 20~30 min after intravenous administration. Therefore all data were collected at this time periods.

We examined the effects of 3 adenosine agonists on WDR cell responses to mechanical and electrical stimulations. All the tested adenosine analogues invariably suppressed activity of WDR cells. As compared to CPCA and NECA, CHA exerted rather weak inhibitory action even at much higher dose(80 μg/Kg). CHA reduced the responses to pinch and C

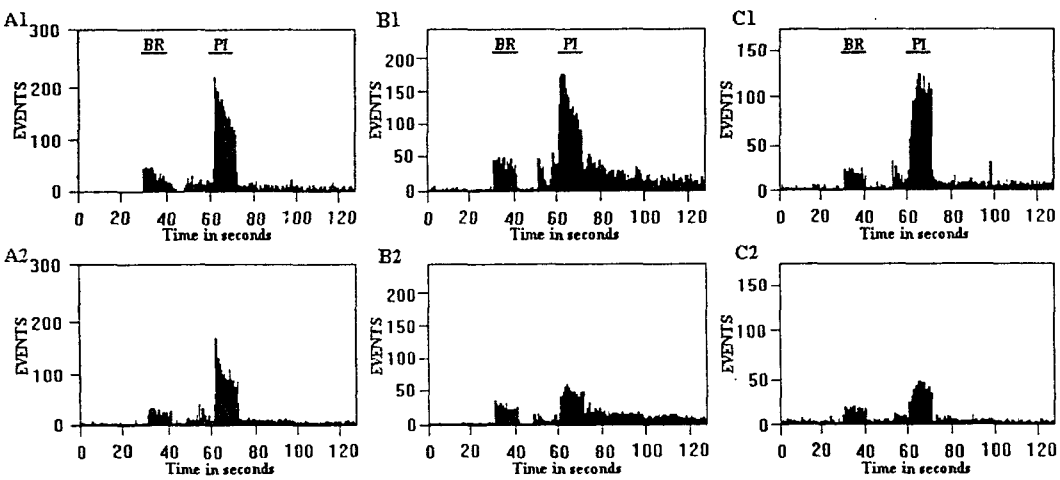

Fig. 1. Inhibition of the responses of WDR cells to mechanical stimulation of the receptive field following intravenous administration of N^6-cyclohexyladenosine (CHA: 80 μg/kg), 5'-(N-cyclopropyl)- carboxamidoadenosine (CPCA: 20 μg/kg), 5'-N-ethylcarboxamidoadenosine (NECA: 20 μg/kg). BR: brush, PI: pinch. Each mechanical stimulus was applied to the receptive field for 10sec. A1, B1 & C1: the control responses of WDR cells to brush and pinch. After intravenous administration of CHA (A2), CPCA (B2) and NECA (C2), the responses of WDR cells to pinch were more strongly depressed than those to brush.

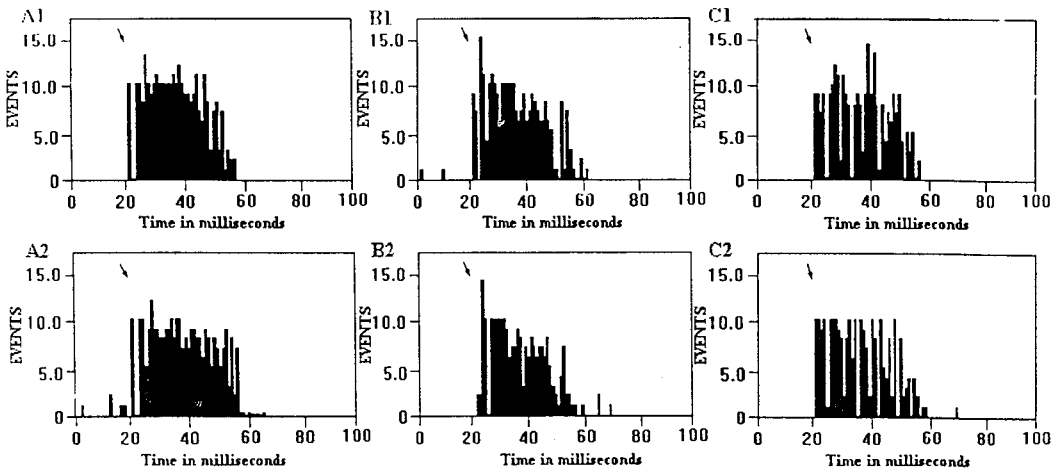

Fig. 2. Effects of intravenously administered adenosine agonists on the A-fiber responses of WDR cells to electrical stimulation of an afferent nerve. Arrows indicate the time at which single stimulus (0.1 msec, 10T) was applied to the afferent nerve. A1, B1 & C1: the control A-fiber responses of WDR cells to electrical stimulation of the common peroneal nerve. There were no significant changes in the A-fiber responses of WDR cells after intravenous administration of CHA(80 μg/kg, A2), CPCA(20 μg/kg, B2) and NECA (20 μg/kg, C2)

