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Involvement of β_2-Adrenergic and μ-Opioid Receptors in Antinociception Produced by Intracerebroventricular Administration of α,β-Methylene-ATP

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ABSTRACT—The present study examined what kind of receptors are involved in the antinociception produced by intracerebroventricular (i.c.v.) administration of α,β-methylene-ATP using antagonists at adrenergic, serotonin or opioid receptors. Antinociceptive effect of α,β-methylene-ATP (10 nmol/rat) was significantly attenuated by subcutaneous pretreatment with propranolol and naloxone, but not phentolamine or methysergide, at a dose of 10 mg/kg. I.c.v. pretreatment with propranolol (100 nmol/rat), butoxamine (100 nmol/rat), ICI-118,551 (100 nmol/rat) and naloxone (30 nmol/rat) significantly attenuated the antinociceptive effect of α,β-methylene-ATP. However, i.c.v. pretreatment with atenolol (100 nmol/rat), naltrindole (30 nmol/rat) or nor-binaltorphimine (30 nmol/rat) did not show any significant effects. These results suggest that supraspinal β_2-adrenergic and μ-opioid receptors are involved in the antinociceptive effect of i.c.v. administered α,β-methylene-ATP.

Keywords: α,β-Methylene-ATP, Antinociception, β_2-Adrenoceptor, μ-Opioid receptor, Intracerebroventricular

Extracellular adenosine 5'-triphosphate (ATP) has been known as a neurotransmitter or neuromodulater in both the peripheral (1, 2) and central (3 – 5) nervous systems. P2 purinoceptors, receptors for ATP, are classified into two subfamilies, ionotropic P2X receptors and metabotropic P2Y receptors, on the basis of their structures and signal transduction systems (6). Up to now, cDNAs for seven subtypes of P2X receptors and six subtypes of P2Y receptors have been cloned as P2 purinoceptors expressed in mammalian cells (7, 8). Recent studies suggest the involvement of ATP and its receptors in peripheral and spinal nociceptive transmission (9, 10). It has been reported that $P2X_3$ purinoceptor mRNA is selectively expressed in capsaicin-sensitive, small diameter afferent neurons of the dorsal root ganglia, which are probably associated with nociception (11). Peripheral administration of ATP and α,β-methylene-ATP have been shown to cause nociceptive responses (12, 13). Furthermore, intrathecal administration of α,β-methylene-ATP produced thermal hyperalgesia, which was blocked by P2 purinoceptor antagonists (14, 15). These findings show that ATP facilitates pain transmission at peripheral and spinal sites, probably via the P2X purinoceptor.

At supraspinal sites, several studies have shown that ATP and its analogues induced fast synaptic currents in the cultured neurons derived from the hippocampus (16) and nucleus of the solitary tract (5) as well as in the slices from the rat medial habenula (17, 18). In addition, it has been reported that ATP modulates the release of some neurotransmitters, such as serotonin, noradrenaline and glutamate, in the central nervous system (19, 20). Thus, ATP is supposed to play various physiological roles in the supraspinal sites. We recently reported that intracerebroventricular (i.c.v.) administration of α,β-methylene-ATP produced antinociceptive effects on mechanical and thermal nociception (21).

It is well known that noradrenergic, serotonergic and opioidergic systems play important roles in pain modulation. In the present study, we examined the involvement of these neuronal systems in the antinociceptive effect of i.c.v. administered α,β-methylene-ATP, as a first step to determine the mechanism of its antinociceptive effect.

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MATERIALS AND METHODS

Animals

All experiments using male Sprague-Dawley rats weighing 220 – 280 g followed the ethical guidelines for investigations of experimental pain in conscious animals (22). Animals were kept at a constant ambient temperature (24 ± 1°C) under a 12-h light and dark cycle with free access to food and water.

Surgical procedure

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 atlas of Paxinos and Watson (23). After the surgery, the animals were returned to the cages and housed individually. They were allowed to recover for 5 to 7 days until the following procedure.

