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Antinociceptive effects of intracerebroventricularly administered P2 purinoceptor agonists in the rat

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Abstract

We examined the effects of adenosine 5′-triphosphate (ATP) and its analogues administered intracerebroventricularly on nociceptive thresholds in rats. Intracerebroventricular (i.c.v.) administration of ATP (10 and 100 nmol/rat), α,β-methylene-ATP (1–30 nmol/rat) and 2′, 3′-*O*-(4-benzoylbenzoyl)-ATP (1–30 nmol/rat) dose-dependently elevated the mechanical nociceptive threshold in the paw pressure test. These antinociceptive effects were rapid and short-lasting, peaking at 5 min and disappearing by 20 min after the administration. However, i.c.v. administration of β,γ-methylene-ATP (1–30 nmol/rat) and UTP (10 and 100 nmol/rat) had no significant effects on the mechanical nociceptive threshold. In other tests, i.c.v. administration of α,β-methylene-ATP (10 and 30 nmol/rat) prolonged the thermal nociceptive latency in the hot plate test, but only a higher dose (30 nmol/rat) of α,β-methylene-ATP prolonged the latency in the tail flick test. α,β-Methylene-ATP produced no motor deficit in the inclined plane test. These results suggest that P2X purinoceptors play an inhibitory role in nociception at the supraspinal level.

Keywords: ATP; α,β-Methylene-ATP; Antinociception; Purinoceptor

1. Introduction

In addition to diverse intracellular roles, extracellular adenosine 5′-triphosphate (ATP) has been established as a neurotransmitter or neuromodulater in both the peripheral (White, 1988; Inomata et al., 1991) and central nervous systems (Edwards et al., 1992; Ueno et al., 1992; Jo and Schlichter, 1999). ATP is contained in synaptic vesicles and co-released with noradrenaline, acethylcholine or other substances (Sneddon et al., 1982; Stone, 1981; Jo and Schlichter, 1999), and then acts on specific receptors, designated as P2 purinoceptors, on the cell surface. P2 purinoceptors are classified into two subfamilies, ionotropic P2X receptors and metabotropic P2Y receptors, on the basis of their structures and signal transduction systems (Ralevic and Burnstock, 1998). cDNAs for seven subtypes of P2X receptors and five subtypes of P2Y receptors have been cloned as P2 purinoceptors expressed in mammalian cells.

Recent studies suggest the involvement of ATP and its receptors in peripheral and spinal nociceptive transmission (Kennedy and Leff, 1995; Burnstock, 1996). It was reported that mRNA of the $P2X_3$ purinoceptor in the dorsal root ganglia is selectively expressed in capsaicin-sensitive, small diameter afferent neurons, which are probably associated with nociception (Chen et al., 1995). Cook et al. (1997) demonstrated that nociceptive, but not non-nociceptive, sensory neurons had $P2X_3$ immunoreactivity in their nerve endings and cell bodies. In electrophysiological studies, ATP and α,β-methylene-ATP, a P2X receptor agonist, evoked inward currents in capsaicin-sensitive, small diameter dorsal root ganglion neurons (Ueno et al., 1999) and spinal dorsal horn neurons (Bardoni et al., 1997; Ping et al., 1998). Furthermore, in vivo studies have provided a body of evidence of the role of P2 purinoceptors at peripheral and spinal sites in nociception. Peripheral administration of ATP and α,β-methylene-ATP have been shown to cause nociceptive responses (Bland-Ward and Humphrey, 1997; Dowd et al., 1998) and facilitate formalin-induced responses (Sawynok and Reid, 1997). Intrathecal administration of α,β-methylene-ATP induced thermal hyperalgesia, which was blocked by P2 purinoceptor antagonists (Driessen et al., 1994; Tsuda et al., 1999). These observations strongly support the idea that ATP plays a crucial role in facilitating pain transmission at peripheral and spinal sites, probably via the P2X purinoceptor.

