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Adenosine 5′-triphosphate inhibits slow depolarization induced by repetitive dorsal root stimulation via P2Y purinoceptors in substantia gelatinosa neurons of the adult rat spinal cord slices with the dorsal root attached

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Abstract

We previously reported that slow depolarization of substantia gelatinosa neurons is evoked by repetitive stimulation of C-fibers of dorsal root in adult rat spinal cord transverse slices with the dorsal root attached, which was considered to be an event relevant to spinal nociception. In the present study, we investigated the effects of adenosine 5′-triphosphate (ATP) and its analogs on the slow depolarization. ATP (10–100 μM) significantly inhibited the amplitude of slow depolarization in a concentration-dependent manner. The inhibitory effect of ATP was not reversed by suramin, an antagonist for some P2-purinoceptors, and was mimicked by a P2Y selective agonist uridine 5′-triphosphate, but not a P2X selective agonist α,β-methylene ATP. These results suggest that ATP inhibits the slow depolarization of substantia gelatinosa neurons relevant to nociceptive transmission in the spinal dorsal horn, via suramin-insensitive P2Y purinoceptors.

Keywords: Adenosine 5′-triphosphate; Substantia gelatinosa; Slow depolarization; Spinal dorsal horn; Uridine 5′-triphosphate; P2Y purinoceptor; Nociceptive transmission; Pain

Nociceptive information from the periphery is carried by primary afferent neurons to the spinal dorsal horn, and is transmitted to the secondary sensory neuron projecting to the upper CNS. Many studies have examined the mechanisms of nociceptive transmission in the superficial dorsal horn of the spinal cord, and reported the involvement of various neurotransmitters or neuromodulators released from primary afferent terminals, such as glutamate, substance P and calcitonin gene related peptide (CGRP) [6]. Recently, adenosine 5′-triphosphate (ATP) was also proposed as another neurotransmitter or neuromodulator for nociception [1,2]. In the spinal dorsal horn, several ionotropic P2X purinoceptors such as $P2X_{2,4,6}$ are expressed in presynaptic terminals of sensory neurons and postsynaptic dorsal horn neurons [2,13], while $P2X_3$ purinoceptors are largely located presynaptically on sensory nerve terminals in lamina II [2,15]. In electrophysiological studies, ATP exhibited excitatory actions on the spinal dorsal horn neurons both in vitro [10,14] and in vivo [18]. Recent studies have shown that approximately half of the cultured spinal dorsal horn neurons used ATP as a fast excitatory neurotransmitter acting at postsynaptic P2X purinoceptors [11]. Furthermore, in vivo studies have shown that intrathecal administration of α,β-methylene-ATP, a P2X purinoceptor agonist, induced thermal hyperalgesia and allodynia, which were blocked by P2 purinoceptor antagonists [5,19]. These findings strongly suggest that ATP plays a crucial role in facilitating spinal nociceptive transmission via the P2X purinoceptors [1,2], although the involvement of G-protein coupled P2Y purinoceptors remains unclear.

On the other hand, substantia gelatinosa neurons in the dorsal horn (lamina II) are known to play important roles in the modulation of spinal nociceptive transmission. We previously reported that repetitive stimulation of the dorsal

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root with an intensity adequate to activate C-fibers, but not A-fibers, induced slow depolarization of substantia gelatinosa neurons in adult rat spinal cord transverse slices with dorsal root attached [16]. The amplitude of slow depolarization was attenuated by [D-Ala^2, N-Me-Phe^4, Gly-ol^5]-enkephalin, a selective μ-opioid receptor agonist, suggesting that this phenomenon is a pain-related excitatory event. In the present study, to examine the roles of ATP on the spinal nociceptive transmission, we investigated the effects of ATP and its analogs on the slow depolarization.