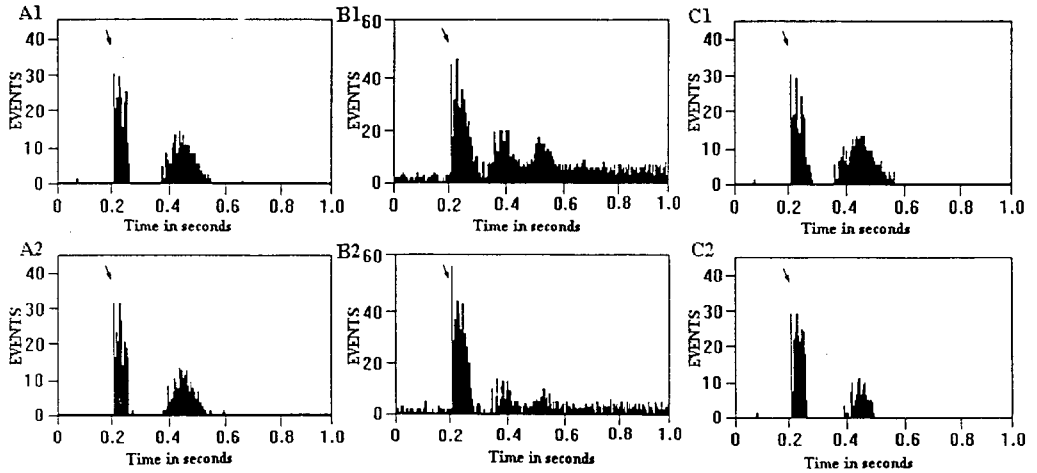

Fig. 3. Depressive actions of intravenously administered adenosine agonists on the C-fiber responses of WDR cells to electrical stimulation of an afferent nerve. Arrows indicate the time at which 3 train stimuli (0.5 msec, 200T) were applied to the afferent nerve. A1, B1 & C1: the control C-fiber responses of WDR cells to electrical stimulation of the common peroneal nerve. CPCA (B2) and NECA (C3) suppressed C-fiber responses more strongly than CHA (A2) did.

fiber stimulation only by 30.9±7.1% (Fig.1 A2) and 23.6±5.3% (Fig. 3A2), respectively while had no significant inhibitory effect on the brush and A fiber responses (Fig. 1A2 & Fig. 2A2). However, CPCA and NECA produced more strong and sustained inhibition of WDR cell responses to pinch and C

