Analgesic Effects of Intrathecal Administration of P2Y Nucleotide Receptor Agonists UTP and UDP in Normal and Neuropathic Pain Model Rats

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ABSTRACT
Recent electrophysiological, behavioral, and biochemical studies revealed that ATP plays a role in facilitating spinal pain transmission via ionotropic P2X nucleotide receptors, although the involvement of metabotropic P2Y nucleotide receptors remains unclear. In the present study, we examined the effects of i.t. administration of P2Y receptor agonists UTP, UDP, and related compounds on nociception in normal rats and tactile allodynia in a neuropathic pain model. In the paw pressure test using normal rats, i.t. administration of UTP (30 and 100 nmol/rat) and UDP (30 and 100 nmol/rat), but not UMP (100 nmol/rat) or uridine (100 nmol/rat), significantly elevated the mechanical nociceptive thresholds, whereas ATP (30 and 100 nmol/rat) and α,β-methylene-ATP (10 and 30 nmol/rat) lowered them. Similarly, in the tail-flick test, UTP (10, 30, and 100 nmol/rat) and UDP (100 nmol/rat) significantly prolonged the thermal nociceptive latency. In the von Frey filament test on normal rats, UTP (100 nmol/rat) and UDP (100 nmol/rat) produced no allodynia to the tactile stimulus, whereas ATP (100 nmol/rat) induced a significant and long-lasting tactile allodynia. In the neuropathic pain model, in which the sciatic nerves of rats were partially ligated, UTP (30 and 100 nmol/rat) and UDP (30 and 100 nmol/rat) produced significant antiallodynic effects. Furthermore, UTP (100 nmol/rat) and UDP (100 nmol/rat) caused no motor deficit in the inclined plane test. Taken together, these results suggest that the activation of UTP-sensitive P2Y2 and/or P2Y4 receptors and the UDP-sensitive P2Y6 receptor, in contrast to P2X receptors, produces inhibitory effects on spinal pain transmission.

The superficial dorsal horn of the spinal cord plays an important role in sensory transmission, including information on pain signaling from the periphery. A body of evidence has indicated the involvement of various neurotransmitters or neuromodulators released from primary afferent terminals, such as glutamate, substance P, and calcitonin gene-related peptide (Fürst, 1999). Recently, ATP has been also proposed as another neurotransmitter or neuromodulator for spinal pain signaling (Kennedy and Leff, 1995; Burnstock, 1996, 2001). Extracellular ATP acts on specific receptors, designated as P2X nucleotide receptors, at the cell surface. P2X nucleotide receptors are classified into two subfamilies, ligand-gated, ionotropic P2X receptors and G protein-coupled, metabotropic P2Y receptors, on the basis of their structures and signal transduction systems. Up to now, cDNAs for seven subtypes of P2X receptors (P2X1–7) and six subtypes of P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12) have been cloned as P2 nucleotide receptors expressed in mammalian cells (Ralevic and Burnstock, 1998; von Kugelgen and Wetter, 2000; Williams and Jarvis, 2000; Nicholas, 2001). P2X receptors have been well documented as targets of extracellular ATP for peripheral and central pain transmission. It was reported that mRNA of the P2X3 receptor in the dorsal root ganglia (DRG) is selectively expressed in capsaicin-sensitive small-diameter afferent neurons, which are probably associated with nociception (Chen et al., 1995). Furthermore, nociceptive, but not non-nociceptive sensory neurons had P2X3 immunoreactivity in their nerve terminal sites and cell bodies (Cook et al., 1997; Vulchanova et al., 1997), whereas several P2X receptors such as P2X2,4,6 were expressed in postsynaptic dorsal horn neurons (Lê et al., 1998; Burnstock, 2000, 2001). Llewellyn-Smith and Burnstock (1998) reported that P2X3 receptor-immunoreactive core terminals were postsynaptic to non-P2X3 receptor-immunoreactive vesicle-containing dendrites and axons in synaptic glomeruli in lamina II of dorsal horn. In electrophysiological studies, ATP and α,β-methylene-ATP, a P2X receptor agonist, evoked inward currents in capsaicin-sensitive small-diameter afferent neurons (Ueno et al., 1999) and spinal dorsal horn neurons (Bardoni et al., 1997; Li et al., 1998). Furthermore, in vivo studies showed that peripheral administration of ATP and α,β-methylene-ATP can cause nociceptive responses and antinociceptive effects on tactile and thermal stimuli.