Drugs and drug administration

(±)-Atenolol, butoxamine hydrochloride, ICI-118,551 ((±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol) hydrochloride, α,β-methylene-ATP lithium salt, methysergide maleate, naloxone hydrochloride, phentolamine hydrochloride and DL-propranolol hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Naltrindole and nor-binaltorphimine (nor-BNI) were synthesized by Dr. H. Nagase (Toray Industries, Kamakura). These drugs were dissolved in 0.9% saline for subcutaneous (s.c.) injections or in phosphate-buffered saline (PBS) for i.c.v. injections. S.c. injections for pretreatment with receptor antagonists were carried out in a volume of 0.5 ml/250 g body weight at 30 min before the i.c.v. injection of α,β-methylene-ATP or its vehicle. Drugs were i.c.v. 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. I.c.v. injection was carried out in a volume of 5 μl at a constant rate of 5 μl/30 s. I.c.v. pretreatments with receptor antagonists were carried out at 15 min before the i.c.v. injection of α,β-methylene-ATP or its vehicle.

A subcutaneous dose of 10 mg/kg of phentolamine, methysergide, propranolol or naloxone was used as an α-adrenergic receptor antagonist, a 5-HT antagonist (24), a β-adrenergic receptor antagonist (25) or an opioid receptor antagonist (26), respectively. Furthermore, i.c.v. injections of propranolol (10 and 100 nmol/rat) as a β-adrenergic receptor antagonist (27), atenolol (100 nmol/rat) as a β_1-adrenergic receptor antagonist (28), butoxamine and ICI-118,551 (100 nmol/rat, each) as β_2-adrenergic receptor antagonists (27, 28), naloxone (10 and 30 nmol/rat) as an opioid receptor antagonist (29), naltrindole (30 nmol/rat) as a δ-opioid receptor antagonist (30) and nor-BNI (30 nmol/rat) as a κ-opioid receptor antagonist (31) were employed.

Measurement of nociceptive threshold

Mechanical nociceptive threshold was evaluated by the paw pressure test using an analgesimeter (Ugo Basile, Milan, Italy) with a cuneate piston after two days of habituation. The piston was put on the right hind paw and the pressure was loaded at a rate of 32 g/s. The pressure that elicited paw withdrawal behavior was determined as a nociceptive threshold. The procedures for the measurement were carried out three times per day for habituation. After two days of habituation, the threshold was measured following two additional habituation procedures, and the value was taken as a control. The control value of nociceptive threshold was 151 ± 5 g (n = 154). Immediately after measuring the control value, the s.c. or i.c.v. pretreatment with receptor antagonists was carried out. The nociceptive threshold was measured at 5, 10, 20, 30 and 60 min after i.c.v. injection of α,β-methylene-ATP.

Statistical analyses

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.

RESULTS

Effects of subcutaneous pretreatment with receptor antagonists

I.c.v. administration of α,β-methylene-ATP (a selective $P2X_1$ and $P2X_3$ agonist, 10 nmol/rat) at 30 min after s.c. pretreatment with saline produced a significant elevation of the mechanical nociceptive threshold (Fig. 1). The effect was rapid and short-lasting, which peaked at 5 min and disappeared by 30 min after i.c.v. administration. The nociceptive thresholds at 5 min after the i.c.v. administration were compared (Fig. 2). In the control group, s.c. pretreated with saline, i.c.v. administered α,β-methylene-ATP elevated the nociceptive threshold to 185 ± 10%. This antinociceptive effect of i.c.v. α,β-methylene-ATP was attenuated by s.c. pretreatments with propranolol and naloxone, but not phentolamine and methysergide, at a dose of 10 mg/kg: the nociceptive thresholds were significantly lowered by propranolol (124 ± 6%) and naloxone (144 ± 8%), but not phentolamine (162 ± 17%) and methysergide (193 ± 5%). In the groups i.c.v. administered with vehicle (PBS), instead of α,β-methylene-ATP, s.c. pretreatment with any receptor antagonists did not change the nociceptive thresholds.