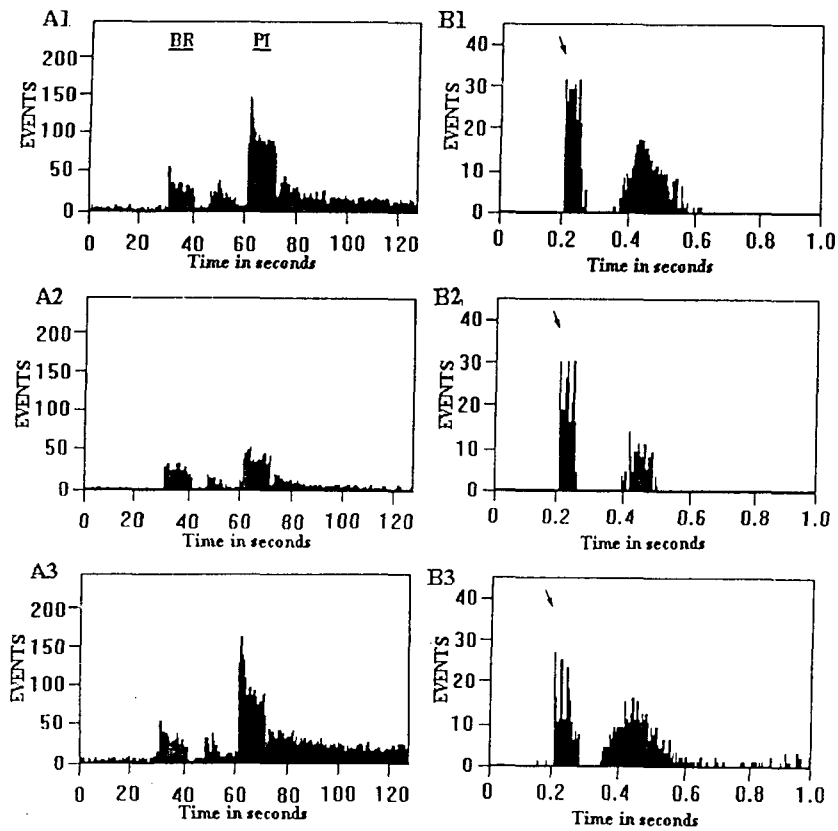

Fig. 4. Changes in the inhibitory effect of CPCA on the responses of WDR cell to mechanical and electrical stimulation before and after intravenous administration of caffeine. BR: brush, PI: pinch. Arrows indicate the time at which 3 train stimuli were applied to afferent nerve. A1 & B1: the control responses of WDR cells to mechanical stimulation of RF (A1) and to C-fiber stimulation (B1). A2 & B2: CPCA(20 μg/kg, i.v.) strongly suppressed pinch-(A2) and C-fiber (B2) responses of WDR cells. A3 & B3: intravenously administered caffeine (25 mg/kg) almost completely antagonized inhibitory action of CPCA on pinch- (A3) and C-fiber (B3) responses of WDR cells.

fiber stimulation than those to brush and A fiber stimulation. After intravenous injection of CPCA, the responses to pinch and C fiber stimulation decreased to 42.8±6.4% (Fig. 1B2) and 47.6±5.4% (Fig. 3B2) of the control responses, respectively. NECA also had comparable inhibitory actions on pinch and C fiber responses (to 43.2±7.1%, and 37.0±6.7% of the control, repectively, Fig. 1C2 & Fig. 3C2). As shown in Fig.4, the inhibitory effects of CPCA on the responses to pinch and C fiber stimulation were almost completely abolished or sometimes exceeded the control level after intravenous administration of caffeine.

As can be seen in Fig.5, WDR cells responded to thermal stimulations with increasing discharges when graded thermal stimuli were applied. CPCA suppressed the thermal responses to about 23.9~31.0% of the control, which were caffeine-reversible. There was no trend that responses to higher temperature were more strongly inhibited than those to lower temperature. Compared to the responses to mechanical and electrical stimulation, thermal responses were suppressed by CPCA more strongly.

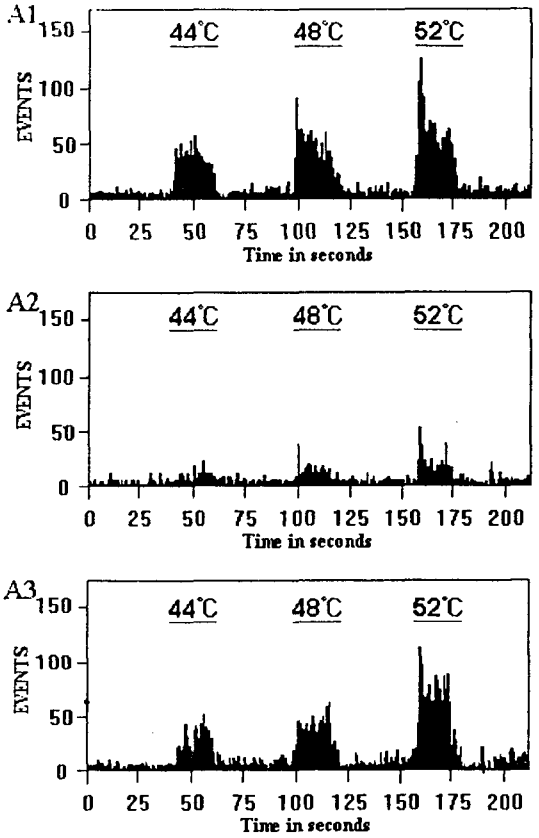

Fig. 5. Inhibitory action of CPCA(20 μg/kg, i.v.) on the thermal responses of WDR cell in the cat. A1: the control responses of WDR cells to graded thermal stimulation (44°C~48°C~52°C). CPCA greatly inhibited thermal responses of WDR cell (A2) which were caffeine-reversible (A3)