ABBREVIATIONS: DRG, dorsal root ganglia; AUC, area under the curve.
nociceptive responses (Bland-Ward and Humphrey, 1997; Dowd et al., 1998; Tsuda et al., 2000) and facilitate formalin-induced responses (Sawynok and Reid, 1997). Intrathecal (i.t.) administration of α,β-methylene-ATP induced thermal hyperalgesia (Driessen et al., 1994; Tsuda et al., 1999) and tactile allodynia (Fukuhara et al., 2000), which were blocked by P2 nucleotide receptor antagonists. These observations strongly support the idea that ATP plays a crucial role in facilitating peripheral and spinal pain transmission via P2X receptors.

The involvement of the widely distributed P2Y receptors in pain transmission has not been well investigated. It has been reported that activation of the P2Y1 receptor contributed to the generation of sensory potential by innocuous somatic stimuli in a *Xenopus laevis* oocyte expression cloning system derived from DRG-derived mRNA (Nakamura and Strittmatter, 1996). Tominaga et al. (2001) reported that extracellular ATP potentiated the responsiveness of vanilloid receptor 1 via P2Y1 receptor in human embryonic kidney 293 cells expressing cloned vanilloid receptor 1 and rat primary sensory neurons. These findings suggest that P2Y receptors are also involved in the generation and/or modulation of pain signaling at peripheral terminals of sensory neurons.

However, little is known about the involvement of P2Y receptors in spinal pain transmission. Recently, we reported that ATP and UTP, a P2Y receptor agonist, inhibited the slow depolarization of substantia gelatinosa neurons evoked by repetitive stimulation of C-fibers of the dorsal root using adult rat spinal cord transverse slices with the dorsal root attached, suggesting that activation of P2Y receptors inhibited spinal pain transmission (Yoshida et al., 2002). In the present study, to address whether P2Y receptors are involved in spinal pain transmission in vivo, we examined the effects of i.t. administration of a P2Y2 and P2Y4 receptor agonist UTP, a P2Y8 receptor agonist UDP, and related compounds on mechanical and thermal nociception in normal rats. Furthermore, because neuropathic pain is associated with damage to peripheral nerves, and no pharmacotherapy has emerged as an optimal treatment (Coderre et al., 1993), we also examined the effects of i.t. administration of P2Y2 receptor agonists on sciatic nerve ligated-induced tactile allodynia in a rat model of neuropathic pain.

**Materials and Methods**

**Animals**

All experiments using male Sprague-Dawley rats weighing 180 to 250 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. They were allowed to acclimate for at least 1 day before use in experiments.

**Drugs and i.t. Administration**

ATP, α,β-methylene-ATP, UTP, UDP, UMP, and uridine were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in phosphate-buffered saline.

For i.t. administration, the skin of the back was incised along the spinous processes at the L2 to L5 level under ether anesthesia and then the wound was sutured. The next day, after extraction of the suture, drug or vehicle was intrathecally administered in a volume of 10 μl to the freely moving rat through a lumbar puncture between L4 and L5 using a 25-gauge stainless steel needle attached to a glass microsyringe. A quick flick of the rat’s tail confirmed the accurate intrathecal position of the needle.

**Behavioral Tests**

**Paw Pressure Test.** The nociceptive threshold of the hind paw to mechanical stimulus was evaluated by paw pressure test using an analgesimeter (Ugo Basile, Milan, Italy) with a cuneate piston. The piston was placed on the ventral surface of the hind paw and the pressure was loaded at a rate of 32 g/s. The pressure that elicited paw withdrawal behavior was determined as the nociceptive threshold. The mechanical nociceptive threshold in untreated (control) rats was 142 ± 0.09 g (n = 102).