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At supraspinal sites, several studies have shown that ATP and its analogues induced fast synaptic currents in the cultured neurons derived from the hippocampus (Inoue et al., 1992) and nucleus of the solitary trace (Ueno et al., 1992) as well as in the slices from the rat medial habenula (Edwards et al., 1997; Sperlagh et al., 1995). In addition, it was reported that ATP enhances or inhibits the release of some neurotransmitters including noradrenaline (Von Kügelgen et al., 1994; Koch et al., 1997b), dopamine (Koch et al., 1997a; Zhang et al., 1995), serotonin (Von Kügelgen et al., 1997; Okada et al., 1999) and glutamate (Koizumi and Inoue, 1997; Inoue, 1998). Thus, ATP is considered to play various physiological roles at supraspinal sites. However, little is known about the involvement of supraspinal ATP and its receptors in nociception. The goal of the present study was to determine the effects of ATP analogues administered i.c.v. on mechanical and thermal nociception and to assess whether the effects observed can be ascribed to a particular type or subtype of P2 purinoceptors through the use of type- or subtype-selective agonists.

2. Materials and methods

2.1. Animals and surgical procedures

All experiments using male Sprague–Dawley rats weighing 220–280 g followed the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). Animals were kept at a constant ambient temperature (24 ± 1°C) under a 12-h light/dark cycle with free access to food and water. Under pentobarbital (50 mg/kg, i.p.) anesthesia, a stainless steel guide cannula (o.d. 0.7 mm) was stereotaxically (P 0.8, L 1.5, H 2.0) implanted on the right side according to the atlast of Paxinos and Watson (1998). After surgery, the animals were returned to cages and housed individually. They were allowed to recover for 5 to 7 days until the following experiments.

2.2. Drugs and drug administration

ATP, α,β-methylene-ATP ($P2X_1$- and $P2X_3$-selective agonist), 2′, 3′-*O*-(4-benzoylbenzoyl)-ATP (Bz-ATP, P2X-selective agonist), β,γ-methylene-ATP ($P2X_1$-selective agonist) and uridine 5′-triphosphate (UTP, P2Y-selective agonist) were purchased from Sigma (St. Louis, MO, USA). These drugs were dissolved in phosphate-buffered saline (PBS) and administered via the injection cannula, which reached the right lateral ventricle (P 0.8, L 1.5, H 4.0) when attached to the guide cannula. The i.c.v. injection was carried out in a volume of 5 μl at a constant rate of 5 μl/30 s. Baclofen (Sigma) was dissolved in saline and administered intraperitoneally. The experimenter was not informed of the names and doses of drugs until the measurements of nociceptive threshold or latency and analyses of the data had been completed.

2.3. Behavioral tests

Mechanical nociceptive threshold was evaluated by the paw pressure test using an analgesimeter (Ugo Basile, Milan, Italy) with a cuneate piston. The piston was put on the right hind paw and the pressure was loaded at a rate of 32 g/s. The pressure, which elicited paw withdrawal behavior, was determined as a nociceptive threshold.

For assessing thermal nociception, the hot plate test was performed by placing the rat on a plate heated to 52°C and measuring the latency to licking a hindpaw or jumping. The cut-off time was 60 s to prevent tissue damage. The tail flick test was performed with the use of a light beam analgesia meter (Ugo Basile). The tail was positioned so that the radiant heat was focused onto the dorsal surface of the tail at 5–6 cm proximal to the tip. The latency to withdrawing the tail was recorded. The cut-off time was 15 s to prevent tissue damage.

The inclined plane test was carried out using a sliding apparatus (Medical Agent, Kyoto, Japan) as previously described (Yonemori et al., 1998) with slight modifications. Each rat was placed on the stainless steel plate inclined at 30°, and the angle of the plate was increased at a rate of 2°/s. The angle at which the animal began to slip down was determined. Untreated (control) animals began to slip down at 46.0 ± 0.8° ($n = 35$). ΔSlope angle for each animals was calculated in accordance with the following formula; Δslope angle (%) = [{(slope angle at which the animal began to slip down after drug administration) − 30°}/{(slope angle at which the animal began to slip down before drug administration) − 30°}] × 100(%).