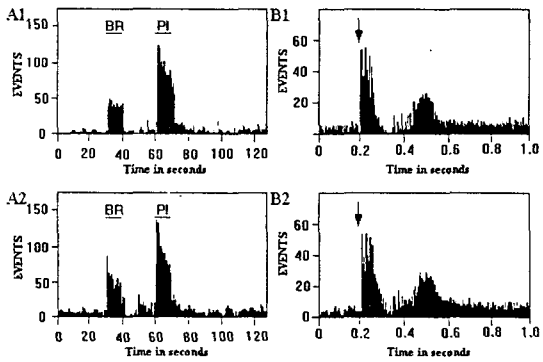

Fig. 6. Effects of CPCA (20 μg/kg, i.v.) on the responses of WDR cells to afferent inputs in the spinal cat. A1 & B1: the control responses of WDR cells to mechanical stimulation of RF (A1) and to C-fiber stimulation (B1). CPCA did not have any inhibitory action on the responses to mechanical (A1) and C-fiber (B2) stimulation in the spinal cat.

To elucidate whether the CPCA-induced inhibition was mediated through spinal and/or supraspinal mechanism, CPCA was intravenously administered into spinal cats or applied directly onto the spinal cord. As shown in Fig.6, the CPCA-induced inhibitory actions on the responses of WDR cells to pinch and C fiber stimulation were attenuated in the spinal cats. The responses to pinch and C fiber stimulation were inhibited to 89.7±5.2% and 82.4 ±4.3% of the control in the spinal cats while reduced to 42.8±6.4% and 47.6±5.4% of the control in the animals with an intact spinal cord, respectively. The spinally applied CPCA also induced inhibitory actions similar to those obtained in the spinal cats (Figure not shown).

In another series of experiments, the CPCA-induced inhibitory effects were examined in the animals with spinal lesion in the ipsilateral or contralateral side of dorsolateral area. In the animals with lesions in spinal cord ipsilateral to recording electrode, CPCA induced small amount of inhibitions which were comparable to those obtained in the spinal cats (Fig.7). On the other hand, strong inhibitory actions of CPCA still were produced in the animals with lesions in the spinal cord contralateral to recording electrode (Fig.8). The responses to pinch and C fiber stimulations were inhibited to 48.18±4.9% and 41.56±6.9% of the control, respectively.

DISCUSSION

Adenosine agonists have been known to be implicated in a number of receptor-mediated phy-

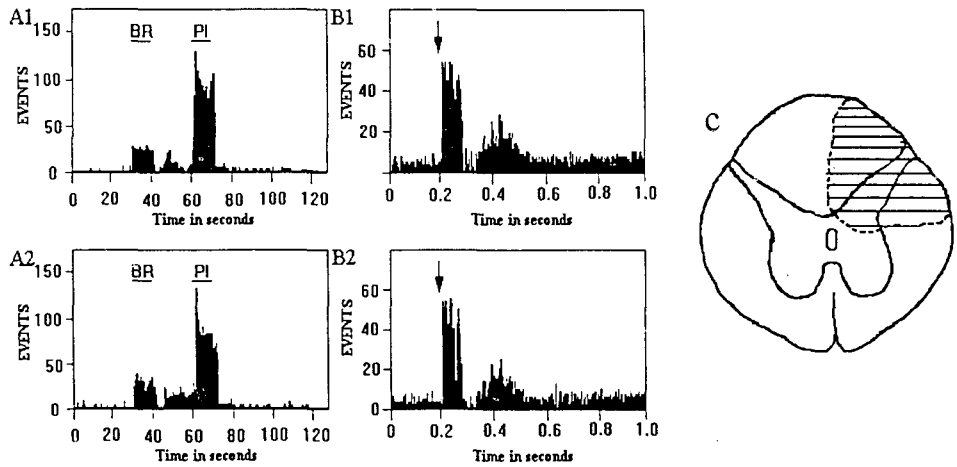

Fig. 7. Changes in the inhibitory action of CPCA (20 μg/kg, i.v.) on the WDR cell responses in the cat with lesion in the ipsilateral dorsolateral funiculus (DLF). A1 & B1: the control responses of WDR cells to mechanical (A1) and C-fiber (B1) stimulation. Most of CPCA-induced inhibitory action was blocked in cats with lesion in ipsilateral DLF (A2 7 B2). C: schematic representation of lesioned area which was located in ipsilateral DLF to recording site.