**Tail-Flick Test.** For assessing nociceptive responses to thermal stimulation, a commercially available light beam analgesia meter was used (Ugo Basile). The tail was positioned so that the radiant heat was focused onto the dorsal surface of the tail at 5 to 6 cm proximal to the tip. The latency until the animal moved its tail from stimulation was recorded automatically. The cutoff time was 15 s to prevent tissue damage after exposure to the stimulation. The thermal nociceptive latency in control rats was 7.1 ± 0.27 s (n = 58).

**von Frey Filament Test.** Tactile allodynia was measured using calibrated von Frey filaments (North Coast Medical, Morgan Hill, CA), as described previously (Yakshe, 1989). Briefly, for testing, animals were individually placed on a wire mesh floor. Cages were mounted in a position that allowed the experimenter access to the bottom of the cage. The tactile stimulus was applied to the middle plantar of the right paw by placing the von Frey filament (0.60 g) perpendicular to the surface of the paw. The von Frey filament was held in this position with enough force to cause a slight bend. The response of animals was graded with a score of 0 for no response; 1 for moderate effort to avoid the probe, such as licking the stimulated paw, and transient vocalization; and 2 for vigorous effort to escape the stimulus, such as jumping, shaking the paw, biting at the probe or the stimulated paw, and frequent and sustained vocalization in response to the probe. One trial involved 10 applications of filaments every 3 or 4 s, each of which was scored as 0, 1, or 2. The trial was evaluated based on a total score of 0 to 20 at culmination.

**Inclined Plane Test.** The inclined plane test was carried out using a sliding apparatus (Medical Agent Co. Ltd., Kyoto, Japan), as described previously (Fukui et al., 2001). Each rat was placed on a 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. Control animal began to slip down at 40.0 ± 0.8° (n = 18). The Δslope angle for each animal was calculated using 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 (%). We previously reported that intraperitoneal administration of baclofen, which has a central effect to cause muscle relaxation, significantly reduced the slope angle, indicating the appropriateness of this procedure (Fukui et al., 2001).

The rats were habituated to the procedure for behavioral testing three times per day. After 2 days of habituation, the threshold, latency, or score was measured after two additional habituation procedures, and the value was taken as a control. Soon after measuring the control value, drug or vehicle was administered intrathecally, and the value was measured at a given time after the i.t. administration.

**Neuropathic Pain Model**

The right sciatic nerve was partially ligated according to the method of Seltzer et al. (1990). Under ether anesthesia, a skin incision was made and the right sciatic nerve was exposed just distal to the branch leading to the posterior biceps femoris/semitendinosus muscles. A tight ligature was made around the dorsal half of the sciatic nerve using 7-0 silk such that one-half to one-third of the sciatic nerve was ligated. The wound was then closed. After recov-
ering from the anesthesia, almost all animals showed guarding of the hind paw, but none presented autotomy. In sham-operated rats, the sciatic nerve was exposed without ligation. Just before the ligation and 7 to 9 days after the surgery, the tactile sensitivity was assessed with von Frey filaments similar to the approach described above for confirmation of allodynia, and rats with an ipsilateral score of more than 10 were regarded as successful models.

**Statistical Analysis**

The mechanical nociceptive threshold in the paw pressure test, the latency in the tail-flick test and the slope angle in the inclined plane test at each time point were presented as means of the percentage of the control ± S.E.M. The scores for the von Frey filament test are presented as the mean ± S.E.M. Statistical analyses were performed by the two-way analysis of variance, Tukey-Kramer multiple comparison test after a one-way analysis of variance, or Dunn’s multiple comparison test after the Kruskal-Wallis test. Differences at \( p < 0.05 \) were considered significant.