The procedures for behavioral testing were carried out three times per day for habituation. After 2 days of habituation, the threshold or latency was measured following two additional habituation procedures, and the value was taken as a control. Soon after measuring the control value, the drugs were administered intracerebroventricularly and the threshold or latency was measured at 5, 10, 20, 30 and 60 min after the intracerebroventricular (i.c.v.) administration.

2.4. Statistical analysis

Statistical analyses were performed by the Dunnett multiple comparisons test following one-way analysis of variance (ANOVA) or Mann–Whitney *U*-test. Differences at $P < 0.05$ were considered significant.

3. Results

3.1. Effects of i.c.v. administration of ATP and its analogues on mechanical nociceptive threshold in the paw pressure test

I.c.v. administration of ATP (100 nmol/rat) slightly, but significantly, elevated the mechanical nociceptive threshold to the paw-pressure stimulation (Fig. 1A). I.c.v.

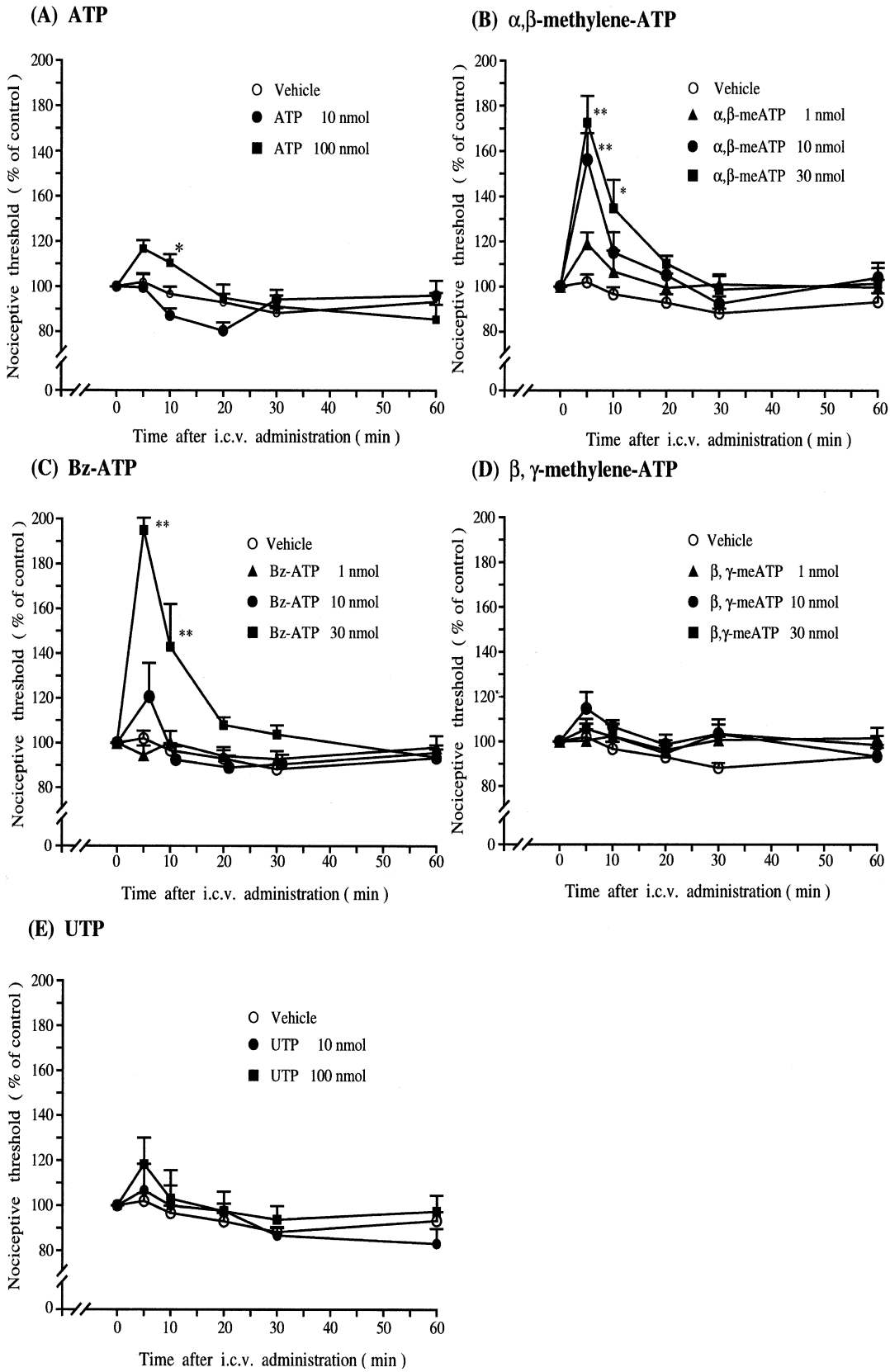