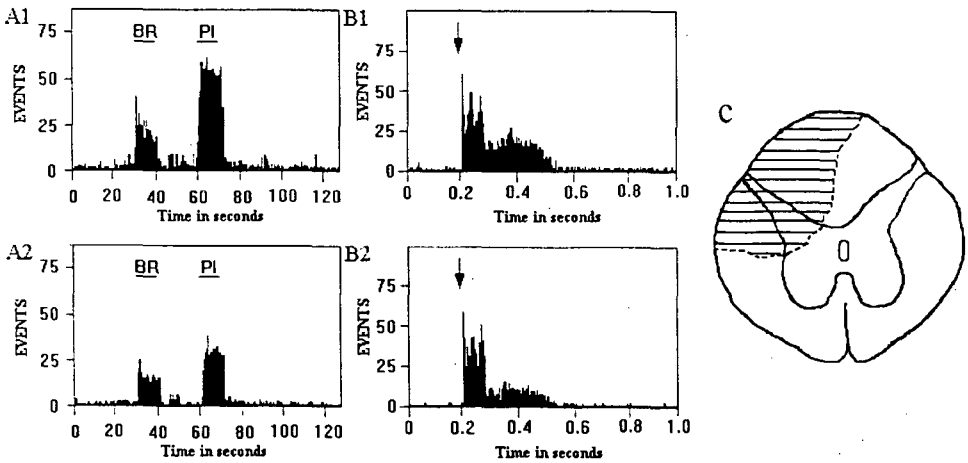

Fig. 8. Effects of CPCA (20 μg/kg, i.v.) on the responses of WDR cells to afferent inputs in the cat with lesion in the contralateral dorsolateral funiculus (DLF). A1 & B1: the control responses of WDR cells to mechanical (A1) and C-fiber (B1) stimulation. Pinch- and C-fiber-induced responses were greatly suppressed even after making lesion in contralateral DLF. C: schematic representation of lesioned area which was located in the contralateral DLF to recording site.

siological actions. In the central nervous system, adenosine agonists protect against ischemic damage (Simpson et al, 1992), alter cyclic AMP concentration (Van Calker et al, 1979) and inhibit neurotransmitter release (Fredholm et al, 1983; Feuerstein et al, 1985; Kirkpatrick & Richardson, 1993), neuronal firing rate (Stone, 1982) and synaptic transmission at neuromuscular junction

(Redman & Silinsky, 1993) and the sympathetic ganglia (Alkadhi et al, 1984).

In the present study,all the tested adenosine agonists invariably produced inhibitory actions on the responses of WDR cells to mechanical, electrical and thermal stimulations, the responses to pinch and C fiber stimulation being more strongly inhibited than those to brush and A fiber stimulation. NECA, known to have similar affinity to A_1 and A_2 receptors, and CPCA, an adenosine A_2 receptor agonist, induced more strong inhibitory actions than CHA, an adenosine A_1 receptor agonists, suggesting strong involvement of A_2-receptor in the mediation of antinociception. Dosage of CHA (80μg/kg) was 4 times those of CPCA and NECA, but the CHA-induced inhibitory action was weaker than those induced by CPCA and NECA. These results obtained in the cat experiment are in sharp contrast with the finding that most inhibitory actions are mediated through adenosine A_1 receptors in the rat (Sawynok et al, 1986; Karlsten et al, 1991; Kirkpatrick & Richardson, 1993; Redman & Silinsky, 1993). However, Yarbrough and McGuffin-Clineschmidt (1981) reported that intrathecally administered CPCA produced much stronger and caffeine-reversible antinociception than adenosine A_1 receptor agonist, CADO in mouse hot plate test.

Though adenosine receptor subtypes and their roles in regulating physiological actions have been well documented in the rat (Linden, 1991; Stone, 1991), few studies have been conducted on the adenosine receptor subtypes and their characteristics in the cat. From the results obtained from the present study, we can not intelligently discuss which type of adenosine receptor is more responsible for the mediation of adenosine-induced antinociception. But differences in species or G protein to be coupled to the adenosine receptor might be possible causes. Another important fact confirmed by this study was that most of the inhibition induced by CPCA was mediated through a supraspinal mechanism while remaining one quarter of the CPCA-induced inhibition was mediated through a spinal mechanism. This is in good agreement with the observation by Herrick-Davis et al (1989) that intracerebroventricullary administered (ICV) adenosine agonists induced stronger antinociception than intraperitoneally administered adenosine agonists. Adenosine and its analogues are known to act through multiple mechanisms. These include an increment in K^+ channel conductance, reduction in Ca^{++} channel conductance and mobilization of Ca^{++} from the intracellular stores.