**Results**

**Paw Pressure Test.** The effects of i.t. administration of UTP, UDP, and its metabolites, as well as ATP and \( \alpha,\beta \)-methylene-ATP, on the mechanical nociceptive threshold for the paw pressure stimulus were examined in normal rats (Fig. 1). Intrathecal administration of vehicle did not alter the threshold. Intrathecal administration of ATP (1–100 nmol/rat) decreased the mechanical nociceptive threshold (Fig. 1A). Significant dose \( [F(4,175) = 11.87, p < 0.0001] \) and time effects \( [F(6,175) = 7.48, p < 0.0001] \) were observed as well as a significant dose × time interaction \( [F(24,175) = 4.17, p < 0.0001] \). The mechanical hyperalgesic effect of ATP was apparently rapid and short-lasting, peaking at 5 to 10 min and disappearing within 30 min of the administration. The dose-response curve of ATP on the mechanical nociceptive threshold is shown in Fig. 1F, where the data are presented as AUC values from 0 to 30 min. ATP at a dose of 100 nmol/rat significantly decreased the AUC values to 79.7 ± 3.0% of control, compared with the group administered with vehicle (\( p < 0.001 \)) and ATP at doses of 1 nmol/rat (\( p < 0.001 \)), 10 nmol/rat (\( p < 0.01 \)), and 30 nmol/rat (\( p < 0.05 \)). Furthermore, ATP at a dose of 30 nmol/rat significantly decreased the AUC values to 89.8 ± 2.8% of control, compared with the group administered with vehicle (\( p < 0.05 \)) and ATP at a dose of 1 nmol/rat (\( p < 0.05 \)).

Similarly, i.t. administration of a P2X1 and P2X3 receptor agonist \( \alpha,\beta \)-methylene-ATP (1–30 nmol/rat) also decreased the mechanical nociceptive threshold, apparently with a time course similar to that for ATP (Fig. 1B). Significant dose \( [F(3,140) = 23.54, p < 0.0001] \) and time effects \( [F(6,140) = 21.16, p < 0.0001] \) were observed as well as a significant dose × time interaction \( [F(18,140) = 6.76, p < 0.0001] \). There was a dose-response effect of \( \alpha,\beta \)-methylene-ATP on the mechanical nociceptive threshold (Fig. 1F). \( \alpha,\beta \)-Methylene-ATP at doses of 10 and 30 nmol/rat significantly decreased the AUC values to 82.4 ± 1.8% (\( p < 0.001 \) versus vehicle and \( p < 0.05 \) versus 1 nmol/rat) and 80.4 ± 3.1% of control (\( p < 0.001 \) versus vehicle and \( p < 0.01 \) versus 1 nmol/rat), respectively.

In contrast, i.t. administration of a P2Y2 receptor agonist UTP (1–100 nmol/rat) produced an elevation in the mechanical nociceptive threshold (Fig. 1C). Significant dose \( [F(4,175) = 26.11, p < 0.0001] \) and time effects \( [F(6,175) = 21.47, p < 0.0001] \) were observed as well as a significant dose × time interaction \( [F(24,175) = 5.12, p < 0.0001] \). The antinociceptive effect of UTP apparently peaked at 5 to 10 min and had disappeared 45 min after the administration. There was a dose-response effect of UTP (Fig. 1F). UTP at doses of 10, 30, and 100 nmol/rat significantly elevated the AUC values to 113.5 ± 3.9% (\( p < 0.05 \) versus vehicle), 116.7 ± 3.4% (\( p < 0.01 \) versus vehicle), and 139.2 ± 1.9% of control (\( p < 0.001 \) versus vehicle, 1, 10, and 30 nmol/rat), respectively.

Similarly, a P2Y2 receptor agonist UDP (10–100 nmol/rat) also elevated the mechanical nociceptive threshold, apparently the time course being similar to that for UTP treatment (Fig. 1D). Significant dose \( [F(3,140) = 75.19, p < 0.0001] \) and time effects \( [F(6,140) = 34.99, p < 0.0001] \) were observed as well as a significant dose × time interaction \( [F(18,140) = 13.75, p < 0.0001] \). There was a dose-response effect of UDP (Fig. 1F). UDP at doses of 30 and 100 nmol/rat significantly elevated the AUC values to 111.1 ± 3.0% (\( p < 0.01 \) versus vehicle and \( p < 0.05 \) versus 10 nmol/rat) and 133.7 ± 2.0% of control (\( p < 0.001 \) versus vehicle, 10 and 30 nmol/rat), respectively. However, UMP (100 nmol/rat) and uridine (100 nmol/rat) had no significant effect on the mechanical nociceptive threshold (Fig. 1, E and F).