Fig. 1. The effects of i.c.v. administration of ATP (A), α,β-methylene-ATP (B), Bz-ATP (C), β,γ-methylene-ATP (D) and UTP (E) on the mechanical nociceptive threshold in the paw pressure test. Drugs were administered intracerebroventricularly at time 0. The nociceptive threshold of each animal before the i.c.v. administration served as the control value (100%). The values are presented as the means of the % of controls ± S.E.M ($n = 6–11$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the vehicle-treated group (multiple comparison by Dunnett multiple comparisons test).

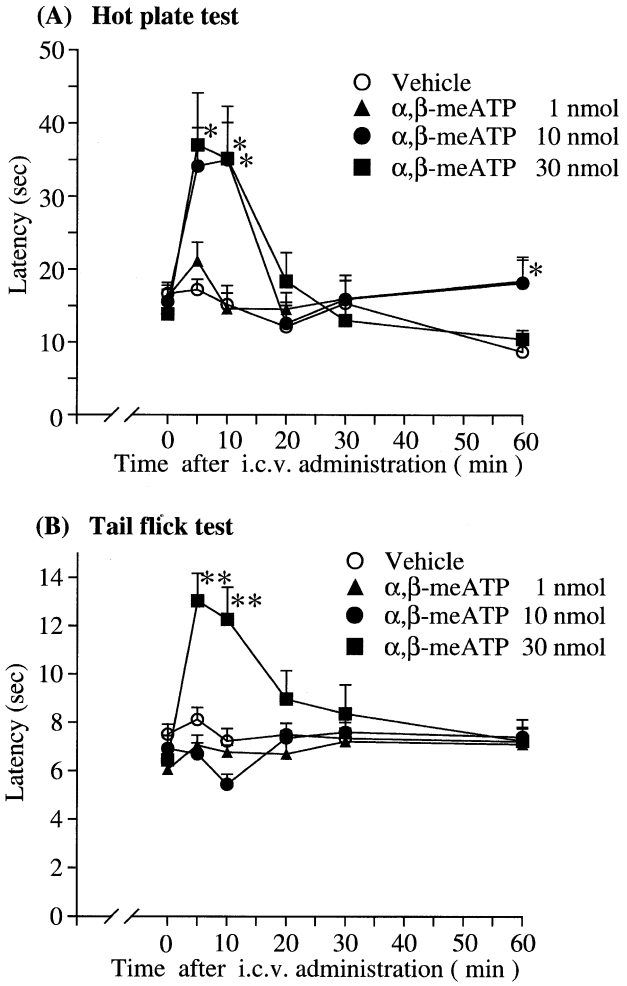

Fig. 2. The effects of i.c.v. administration of α,β-methylene-ATP on the thermal nociceptive latency in the hot plate test (A) and tail flick test (B). Drugs were administered intracerebroventricularly at time 0. The values are presented as the means ± S.E.M. of the latency (s) ($n = 6–8$). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the vehicle-treated group (multiple comparison by Dunnett multiple comparisons test).