The spinal mechanism of adenosine can be easily explained by pre- and postsynaptic mechanisms. In the presynaptic nerve terminals and the postsynaptic neurons (Trussel & Jackson, 1985; Gerber et al, 1989; Thompson et al, 1992; Li & Perl, 1994), adenosine is known to produce hyperpolarization accompanied by a steady-state outward current in association with an increase in ionic conductance, which is antagonized by tetraethylammonium and cesium. This hyperpolarization induced by the activation of K^+ channel prevents the opening of voltage-sensitive Ca^{++} channels (Quast & Cook, 1989). Adenosine and its agonists also dose-dependently suppress Ca^{++} uptake and ω-conotoxin-sensitive Ca^{++} current measured in a variety of neurons (Kasai & Aosaki, 1989; Zhu & Ikeda, 1993; Wu & Saggau, 1994) and also postsynaptically depress the excitatory postsynaptic potentials (Gerber et al, 1989; Li & Perl, 1994). All these factors together can reduce neurotransmitter release from the presynaptic terminals and in turn inhibit synaptic transmission. Santicioli et al (1992, 1993) demonstrated in in vitro experiments that adenosine suppressed the release of SP-like and CGRP-like immunoreactivities from the capsaicin-sensitive primary afferent fibers in the dorsal spinal cord slices but not in the ventral spinal cord preparations. In fact, these two substances have been known to be nociceptive neurotransmitters. Others also reported that adenosine agonists inhibited neurotransmitter release from the terminals of various

kinds of neurons (Fredholm et al, 1983; Feuerstein et al, 1985; Poli et al, 1991; Kirkpatrick & Richardson, 1993).

The known pre- and postsynaptic mechanisms can not satisfactorily explain the supraspinally mediated CPCA-induced inhibition observed in the present experiment. This supraspinally induced inhibitory action is suggested to be transmitted to the spinal neurons through dorsolateral area of the spinal cord ipsilateral to the recording electrode. To explain the mechanism by which ICV morphine induces antinociception, Zambotti et al (1982) and Moreau and Field (1986) proposed a hypothesis that the periaqueductal gray (PAG) neurons which are implicated in the descending inhibitory control have synaptic connections with the GABAergic inhibitory neurons and then are under the tonic inhibitory action of these neurons. They suggested that morphine suppressed these GABAergic neurons and in turn PAG neurons were disinhibited. Adenosine (i.c.v.) has been known not only to induce antinociceptive action (Ocana & Baeyens, 1994) but also to activate K^+ channels (Trussell & Jackson, 1985; Gerber et al, 1989), consequently suppressing the K^+-evoked GABA release from the cerebrocortical slices (O'Regan et al, 1992). ICV cromakalim, a K^+ channel opener, is reported to produce antinociception and also increased the spinal noradrenaline turnover (Narita et al, 1993). Norepinephrine has been known to be one of the neurotransmitters released from the nerve endings which is implicated in descending inhibition. Although we could not find any study on the effect of adenosine on the serotonergic descending inhibitory control system, the possibility that adenosine may alter serotonergic descending inhibitory mechanism can not be ruled out. Putting all these informations together, adenosine may lead to the suppression of inhibitory GABAergic neurons through activation of K^+ channels. The PAG neurons and other neurons responsible for the descending inhibitory control are released from inhibition of GABAergic neurons. If this is the case, the descending inhibitory control system can be relatively activated and signal transmissions at the spinal cord may be suppressed. But the above hypothesis and the exact supraspinal site(s) on which CPCA acts need to be investigated further in the near future.

In conclusion, this study shows that adenosine agonists strongly suppress the responses of WDR cells to pinch, C fiber stimulation and thermal stimulation mainly through supraspinal adenosine A_2-receptors.