**Tail-Flick Test.** The effect of i.t. administration of UTP and UDP on the thermal nociceptive latency was examined in the tail-flick test using normal rats (Fig. 2). Intrathecal administration of vehicle did not alter the thermal nociceptive latency. Intrathecal administration of UDP (1–100 nmol/rat) prolonged the latency (Fig. 2A). Significant dose \( [F(4,175) = 6.9, p < 0.0001] \) and time effects \( [F(6,175) = 4.96, p < 0.0001] \) were observed as well as a significant dose × time interaction \( [F(24,175) = 2.75, p < 0.0001] \). The antinociceptive effect of UTP apparently peaked at 5 to 10 min and disappeared with 45 min of the administration. The dose-response curve of UTP on the thermal nociceptive latency is shown in Fig. 2C, where the data are presented as AUC values from 0 to 15 min. UTP at doses of 30 and 100 nmol/rat significantly prolonged the AUC values to 139.7 ± 7.1% (\( p < 0.01 \) versus vehicle and \( p < 0.05 \) versus 1 nmol/rat) and 143.6 ± 7.1% of control (\( p < 0.01 \) versus vehicle and 1 nmol/rat), respectively.

Similarly, UDP (1–100 nmol/rat) also prolonged the latency, and the time course was apparently similar to that of UTP (Fig. 2B). Significant dose \( [F(4,175) = 4.97, p = 0.0008] \) and time effects \( [F(6,175) = 8.82, p < 0.0001] \) were observed as well as a significant dose × time interaction \( [F(24,175) = 4.18, p < 0.0001] \). There was a dose-response effect of UDP on the thermal nociceptive latency (Fig. 2C). UDP at a dose of 100 nmol/rat significantly elevated the AUC values to 136.8 ± 5.2% (\( p < 0.05 \) versus vehicle and 1 nmol/rat).

**von Frey Filament Test in Normal Rats.** The effects of i.t. administration of UTP, UDP, and ATP on the allodynia to the tactile stimulus applied to the skin of the hind paw with von Frey filaments (0.60 g) in normal rats were examined (Fig. 3). Intrathecal administration of vehicle did not change the allodynic scores of the von Frey filament test. Intrathecal administration of ATP (100 nmol/rat) significantly increased the allodynic scores compared with the group administered vehicle. The tactile allodynia induced by ATP developed gradually, apparently reached a maximum at 45 min after the administration (15.3 ± 1.8), and lasted for up to 120 min.

On the other hand, UTP (100 nmol/rat) and UDP (100 nmol/rat) had no effect on the allodynic scores for the von Frey filament test.
Fig. 1. Effects of i.t. administration of ATP (A), α,β-methylene-ATP (B), UTP (C), UDP (D), UMP and uridine (E) on the mechanical nociceptive threshold of normal rats in the paw pressure test. Drugs were administered intrathecally at time 0. The mechanical nociceptive threshold of each animal before the i.t. administration served as the control value (100%). F, magnitudes of the effects of ATP, α,β-methylene-ATP, UTP, UDP, UMP, and uridine are presented as the AUC values from 0 to 30 min after i.t. administration. The AUC value of vehicle-treated group was assigned as a value of 100%. These values are presented as the mean ± S.E.M. (n = 6). *p < 0.05; **p < 0.01; ***p < 0.001 compared with the vehicle-treated group; †p < 0.05; ††p < 0.01; †††p < 0.001 compared with the each drugs at a dose of 1 nmol-treated group; ‡p < 0.05; ‡‡p < 0.01; ‡‡‡p < 0.001 compared with the each drugs at a dose of 10 nmol-treated group; †††p < 0.001 compared with the each drugs at a dose of 30 nmol-treated group (Tukey-Kramer multiple comparison test).
von Frey Filament Test in the Neuropathic Pain Model. The effects of i.t. administration of UTP and UDP on the tactile allodynia induced by ligation of the sciatic nerve were examined in a rat model of neuropathic pain (Fig. 4). Before surgery, the rats showed no response to the tactile stimulus applied to the skin of the hind paw with von Frey filaments (0.60 g). After ligation, the allodynic scores of the ipsilateral paw for the same tactile stimulus were increased to 15.0 (± 0.4), (n = 29), which is regarded as the presentation of neuropathic pain, whereas the sham-operated rats showed no significant change in the scores (0.53 ± 0.12, n = 32). Intrathecal administration of UTP at 30 and 100 nmol/rat in the neuropathic pain model rats significantly reduced the extent of the tactile allodynia. The antiallodynic effect of UTP apparently peaked at 5 to 10 min and had disappeared 45 min after the administration. The apparent maximal effect of UTP at 100 nmol/rat was obtained at 10 min (2.2 (± 0.7), p < 0.01), compared with the group administered vehicle (17.0 ± 1.0). On the other hand, i.t. administration of UTP at a dose of 100 nmol/rat to the sham-operated rats had no significant effects on the allodynic scores. Similarly, UDP at 30 and 100 nmol/rat transiently and significantly suppressed the allodynia induced by ligation of the sciatic nerve, and the time course of the effect resembled that for UTP treatment. The apparent maximal effect of UDP at 100 nmol/rat was obtained at 10 min (1.0 ± 0.7, p < 0.01). On the other hand, UDP at 100 nmol/rat had no significant effect on the alldyinic scores in the sham-operated rats.