The methods for obtaining adult rat spinal cord transverse slices with dorsal root attached and for patch-clamp recordings from substantia gelatinosa neurons were as described previously [16,21]. In brief, male adult Sprague–Dawley rats (160–260 g) were anesthetized with urethane (1 g/kg, i.p.) and the lumbosacral spinal cords (L1–S3) were removed. In artificial cerebrospinal fluid (aCSF) at 4°C (composition (mM): 117 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25.0 $NaHCO_3$, 11.5 glucose, equilibrated continuously with 95% O_2–5% CO_2), all ventral and dorsal roots except L5 or L6 on one side were cut and the pia-arachnoid membrane was removed. Transverse slices with dorsal root attached (thickness of 500–550 μm) were prepared with a vibratome and were superfused in the recording chamber with 36 ± 1°C aCSF (20 ml/min) for 1 h. Recordings were carried out at a rate of 12 ml/min. Blind whole cell patch clamp recordings were performed from substantia gelatinosa neurons in current clamp mode. The recording pipette was made of a fiber-filled glass and filled with a solution of the following composition (mM): 135 potassium gluconate, 5 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, 5 HEPES, 5 Mg-ATP. The pH and osmolarity of the solution were adjusted to 7.1 and 285 mOsm, respectively.

The induction of slow depolarization was performed as previously described [16]. To distinguish slow depolarization from the summation of the excitatory postsynaptic potentials (EPSPs) mediated by non-NMDA receptors, all experiments were performed in the presence of 6-cyano-7-quinoxaline-2,3-dione disodium (CNQX; Tocris Cookson, Bristol, UK), a non-NMDA receptor antagonist, at a dose of 20 μM during the measurement of slow depolarization amplitude. A repetitive electrical stimulation of the dorsal root was given by ten trains (20 rectangular pulses at 100 Hz/train) with an inter-trains interval of 2 s using a suction electrode. Pulse intensity and duration to activate C-fibers were 5 V–400 μs. The amplitude of slow depolarization was determined as the difference between the membrane potential immediately before the first train and that immediately before the fifth one. When the amplitude was more than 2 mV, slow depolarization was judged to be evoked. When slow depolarization was evoked by the first repetitive stimulation in a slice preparation, the second repetitive stimulation was administered at 15 min or more after the first one.

ATP, α,β-methylene-ATP and uridine 5′-triphosphate (UTP) were purchased from Sigma (St. Louis, MO, USA). These drugs were dissolved in aCSF and applied from 3 min before the second ten train repetitive stimulation to the end of experiment. Suramin hexasodium was purchased from Research Biochemicals International (Natick, MA, USA) and applied 2 min before and during the application of ATP.

Whole-cell patch clamp recordings were obtained from 285 substantia gelatinosa neurons. In the presence of 20 μM CNQX, on 51 neurons (17.9%), slow depolarization was evoked by ten trains repetitive stimulation of the dorsal roots. The mean amplitude of slow depolarization was 5.37 ± 0.39 mV. Then we examined the effects of ATP on the slow depolarization. Immediately after bath application of 50 and 100 μM ATP, the membrane potential was hyperpolarized by an average of 4.6 mV in five of 14 neurons and 7.3 mV in three of six neurons, respectively, while 10 μM ATP had no effect. When the membrane potential was hyperpolarized, the second repetitive stimulation was given after recovering to the previous level of the membrane potential by a depolarizing current pulse. In the neurons administered ATP (10, 50 and 100 μM), the amplitude of slow depolarization evoked by the second repetitive stimulation was attenuated in a concentration-dependent manner (Figs. 1a and 2). The significant inhibitory effects of ATP