administration of α,β-methylene-ATP (1–30 nmol/rat) produced a dose-dependent elevation of the mechanical nociceptive threshold (Fig. 1B). A significant elevation of the threshold was observed at 5 min after i.c.v. administration of α,β-methylene-ATP at doses of 10 and 30 nmol/rat (156 ± 12% and 173 ± 12% of control, respectively) and at 10 min at a dose of 30 nmol/rat (135 ± 13% of control) compared with the group administered with vehicle (102 ± 3% and 97 ± 3% of control, respectively). The effect was rapid and short-lasting, which peaked at 5 min and disappeared by 20 min after i.c.v. administration. Similarly, Bz-ATP (1–30 nmol/rat) dose-dependently and transiently elevated the mechanical nociceptive threshold (Fig. 1C). A significant elevation was observed at 5 and 10 min after i.c.v. administration of Bz-ATP at a dose of 30 nmol/rat (195 ± 5% and 143 ± 19% of control, respectively) compared with the group administered with vehicle. In contrast, i.c.v. administration of β,γ-methylene-ATP (1–30 nmol/rat) and UTP (10 and 100 nmol/rat) did not significantly elevate the mechanical nociceptive threshold (Fig. 1D,E).

3.2. Effect of i.c.v. administration of α,β-methylene-ATP on thermal nociceptive latency in the hot plate test and the tail flick test

In the hot plate test, i.c.v. administration of α,β-methylene-ATP (10 and 30 nmol/rat) produced a rapid and short-lasting prolongation of thermal nociceptive latency (Fig. 2A). A significant effect was observed at 5 min after

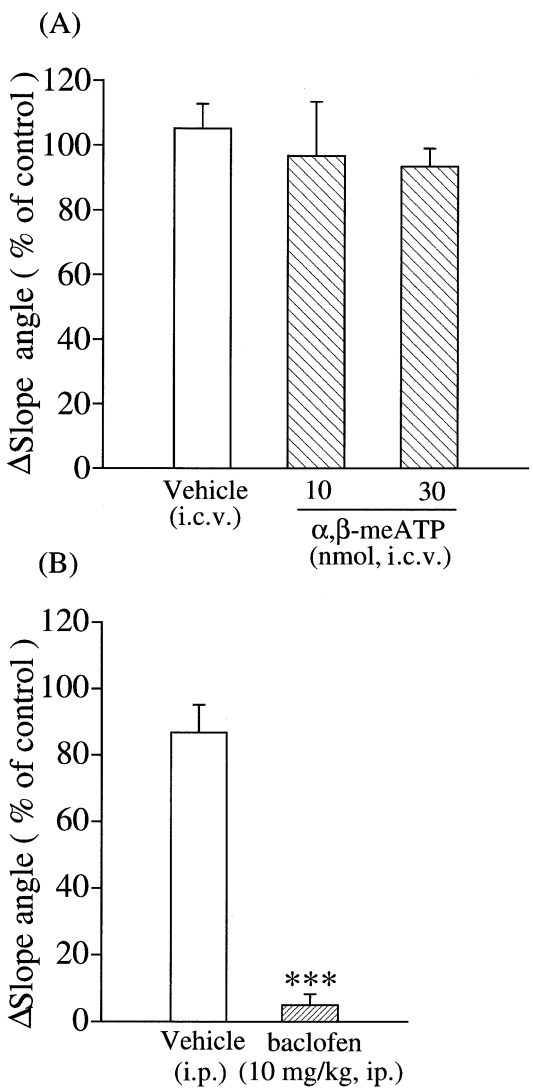

Fig. 3. The effects of i.c.v. administration of α,β-methylene-ATP (A) and baclofen (B) on the motor function in the inclined plane test. The test was carried out at 5 min after i.c.v. administration of α,β-methylene-ATP or vehicle, and at 30 min after i.p. administration of baclofen or vehicle. The values are presented as the means of Δslope angle ± S.E.M. ($n = 6–8$). $^{***}P < 0.001$ compared with the group administered intraperitoneally with vehicle (Mann–Whitney *U*-test).

i.c.v. administration of α,β-methylene-ATP at a dose of 30 nmol/rat (37.0 ± 7.1 s, $P < 0.05$) and at 10 min at doses of 10 and 30 nmol/rat (35.0 ± 5.1 s, $P < 0.05$ and 35.1 ± 7.1 s, $P < 0.05$, respectively) compared with the group administered with vehicle (17.2 ± 1.4 and 15.2 ± 1.6 s, respectively). In the tail flick test, only a high dose (30 nmol/rat) of α,β-methylene-ATP prolonged the latency (Fig. 2B). A significant effect was observed at 5 and 10 min after i.c.v. administration of α,β-methylene-ATP at a dose of 30 nmol/rat (13.0 ± 1.1 s, $P < 0.05$ and 12.3 ± 1.3 s, $P < 0.05$, respectively) compared with the group administered with vehicle (8.1 ± 0.5 s and 7.2 ± 0.5 s, respectively).