Fig. 2. Effects of i.t. administration of UTP (A) and UDP (B) on the thermal nociceptive latency of normal rats in the tail-flick test. Drugs were administered intrathecally at time 0. The thermal nociceptive latency of each animal before the i.t. administration served as the control value (100%). C, magnitudes of the effects of UTP and UDP are presented as the AUC values from 0 to 15 min after i.t. administration. The AUC value of vehicle-treated group was assigned as a value of 100%. These values are presented as the mean ± S.E.M. (n = 5–6). *p < 0.05; **p < 0.01 compared with the vehicle-treated group; †p < 0.05; ††p < 0.01 compared with each drugs at a dose of 1 nmol-treated group (Tukey-Kramer multiple comparison test).
Inclined Plane Test. To assess the effects of UTP and UDP on motor function, we carried out the inclined plane test in normal rats. Intrathecal administration of vehicle did not alter the slope angle. Intrathecal administration of UTP (100 nmol/rat) and UDP (100 nmol/rat), which showed significant analgesic effects, had no effect on the slope angle (Fig. 5).

**Discussion**

Electrophysiological, behavioral, and biochemical studies suggest that ATP plays a role in facilitating pain transmission from peripheral sites to the spinal cord via ionotropic P2X receptors (Burnstock, 2000, 2001). It has been reported that α,β-methylene-ATP caused depolarization of small-diameter, capsaicin-sensitive sensory afferent neurons (Ueno et al., 1999), and that ATP facilitated glutamate release from sensory afferent neurons via presynaptic P2X receptors (Gu and MacDermott, 1997), suggesting that P2X receptors, located presynaptically on primary sensory afferent terminals, have the potential to facilitate pain transmission. Furthermore, at the spinal sites, in vivo studies have shown that i.t. administration of α,β-methylene-ATP, a potent and selective agonist for P2X₁ and P2X₃ receptors, induced thermal hyperalgesia (Driessen et al., 1994; Tsuda et al., 1999). The present study also showed that i.t. administration of ATP and α,β-methylene-ATP to normal rats dose dependently decreased the mechanical nociceptive threshold in the paw pressure test, which was rapid and short-lasting. It has been reported that the thermal hyperalgesia induced by α,β-methylene-ATP was antagonized by a P2 nucleotide receptor antagonist, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonate, and a selective P2X₁, P2X₃, and heteromultimeric P2X₂/₃ receptor antagonist, 2′,3′-O-(2,4,6-trinitrophenyl) adenosine 5′-triphosphate, and that i.t. administration of β,γ-methylene-1-ATP, a potent agonist for the P2X₁ receptor, failed to produce a thermal hyperalgesia (Tsuda et al., 1999). Furthermore, immunohistochemical studies have revealed that P2X₃ receptors in the spinal cord are localized in the central presynaptic terminals of primary sensory neurons, but not in the cell bodies of superficial dorsal horn neurons (Cook et al., 1997; Vulchanova et al., 1997;
tors, and that of UDP via the P2Y6 receptor. However, we have cloned receptors, namely, P2Y1,2,4,6,11,12, which produce these antinociceptive effects. Indeed, we found that (Zimmermann, 1996), it is possible that metabolites of UTP are metabolized by ectonucleotidases and other ectoenzymes in the tail-flick test. Because extracellular UTP is readily metabolized by nucleotidases and other enzymes (Zimmerman, 1996), it is possible that metabolites of UTP produce these antinociceptive effects. In the present study, we found that i.t. administration of a P2Y agonist urea to normal rats dose dependently elevated the mechanical nociceptive threshold in the paw pressure test, and prolonged the thermal nociceptive latency in the tail-flick test. Because extracellular UTP is readily metabolized by ectonucleotidases and other enzymes (Zimmerman, 1996), it is possible that metabolites of UTP produce these antinociceptive effects. Indeed, we found that i.t. administration of UDP, a metabolite of UTP, also elevated the mechanical nociceptive threshold, whereas UMP and uridine acts on P2 nucleotide receptors on the central presynaptic terminals of sensory afferent neurons.