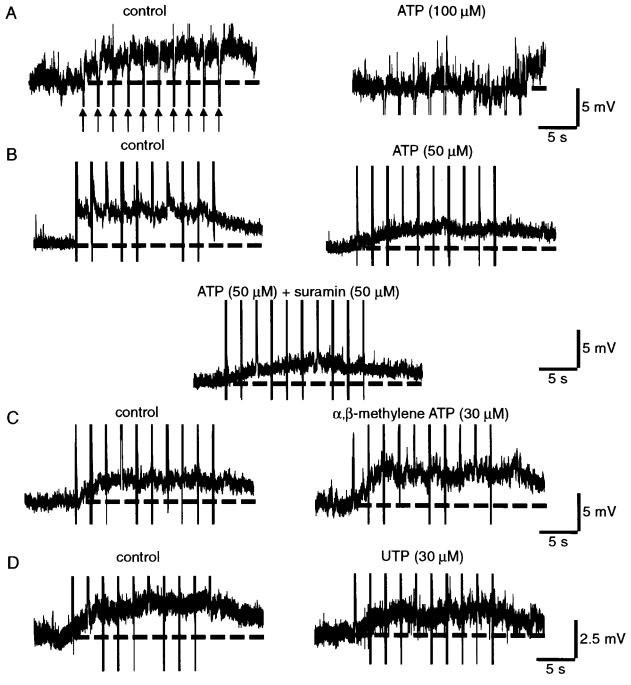

Fig. 1. Effects of ATP and its related analogs on the slow depolarization evoked by ten repetitive stimulation trains of C-fibers of the dorsal root in adult rat spinal cord transverse slices with the dorsal root attached. Tracers show representative results. An arrow indicates a train (20 pulses: 100 Hz for 0.2 s) of stimulation. (A) Effect of ATP on slow depolarization in a neuron. ATP (100 μM) inhibited the amplitude of slow depolarization. (B) Effect of suramin on the inhibition of slow depolarization by ATP. Suramin (50 μM) was applied 2 min before and during the application of ATP (50 μM). (C, D) Effects of α,β-methylene-ATP (30 μM) and UTP (30 μM) on the slow depolarization, respectively.

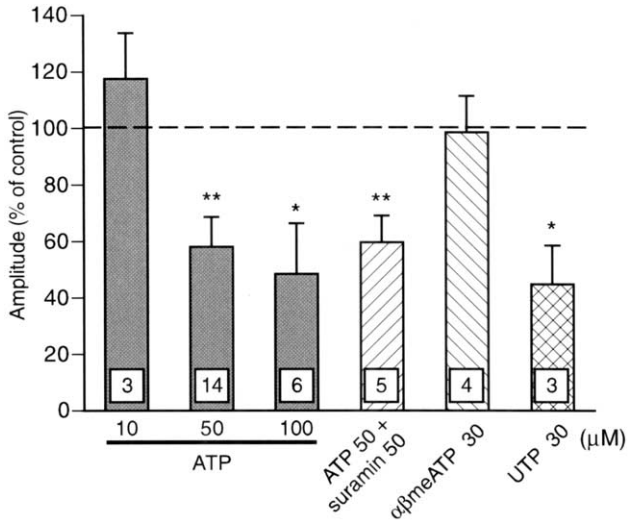

Fig. 2. The effects of ATP and its related analogs on the amplitude of slow depolarization. The amplitude of slow depolarization was determined as the difference between the membrane potential immediately before the first train and that immediately before the fifth one. In each experiment, the amplitude of slow depolarization evoked by the first repetitive stimulation was assigned a value of 100% as a control. Values are presented as means ± SEM. Numerals in each square indicate the number of experiments. $*P < 0.05$, $**P < 0.01$, compared with each control (Student's *t*-test).

were observed at the doses of 50 and 100 μM (58.2 ± 10.5% of the control level, $n = 14$ and 47.6 ± 18.1% of the control level, $n = 6$, respectively). However, the inhibitory effect of ATP (50 μM) was not antagonized by suramin (50 μM), an antagonist for some P2 purinoceptors (58.4 ± 9.8% of the control level, $n = 5$) (Figs. 1b and 2), although the hyperpolarization by ATP was not observed. The bath application of suramin alone did not affect the amplitude of slow depolarization (data not shown). We next examined the effects of a P2X purinoceptor selective agonist α,β-methylene ATP and a P2Y purinoceptor selective agonist UTP on the slow depolarization. Unlike ATP, the bath application of α,β-methylene ATP or UTP did not affect the membrane potential. On the other hand, UTP (30 μM) significantly inhibited the amplitude of slow depolarization (43.5 ± 13.8% of the control level, $n = 3$), while α,β-methylene ATP (30 μM) had no effect (95.4 ± 15.2% of the control level, $n = 4$) (Figs. 1c,d and 2).