3.3. Effect of i.c.v. administration of α,β-methylene-ATP on motor function in the inclined plane test

To assess the effect of α,β-methylene-ATP on motor function, we carried out the inclined plane test (Fig. 3A). At 5 min after i.c.v. administration, a time point when significant effects of α,β-methylene-ATP on the nociceptive threshold and latency were observed, i.c.v. administration of α,β-methylene-ATP (10 and 30 nmol/rat) had no significant effects on the slope angle. However, intraperitoneal administration of baclofen (10 mg/kg), which is well known to have a central effect to cause muscle relaxation, significantly reduced the slope angle at 30 min after i.p. administration, indicating the appropriateness of the inclined plane test to assess motor function.

4. Discussion

The findings of the present study showed that i.c.v. administration of ATP to rats elevated the mechanical nociceptive threshold in the paw pressure test. A greater elevation was observed when α,β-methylene-ATP or Bz-ATP was administered i.c.v., whereas β,γ-methylene-ATP and UTP had no significant effects on mechanical nociception. I.c.v. administration of α,β-methylene-ATP at doses showing antinociceptive effects had no effects on motor function in the inclined plane test, indicating that the elevation of mechanical nociceptive threshold was likely due to an antinociceptive effect of α,β-methylene-ATP, rather than a deficit of motor function. ATP is rapidly metabolized in vivo by ecto-ATPase to adenosine which is reported to produce the antinociceptive effects both at spinal and supraspinal sites through P1 purinoceptors (Sawynok, 1998, 1999). These observations raised concerns that the antinociception produced by i.c.v. administration of ATP was due to the adenosine generated from ATP, but not due to ATP itself. However, the present finding that α,β-methylene-ATP, which is a stable ATP analogue resistant to ecto-ATPase metabolism, produced a stronger effect than ATP suggests that the antinociceptive effect of i.c.v. ATP was mediated through P2 purinoceptors (ATP receptors), but not through P1 purinoceptors (adenosine receptors). We tried to confirm the involvement of P2 purinoceptors in the antinociception by using the P2 purinoceptor antagonists, suramin and pyridoxal-phosphate-6-azophenyl-2′, 4′-disulphonate tetrasodium (PPADS). However, it was not possible to determine the effects of these antagonists on the antinociception produced by ATP analogues, because i.c.v. administration of suramin or PPADS at doses of 10 and 30 nmol/rat induced abnormal behaviors such as convulsion, which started immediately or soon after i.c.v. administration and continued for more than 1 h (unpublished observation).

P2 purinoceptors have been classified into two families, P2X and P2Y, and cDNA clonings for seven P2X subtypes and five P2Y subtypes are reported for mammalian systems (Williams and Jarvis, 2000). Since subtype-selective agonists or antagonists are not available for each subtype, the P2 receptor subtype(s) involved in the antinociceptive effect of i.c.v. ATP needs to be judged from the order of potency of several P2 purinoceptor agonists. In the present study, α,β-methylene-ATP and Bz-ATP were more effective than ATP. α,β-Methylene-ATP acts on P2X, but not P2Y, purinoceptors (Burnstock, 1990). Although Bz-ATP has been used as a $P2X_7$ selective agonist (Surprenant et al., 1996), it was recently shown to be a potent agonist not only for $P2X_7$ subtype, but also for several other subtypes, such as $P2X_1$ and $P2X_3$ purinoceptors (Bianchi et al., 1999). Furthermore, UTP, which is a potent agonist for several P2Y purinoceptors, did not show the antinociceptive effect. These findings suggest that the antinociceptive effects of ATP and its analogues are mediated by P2X, but not P2Y, purinoceptors.