In the present study, we found that i.t. administration of a P2Y agonist urea to normal rats dose dependently elevated the mechanical nociceptive threshold in the paw pressure test, and prolonged the thermal nociceptive latency in the tail-flick test. Because extracellular UTP is readily metabolized by ectonucleotidases and other enzymes (Zimmerman, 1996), it is possible that metabolites of UTP produce these antinociceptive effects. In the present study, we found that i.t. administration of UDP, a metabolite of UTP, also elevated the mechanical nociceptive threshold, whereas UMP and uridine acts on P2 nucleotide receptors on the central presynaptic terminals of sensory afferent neurons. Furthermore, we showed that i.t. administration of UDP, a metabolite of UTP, also elevated the mechanical nociceptive threshold, whereas UMP and uridine acts on P2 nucleotide receptors on the central presynaptic terminals of sensory afferent neurons.

Furthermore, we showed that i.t. administration of UTP and UDP at a dose showing maximum antinociceptive effects (100 nmol/rat) did not affect motor function in the inclined plane test, indicating that the elevation of mechanical nociceptive threshold and prolongation of the thermal nociceptive latency were likely due to antinociceptive effects of UTP and UDP, rather than deficits of motor function. Taken together, i.t. administration of UTP and UDP had antinociceptive effects on acute mechanical and thermal nociception in normal rats via P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors, whereas ATP and $\alpha$-methyleno-ATP lead to the induction of mechanical hyperalgesia, maybe via the P2X$_r$ receptor, as well as thermal hyperalgesia as reported previously (Driessen et al., 1994; Tsuda et al., 1999).

It has been reported that intraplantar and i.t. administrations of $\alpha$-methyleno-ATP induced tactile allodynia, a condition characterized by nociceptive behavioral responses to normally innocuous stimuli conducted by A$\beta$ fibers (Fukuhara et al., 2000; Tsuda et al., 2000). In the present study, we showed that i.t. administration of ATP, but not UTP or UDP, also induced tactile allodynia in the von Frey filament test. The tactile allodynia induced by ATP developed gradually and lasted for up to 120 min after the administration, whereas the hyperalgesic effects of ATP and $\alpha$-methyleno-ATP were rapid and short-lasting. Furthermore, we observed that the ATP-induced tactile allodynia remained even 2 days after the administration (data not shown). These findings suggest that the mechanism of spinal ATP-induced tactile allodynia was different from that of spinal ATP-induced hyperalgesia. Because the $\alpha$-methyleno-ATP-induced tactile allodynia was reported to be blocked by pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (Fukuhara et al., 2000), it is surmised that the ATP-induced tactile allodynia may also be mediated via P2X receptors in the spinal cord. At least, UTP-sensitive P2Y$_2$ and P2Y$_4$ receptors and UDP-sensitive P2Y$_6$ receptors do not contribute to the induction of tactile allodynia. On the other hand, it has been reported that systemic administration of P2 nucleotide receptor antagonists produced antiallodynic effects in rat models of neuropathic pain (Park et al., 2000) and postoperative pain (Tsuda et al., 2001). Furthermore, a recent study has shown that the expression of the P2X$_r$ receptor in DRG neurons and superficial laminae of the spinal cord is up-regulated in the rat neuropathic pain model by chronic constriction of the sciatic nerve (Novakovic et al., 1999). Taken together, these findings support the hypothesis that endogenous ATP in the spinal cord plays a role in the development and/or maintenance of neuropathic pain, probably via P2X receptors.