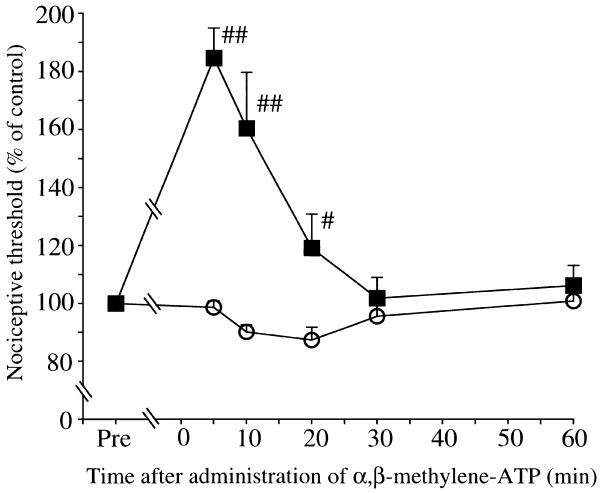

Fig. 1. Effects of i.c.v. administration of α,β-methylene-ATP on the mechanical nociceptive threshold in the paw pressure test. α,β-Methylene-ATP (10 nmol/rat) (squares) or PBS (circles) was i.c.v. administered 30 min after s.c. pretreatment with saline. The nociceptive threshold of each animal before 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). $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the group i.c.v. administered with vehicle (PBS).

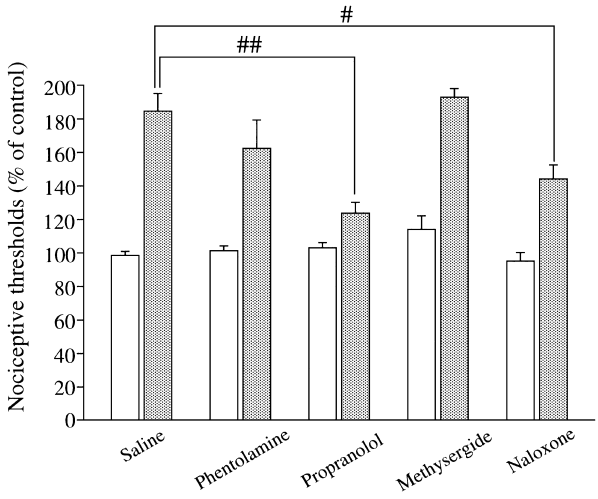

Fig. 2. Effects of s.c. pretreatment with receptor antagonists on the antinociception produced by i.c.v. administration of α,β-methylene-ATP. α,β-Methylene-ATP (10 nmol/rat) (closed bars) or PBS (open bars) was i.c.v. administered 30 min after the s.c. pretreatment with receptor antagonists (10 mg/kg). The nociceptive thresholds at 5 min after the i.c.v. administration of α,β-methylene-ATP were compared. 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). $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared with the group s.c. pretreated with vehicle (saline).

Effects of intracerebroventricular pretreatment with β-adrenergic receptor antagonists

I.c.v. pretreatment with propranolol (10 and 100 nmol /rat) dose-dependently attenuated the antinociceptive effect

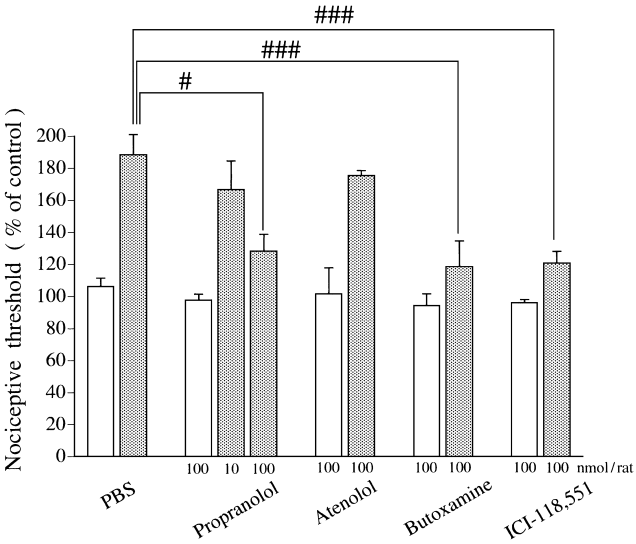

Fig. 3. Effects of i.c.v. pretreatment with β-adrenoceptor antagonists on the antinociception produced by i.c.v. administration of α,β-methylene-ATP. α,β-Methylene-ATP (10 nmol/rat) (closed bars) or PBS (open bars) was i.c.v. administered 15 min after i.c.v. pretreatment with β-adrenoceptor antagonists. The nociceptive thresholds at 5 min after i.c.v. administration of α,β-methylene-ATP were compared. The nociceptive threshold of each animal before the i.c.v. pretreatment 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 group i.c.v. pretreated with vehicle (PBS).