REFERENCES

Alkadhi KA, Brown TR & Sabouni MH (1984) Inhibitory effect of adenosine on transmission in sympathetic ganglia. *Naunyn-Schmiedeberg's Arch Pharmacol* **328,** 16-19

Choi CH, Lee ML, Kim JH, Shin HK & Kim KS (1993) Inhibitory effects of adenosine agonists on responsiveness of the dorsal horn cell in anesthetized cats. *Abstr Kor Physiol Soc* **45,** 28

De Mendonca A & Ribeiro JA (1993) Adenosine inhibits the NMDA receptor-mediated excitatory postsynaptic potential in the hippocampus. *Brain Res* **606,** 351-356

De Sarr G, De Sarro A & Meldrum BS (1991) Anticonvulsant action of 2-chloroadenosine injected focally into the inferior colliculus and substantia nigra. *Europ J Pharmacol* **194,** 145-152

DeLander GE & Hopkins CJ(1987) Involvement of A_2 adenosine receptors in spinal mechanism of antinociception. *Europ J Pharmacol* **139,** 215-223

Fastbom J, Post C & Fredholm BB(1990) Antinociceptive effects and spinal disinhibition of two adenosine receptor agonists after intrathecal administration. *Pharmacol Toxicol* **66,** 69-75

Feuerstein TJ, Hertting G & Jackisch R (1985) Modulation of hippocampal serotonin (5-HT) release by endogenous adenosine. *Europ J Pharmacol* **107,** 233-242

Fredholm BB, Jonzon B & Lindgren E (1983) Inhibition of noradrenaline release from hippocampal slices by a stable adenosine analogue. *Acta Physiol Scand* (Suppl) **515,** 7-10

Gerber U, Greene RW, Haas HL & Stevens DR (1989) Characterization of inhibition mediated by adeno-

sine in the hippocampus of the rat in vitro. *J Physiol* **417,** 567-578

Goncalves ML, Pinto F & Ribeiro, JA (1991) Effects of adenosine on $^{45}Ca^{2+}$ uptake by electrically stimulated rat brain synaptosomes. *J Neurochem* **56,** 1769-1773

Gross RA, Macdonald RL & Ryan-Jastrow T (1989) 2-chloroadenosine reduces the N calcium current of cultured mouse sensory neurons in a pertussis toxin-sensitive manner. *J Physiol* **411,** 585-595

Herrick-Davis K, Chippari S, Luttinger D & Ward SJ (1989) Evaluation of adenosine agonists as potential analgesics. *Europ J Pharmacol* **162,** 365-369

Holmgren M., Hednar T, Nordberg G & Mellstrand T (1983) Antinociceptive effects in the rat of an adenosine analogue, N^6-phenylisopropyladenosine. *J Pharm Pharmacol* **35,** 679-680

Karlsten R, Post C, Hide I & Daly JW (1991) The antinociceptive effects of intrathecally administered adenosine analogs in mice corelates with the affinity for the A_1-adenosine receptor. *Neurosci Lett* **121,** 267-270

Kasai H & Aosaki T (1989) Modulation of Ca-channel current by an adenosine analog mediated by a GTP-binding protein in chick sensory neurons. *Pflügers Arch* **44,** 145-149

Kim C, Kim JH, Shin HK & Kim KS (1994) Supraspinally mediated inhibitory action of adenosine on the responsiveness of cat dorsal cell. *Abst Kor Neurobiol* 3, 4

Kirkpatrick KA & Richardson PJ (1993) Adenosine receptor-mediated modulation of acetylcholine release from rat striatal synaptosomes. *Brit J Pharmacol* **110,** 949-954

Kirsch GE, Codina J, Birnbaumer L & Brown AM (1990) Coupling of ATP-sensitive K^+-channels to A1 receptors by G proteins in the rat ventricular myocytes. *Am J Physiol* **259,** H820-H826

Lee ML, Lee K, Kim JH, Shin HK & Kim KS (1993) Antinociceptive actions of adenosine and its analogues on the tonic pain in the rat. *Abstr Kor Soc Neurobiol* 2, 3

Li J & Perl ER (1994) Adenosine inhibition of synaptic transmission in the substantia gelatinosa. *J Neurophysiol* **72,** 1611-1621

Linden J (1991) Structure and function of A_1 adenosine receptors. *FASEB J* **5,** 2668-2676

Moreau JL & Fields HL (1986) Evidence for GABA involvement in midbrain control of medullary neurons that modulate nociceptive transmission. *Brain Res* **397,** 37-46

Narita M, Takamori K, Kawashima N, Funada M, Kamei J, Suzuki T, Misawa M & Nagase H (1993) Activation of central ATP-sensitive potassium channels produces the antinociception and spinal noradrenaline turnover-enhancing effect in mice. *Psychopharmacology* **113,** 11-14