α,β-Methylene-ATP was reported to be quite selective to $P2X_1$ and $P2X_3$ purinoceptors (Garcia-Guzman et al., 1997; Burnstock, 1997; Williams and Jarvis, 2000). As β,γ-methylene-ATP, which is considered to be selective for a $P2X_1$ purinoceptor (Trezise et al., 1995), did not show the antinociceptive effect, it is likely that the antinociceptive effects of ATP and its analogues are mediated by $P2X_3$ purinoceptors. This concept may also explain the rapid and short-lasting antinociceptive effects of ATP analogues, because the $P2X_3$ purinoceptor was reported to be rapidly desensitized after activation, while other purinoceptors, such as $P2X_2$, $P2X_4$, $P2X_5$, $P2X_6$ and $P2X_7$ purinoceptors, show sustained depolarizing currents (Williams and Jarvis, 2000).

Our results suggesting specific involvement of $P2X_3$ purinoceptors are difficult to reconcile with previous observations (Collo et al., 1996; Vulchanova et al., 1996; Kidd et al., 1998) that failed to detect mRNA and protein for $P2X_3$ or $P2X_1$ purinoceptors in the adult rat brain. Bo and Burnstock (1994) reported [^{3}H] α,β-methylene-ATP binding sites in widespread regions of the rat brain, suggesting the existence of a novel α,β-methylene-ATP sensi-

tive $P2X_3$-like purinoceptor in the brain. More recently, a $P2X_8$ purinoceptor was cloned from embryonic chick skeletal muscle (Bo et al., 2000); this was α,β-methylene-ATP sensitive and abundantly expressed in the chick brain. Thus, a novel $P2X_3$-like purinoceptor, such as chick $P2X_8$ purinoceptor, might be expressed in the rat brain and involved in the antinociceptive effects of ATP and its analogues.

In addition to the mechanical antinociceptive effect, i.c.v. administration of α,β-methylene-ATP showed a thermal antinociceptive effect. α,β-Methylene-ATP produced these antinociceptive effects probably by acting at supraspinal sites, since the intrathecal injection of α,β-methylene-ATP was reported to induce thermal hyperalgesia (Tsuda et al., 1999). At a spinal level, the activation of P2X purinoceptors has been suggested to evoke the release of glutamate from the terminals of primary sensory neurons (Gu and MacDermott, 1997), which leads to the generation of hyperalgesia through the activation of ionotropic glutamate receptors (Tsuda et al., 1999). However, the mechanism involved in the antinociceptive effect of ATP at a supraspinal level remains unclear. Descending noradrenergic and serotonergic systems are well known to play important roles in antinociception. ATP and its analogues have been shown to cause depolarization of locus coeruleus noradrenergic neurons (Fröhlich et al., 1996; Shen and North, 1993; Harms et al., 1992), and to increase extracellular serotonin levels in the rat hippocampus (Okada et al., 1999). In the present study, a high dose of α,β-methylene-ATP showed antinociception in the tail flick test. From those observations, it is suggested that the antinociceptive effects of ATP analogues, at least, at a high dose might be due to the activation of the descending noradrenergic and/or serotonergic systems. However, a lower dose of α,β-methylene-ATP exhibited antinociceptive effects in the paw pressure and hot plate tests, but not the tail flick test, suggesting that the descending noradrenergic and/or serotonergic systems were not involved in the effects of α,β-methylene-ATP at the lower dose. Further studies are necessary to elucidate the mechanism of the supraspinal antinociceptive effects of ATP and its analogues.

The present results showed supraspinal antinociceptive effects of ATP and its analogues, which were rapid and short-lasting, and probably mediated through P2X purinoceptors. Interestingly, the time course of the antinociceptive effect was very similar to that of α,β-methylene-ATP-induced nociceptive response or hyperalgesia which is mediated through peripheral or spinal P2X purinoceptors (Bland-Ward and Humphrey, 1997; Hamilton et al., 1999; Tsuda et al., 1999), while the directions of the effects are opposite to each other. The investigation of the receptor subtype(s) and the neural mechanism involved in the supraspinal antinociceptive effect of ATP may be useful for further understanding supraspinal mechanisms of pain transmission and modulation.

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