Finally, we found that i.t. administration of UTP and UDP suppressed the tactile allodynia induced by ligation of the sciatic nerve. The antiallodynic effects of UTP and UDP showed similar time courses to their thermal and mechanical antinociceptive effects. These findings suggest that the activation of spinal P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors could reduce neuropathic pain, in contrast to P2X receptors.

A few studies have suggested that P2Y receptors contribute to pain transmission (Nakamura and Strittmatter, 1996; Tominaga et al., 1997). However, these studies examined the involvement of P2Y$_2$ receptors, particularly the P2Y$_2$ receptor, in facilitating the pain transmission at the peripheral sites. On the other hand, the present study indicated that i.t. administrations of ATP and UDP, which do not act on the P2Y$_2$ receptor, produced analgesic effects, probably via spinal UTP-sensitive P2Y$_2$ and/or P2Y$_4$ receptors and UDP-sensitive P2Y$_6$ receptor. Supporting the present findings, we recently reported that ATP and UTP, but not $\alpha$-methyleno-ATP, inhibited a slow depolarization of substantia gelatmosa neurons relevant to spinal nociception by activation of spinal suramin-insensitive P2Y$_2$ receptors (Yoshida et al., 2002). Furthermore, our preliminary experiments with a reverse
transcription-polymerase chain reaction-based method showed that P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptor mRNAs were expressed in the spinal cord, and the P2Y$_2$ and P2Y$_6$ receptor mRNAs were expressed in DRG. These findings suggest that these P2Y receptors expressed in pre- and/or postsynaptic sites might be involved in the signaling pathway through nucleotide receptors in the spinal cord, as well as previous articles about P2X$_3$ receptor (Llewellyn-Smith and Burnstock, 1998). Additional investigations are needed to elucidate the inhibitory role of P2Y receptors in the spinal pain transmission.

Koizumi and Inoue (1997) have indicated that activation of suramin-insensitive G protein-coupled P2Y receptors attenuated calcium oscillations by inhibiting glutamate release from the presynaptic terminal in cultured rat hippocampal neurons. Recently, it was reported that ATP facilitates inhibitory GABAergic and glycinegic transmission at dorsal horn interneuron synapses (Hugel and Schlichter, 2000; Rhee et al., 2000), although these inhibitory effects are postulated to be mediated via P2X receptors. These findings suggest that P2 nucleotide receptors also play an inhibitory role in neurotransmission, which is consistent with the present findings.

It was reported that P2Y receptors are expressed in astrocytes (Fam et al., 2000) and that UTP increased the intracellular Ca$^{2+}$ concentration in >99% of dorsal horn astrocytes via P2Y receptors (Idestrup and Salter, 1998). Because these signals may cause neighboring neurons to modulate the transmission of nociceptive information (Fam et al., 2000), it is also possible that the analgesic effects of UTP and UDP are due to the effects on glial cells.

At the supraspinal level, however, we previously reported that intracerebroventricular administration of UTP (100 nmol) had no effect on the mechanical nociceptive threshold (Fukui et al., 2001), indicating that the analgesic effects of UTP and UDP might be attributed to the activation of spinal, but not supraspinal, P2Y receptors.

In summary, the present data showed that UTP and UDP had mechanical and thermal antinociceptive effects in normal rats and antiallodynic effects in a neuropathic pain model, probably via spinal P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors, whereas ATP and $\alpha$-$\beta$-methylene-ATP produced mechanical hyperalgesia and tactile allodynia via P2X receptors. These results support that P2Y receptor agonists may have potential in the development of a new class of analgesics.