In the present study, we found that ATP inhibited slow depolarization of substantia gelatinosa neurons evoked by the repetitive stimulation of the dorsal root. The inhibitory effect of ATP on the slow depolarization was not antagonized by suramin, an antagonist for some P2 purinoceptors. Adenosine, a metabolite of ATP, is well known to be an inhibitory neurotransmitter through P1 purinoceptors, and has been suggested to be responsible for some inhibitions observed with ATP [1]. Although the effect of adenosine receptor antagonists on the inhibitory action of ATP remains to be examined, a P2Y selective agonist UTP, which is not an adenosine derivative, markedly inhibited the slow depolarization, suggesting the involvement of P2Y purinoceptors. However, we cannot rule out the possibility that the inhibitory effect of ATP is partly mediated via P1 purinoceptors. Taken together, these findings suggest that ATP probably acts on suramin-insensitive P2Y purinoceptors such as $P2Y_4$, whose response has been reported to be pertussis toxin-sensitive [3], to produce the inhibitory effects on the slow depolarization. This is supported by the finding that $P2Y_4$ purinoceptor is expressed in the rat spinal cord [20].

In contrast, a body of evidence suggests that ATP acts as a fast excitatory neurotransmitter or neuromodulator to facilitate the nociceptive information via P2X purinoceptors in the spinal cord [1,2]. Gu and MacDermott have shown that ATP facilitated glutamate release from primary sensory afferent neurons via presynaptic P2X purinoceptors to evoke an inward current in the spinal dorsal horn neuron [7]. Furthermore, ATP was reported to enhance glutamate-induced responses in substantia gelatinosa neurons [14]. However, the present experiments were performed in the presence of CNQX to distinguish slow depolarization from the summation of the EPSPs mediated by non-NMDA receptors. It is suggested that the absence of excitatory effects of ATP in the present preparations might be due to the blockade of the excitatory components of glutamate by CNQX. On the other hand, it has been suggested that approximately half of the cultured dorsal horn neurons were directly excited by ATP via postsynaptic P2X purinoceptors [11], that is, the remaining half of the dorsal horn neurons are not excited postsynaptically by ATP. The dorsal horn neurons in which slow depolarization was evoked in the present study (17.9%) might be those which were not excited by ATP.

There are a few studies that suggested ATP plays an inhibitory role in neurotransmission, which are consistent with the present findings. For example, in rat cultured hippocampal neurons, ATP attenuated calcium oscillations by inhibiting glutamate release from the presynaptic terminal via suramin-insensitive G-protein coupled P2 purinoceptors [12]. Recently, it was reported that ATP facilitates inhibitory GABAergic and glycinergic transmission at the dorsal horn interneuron synapses [8,17], although these inhibitory effects were postulated to be mediated via P2X purinoceptors. It is likely that the facilitation of inhibitory GABAergic and glycinergic interneurons by ATP indirectly inhibited slow depolarization of substantia gelatinosa neurons. Indeed, we found that both muscimol and baclofen inhibited the slow depolarization via presynaptic GABA receptors (our unpublished data).

Furthermore, it was reported that P2Y purinoceptors are expressed in astrocytes [4], and that ATP and its analogs increased the intracellular Ca^{2+} concentration in >99% of dorsal horn astrocytes via P2Y purinoceptors [9]. These signals may affect the neighboring neurons to modulate the transmission of nociceptive information [4]. Thus, it is

also possible that the inhibitory effects of ATP on the slow depolarization are due to the effects on glial cells around the recording neurons.

On our preliminary experiments, it was shown that intrathecal administration of UTP induced antinociception in the rat paw pressure test and tail flick test, and reduced mechanical allodynia in neuropathic-pain model rats.

In summary, the above described findings suggest that ATP inhibits the slow depolarization of substantia gelatinosa neurons evoked by the repetitive stimulation of the dorsal root via suramin-insensitive P2Y purinoceptors and plays a role in spinal antinociception. Additional investigations are needed to elucidate the inhibitory roles of ATP in the spinal nociceptive transmission.

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