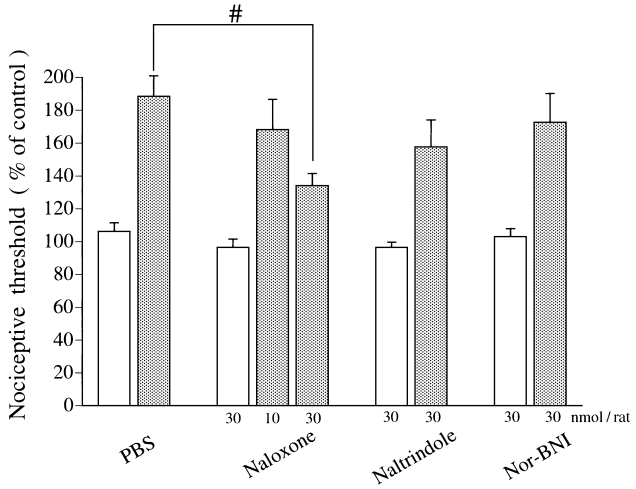

Fig. 4. Effects of i.c.v. pretreatment with opioid receptor antagonists on the antinociception produced by i.c.v. administration of α,β-methylene-ATP. α,β-Methylene-ATP (10 nmol/rat) (closed bars) or PBS (open bars) was i.c.v. administered 15 min after i.c.v. pretreatment with opioid receptor antagonists. The nociceptive thresholds at 5 min after i.c.v. administration of α,β-methylene-ATP were compared. The nociceptive threshold of each animal before the i.c.v. pretreatment served as the control value (100%). The values are presented as the means of the % of controls ± S.E.M. (n = 6 – 11). $^{\#}P<0.05$, compared with the group i.c.v. pretreated with vehicle (PBS).

of α,β-methylene-ATP (10 nmol/rat). I.c.v. pretreatment with 100 nmol of propranolol significantly lowered the nociceptive threshold (128 ± 10%) compared with the control group pretreated with PBS (Fig. 3). I.c.v. pretreatment with butoxamine and ICI-118,551, but not atenolol, at a dose of 100 nmol/rat significantly attenuated the antinociceptive effect of i.c.v. α,β-methylene-ATP (Fig. 3). Butoxamine and ICI-118,551 lowered the nociceptive threshold to 119 ± 7% and 121 ± 2%, respectively. In the groups i.c.v. administered with vehicle (PBS), instead of α,β-methylene-ATP, i.c.v. pretreatments with any β-adrenoceptor antagonists did not change the nociceptive thresholds.

Effects of intracerebroventricular pretreatment with opioid receptor antagonists

I.c.v. pretreatments with naloxone (10 and 30 nmol/rat) dose-dependently attenuated the antinociceptive effect of α,β-methylene-ATP (10 nmol/rat). I.c.v. pretreatment with 30 nmol of naloxone significantly lowered the nociceptive threshold (134 ± 7%) compared with the control group pretreated with PBS (Fig. 4). However, i.c.v. pretreatments with naltrindol and nor-BNI failed to attenuate the antinociceptive effect of α,β-methylene-ATP (Fig. 4). In the groups i.c.v. administered with vehicle (PBS), instead of α,β-methylene-ATP, i.c.v. pretreatments with any opioid receptor antagonists did not change the nociceptive thresholds.

DISCUSSION

In the present study, we investigated the involvement of adrenergic, serotonin and opioid receptors in the antinociceptive effect of α,β-methylene-ATP. I.c.v. administration of α,β-methylene-ATP dose-dependently (1 – 30 nmol/rat) produced antinociception with a submaximal effect at a dose of 10 nmol/rat (21). Thus, we examined the effects of pretreatment with receptor antagonists on the antinociception produced by i.c.v. injection of 10 nmol of α,β-methylene-ATP.