O'Regan MH, Simpson RE, Perkins LM & Phillis JW (1992) Adenosine receptor agonists inhibit the release of γ-aminobutyric acid (GABA) from the ischemic rat cerebral cortex. *Brain Res* **582,** 22-26

Ocana M & Baeyens JM (1994) Role of ATP-sensitive K^+-channels in antinociception induced by R-PIA, an adenosine A1 receptor agonist. *Naunyn- Schmiedeberg's Arch Pharmacol* **350,** 57-62

Poli A, Lucchi R, Vibio M & Barnabei O (1991) Adenosine and glutamate modulate each others release from rat hippocampal synaptosomes. *J Neurochem* **57,** 298-306

Quast U & Cook NS (1989) Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *TIPS* **10,** 431-435

Redman RS & Silinsky EM (1993) A selective adenosine antagonist (8-Cyclopentyl-1,3- dipropylxanthine) eliminates both neuromuscular depression and the action of exogenous adenosine by an effect on A_1 receptors. *Mol Pharmacol* **44,** 835-840

Santicioli P, Del Bianco E & Maggi CA (1993) Adenosine A1 receptors mediate the presynaptic inhibition of calcitonin gene-related peptide release by adenosine in the rat spinal cord. *Europ J Pharmacol* **231,** 139-142

Santicioli P, Del Bianco E, Tramontana M & Maggi CA (1992) Adenosine inhibits action potential- dependent release of calcitonin gene-related peptide- and substance P-like immunoreactivities from primary afferents in rat spinal cord. *Neurosci Lett* **144,** 211-214

Sawynok J, Sweeney MI & White TD (1986) Classification of adenosine receptors mediating antinociception in the rat spinal cord. *Brit J Pharmacol* **88,** 923-930

Scholz KP & Miller RJ (1991) Analysis of adenosine actions on Ca^{2+} currents and synaptic transmission in cultured rat hippocampal pyramidal neurons. *J*

Physiol **435,** 373-393

Simpson RE, O'Regan MH, Perkins LM. & Phillis JW (1992) Excitatory transmitter amino acid release from the ischemic rat cerebral cortex: Effects of adenosine receptor agonists and antagonists. *J Neurochem* **58,** 1683-1690

Stone TW (1982) Purine receptors involved in the depression of neuronal firing in cerebral cortex. *Brain Res* **248,** 367-370

Stone TW (1985) Summary of a symposium discussion on purine receptor nomenclature, *in:* Stone TW(ed.), Purines: Pharmacology and physiological roles, VCH Verlagsgesellschaft, Weinheim, P.1.

Stone TW (1991) Receptors for adenosine and adenine nucleotides. *Gen. Pharmacol* **22,** 25-31

Thompson SM, Haas HL & Gahwiler BH (1992) Comparison of the actions of adenosine at pre- and postsynaptic receptors in the rat hippocampus in vitro. *J Physiol* **451,** 347-363

Trussell LO & Jackson MB (1985) Adenosine-activated potassium conductance in cultured striatal neurons. *Proc Natl Acad Sci USA* **82,** 4857-4861

Van Calker D, Muller M. & Hamprecht B (1979) Adenosine regulates via two different types of receptor the accumulation of cyclic AMP in cultured brain cells. *J Neurochem* **33,** 999-1005

Vapaatalo H, Onken D, Neuronen PJ & Wereerman E (1975) Stereospecificity in some central and circulatory effects of phenylisopropyladenosine (PIA). *Arzneim-Forsch* **25,** 407-410

Wu LG & Saggau P (1994) Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. *Neuron* **12,** 1139-1148

Wu PH, Phillis JW & Thierry DL (1982) Adenosine receptor agonists inhibit K^+-evoked Ca^{2+} uptake by rat brain cortical synaptosomes. *J Neurochem* **39,** 700-708

Yarbrough GG & McGuffin-Clineschmidt JC (1981) In vivo behavioral assessment of central nervous purinergic receptors. *Europ J Pharmacol* **76,** 137-144

Zambotti F, Zonta N, Parenti M, Tommasi R, Vicentini L, Conci F & Mantegazza P (1982) Periaqueductal gray matter involvement in the muscimol-induced decrease of morphine antinociception. *Naunyn-Schmiedeberg's Arch. Pharmacol* **318,** 368-369

Zhu YU & Ikeda SR (1993) Adenosine modualtes voltage-gated Ca^{2+} channels in adults rat sympathetic neurons. *J Neurophysiol* **70,** 610-620