S.c. pretreatment with propranolol, but not phentolamine, significantly attenuated the antinociception by α,β-methylene-ATP, suggesting that the β-adrenergic receptors, but not α-adrenergic receptors, are involved in the antinociception by α,β-methylene-ATP. Although propranolol has been reported to have an affinity for some serotonin receptor subtypes (32), the involvement of serotonin receptors in the antinociception by α,β-methylene-ATP is unlikely because s.c. pretreatment with methysergide, a non-selective serotonin receptor antagonist, did not show any significant attenuation of antinociception by α,β-methylene-ATP. Significant attenuation of antinociceptive effect of α,β-methylene-ATP by naloxone indicates the involvement of opioid receptors.

Descending noradrenergic and serotonergic systems are well known to play important roles in antinociception. However, it is unlikely that these systems are involved in the antinociception produced by i.c.v. α,β-methylene-ATP, because our results showed that α-adrenergic receptors and serotonin receptors were not important for the antinociception produced by i.c.v. α,β-methylene-ATP, while these receptors have been reported to play crucial roles in the descending pain inhibitory system (33). Furthermore, our previous study showed that a higher dose of i.c.v. α,β-methylene-ATP was necessary to produce antinociception in the tail flick test in which spinal reflexes are concerned than in the paw pressure and hot plate tests in which integrative responses in the brain are concerned (21). These findings suggest that the antinociceptive effect of i.c.v. α,β-methylene-ATP is mediated via the supraspinal mechanism rather than the spinal one.

To determine the subtypes of β-adrenergic and opioid receptors involved in the antinociceptive effect of α,β-methylene-ATP at the supraspinal site, effects of i.c.v. pretreatment with subtype-selective antagonists on the antinociception produced by α,β-methylene-ATP were examined. I.c.v. pretreatment with propranolol dose-dependently attenuated the antinociceptive effect of i.c.v. α,β-methylene-ATP. I.c.v. pretreatment with 100 nmol/rat of propranolol significantly lowered the nociceptive threshold. I.c.v. pretreatment with butoxamine and ICI-118,551, but not atenolol, significantly attenuated the antinociception produced by α,β-methylene-ATP at a dose of 100 nmol/rat. These results suggest that β_2-, but not β_1-, adrenergic receptors are involved in the antinociceptive effect of α,β-methylene-ATP. Yao et al. (34) demonstrated widespread distribution patterns of $P2X_1$ and $P2X_3$ receptors throughout the rat hindbrain including the nucleus of the solitary tract, medial vestibular nucleus, medial and lateral parabrachial nuclei, rostral ventrolateral medulla and locus coeruleus; and these subtypes were shown to colocalize with tyrosine hydroxylase in A1, A2, A5, C1 and C2 regions. Furthermore, α,β-methylene-ATP has been reported to excite locus coeruleus neurons (35, 36). Thus, it is likely that i.c.v. administered α,β-methylene-ATP induces the release of noradrenaline and/or adrenaline, and then the released noradrenaline/adrenaline acts on supraspinal β_2-adrenergic receptors to produce antinociception.

I.c.v. pretreatment with naloxone, but not naltrindole and nor-BNI, attenuated the antinociceptive effect of i.c.v. α,β-methylene-ATP. Naloxone binds a μ-opioid receptor with higher affinity than δ- and κ-opioid receptors. Naltrindole and nor-BNI are subtype-selective agonists for δ- and κ-opioid receptors, respectively. Thus, μ-, rather than δ- and κ-, opioid receptors are probably involved in the antinociception produced by i.c.v. α,β-methylene-ATP. Although the mechanism for activation of endogenous opioid

system by α,β-methylene-ATP remains unclear, it is likely that α,β-methylene-ATP stimulates the supraspinal opioidergic neurons because of the widespread distribution of $P2X_1$ and $P2X_3$ receptors in the brain (34).

The present results show that supraspinal β_2-adrenergic receptors and μ-opioid receptors are involved in the antinociceptive effect of i.c.v. administered α,β-methylene-ATP. Since the effects of both β_2-adrenergic and μ-opioid receptor antagonists were partial, it should be examined whether these antagonists additively antagonize the antinociceptive effect of α,β-methylene-ATP or not. Further studies are necessary to elucidate the supraspinal mechanisms by which α,β-methylene-ATP produces antinociception.

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