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Enzymatic conversion of ATP to adenosine contributes to ATP-induced inhibition of glutamate release in rat medullary dorsal horn neurons

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ABSTRACT

Purine nucleotides, such as ATP and ADP, activate ionotropic P2X and metabotropic P2Y receptors to regulate neurotransmitter release in the peripheral as well as central nervous system. Here we report another type of ATP-induced presynaptic modulation of glutamate release in rat medullary dorsal horn neurons. Glutamatergic excitatory postsynaptic currents (EPSCs) induced by electrical stimulation of trigeminal tract were recorded from horizontal brain stem slices using a whole-cell patch clamp technique. ATP decreased the amplitude of glutamatergic EPSCs in a reversible and concentration dependent manner and increased the paired-pulse ratio. In addition, ATP reduced the frequency of miniature EPSCs without affecting the current amplitude, suggesting that ATP acts presynaptically to reduce the probability of glutamate release. The ATP-induced decrease in glutamatergic EPSCs was not affected by P2X and P2Y receptor antagonists, but was completely blocked by DPCPX, a selective adenosine A_1 receptor antagonist. The ATP-induced decrease in glutamatergic EPSCs was also inhibited by an inhibitor of tissue nonspecific alkaline phosphatase but not by inhibitors of other enzymes such as ecto-nucleoside triphosphate diphosphohydrolases and ecto-5'-nucleotidases. The results suggest that exogenously applied purine nucleotides are rapidly converted to adenosine by specific enzymes, and subsequently act on presynaptic A_1 receptors to inhibit glutamate release from primary afferent terminals. This type of modulation mediated by purine nucleotides may play an important role in regulating nociceptive transmission from orofacial tissues.

1. Introduction

ATP is an extracellular signaling molecule that acts as a fast neurotransmitter in the peripheral and central nervous system (Burnstock, 2007). ATP is involved in a number of physiological functions, such as neuroprotection, locomotion, development, and pain, through the activation of multiple types of P2 receptors (e.g., ionotropic P2X and metabotropic P2Y receptors) expressed on neuronal membranes (Ralevic and Burnstock, 1998; Burnstock, 2007). P2 receptors are also expressed on presynaptic nerve terminals, and regulate the release of a variety of neurotransmitters (Rodrigues et al., 2005; Dorostkar and Boehm, 2008; Gonçalves and Queiroz, 2008). Moreover, extracellular ATP can be rapidly hydrolyzed to ADP, AMP, and adenosine via multiple types of extracellular

Abbreviations: αβ-me-ATP, αβ-methylene-ATP; APV, DL-2-amino-5-phosphonovaleric acid; ARL67156, 6-N,N-diethyl-β-γ-dibromomethylene-D-adenosine-5′-triphosphate; BBG, Brilliant Blue G; Bz-ATP, 2′-3′-*O*-(4-benzoylbenzoyl)-ATP; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DRG, dorsal root ganglia; ENT1, equilibrative nucleoside transporter 1; EPSCs, excitatory postsynaptic currents; K–S test, Kolmogorov–Smirnov test; mEPSCs, miniature EPSCs; MRS2179, 2'-deoxy-N^6-methyl adenosine 3′,5'-diphosphate; MRS2211, 2,2-dimethyl-propionic acid 3-(2-chloro-6-methylaminopurin-9-yl)-2-(2,2-dimethyl-2-[(2-chloro-5-nitrophenyl)azo]-5-hydroxy-6-methyl-3-[(phosphonooxy)methyl]-4-pyridinecarboxaldehyde; MRS2395, propionyloxymethyl)-propyl ester; NBMPR, *S*-(4-nitrobenzyl)-6-thioinosine; NT5E, ecto-5'-nucleotidases; NTPDases, ecto-nucleoside triphosphate diphosphohydrolases; PAP, prostatic acid phosphatase; PPADS, pyridoxalphosphate-6-azophenyl-2′,4'-disulfonic acid; PPR, paired-pulse ratio; SR95531, 6-imino-3-(4-methoxyphenyl)-1(6H)-pyridazinebutanoic acid; TG, trigeminal ganglia; TNAP, tissue nonspecific alkaline phosphatase; TTX, tetrodotoxin; VDCCs, voltage-dependent Ca^{2+} channels.

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enzymes, including ecto-nucleoside triphosphate diphosphohydrolases (NTPDases), ecto-5′-nucleotidases (NT5E, also known as CD73), prostatic acid phosphatase (PAP), and tissue nonspecific alkaline phosphatase (TNAP) (Dunwiddie et al., 1997; Cunha et al., 1998; Zimmermann, 2000; Zylka et al., 2008; Street et al., 2013). Because NT5E and PAP hydrolyze AMP to adenosine (Zylka et al., 2008; Sowa et al., 2010), these enzymes require ATP or ADP to first be degraded to AMP by NTPDases. In contrast, TNAP can hydrolyze ATP, ADP, and AMP to adenosine (Zimmermann, 2000; Street et al., 2013). The resultant adenosine activates P1 adenosine receptors to modulate the excitability of peripheral and central neurons (Fredholm et al., 2005).

ATP is closely involved in nociceptive transmission, because sensory neurons, such as dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons, and primary afferents express multiple types of P2X, P2Y, and adenosine receptors (Gerevich and Illes, 2004; Donnelly-Roberts et al., 2008; Burnstock, 2009a, 2009b). In addition, endogenous ATP can be released from primary afferent terminals and interneurons onto spinal dorsal horn neurons (Bardoni et al., 1997; Jo and Schlichter, 1999), and it acts presynaptically to modulate excitatory sensory synaptic transmission. For example, ATP acts on presynaptic $P2X_1$ and/or $P2X_3$ receptors and facilitates spontaneous glutamate release onto spinal dorsal horn neurons (Gu and MacDermott, 1997; Nakatsuka and Gu, 2001), suggesting that ATP via the activation of P2X receptors plays a pronociceptive role in the spinal cord. In contrast, the activation of $P2Y_1$ receptors reduces membrane currents mediated by voltage-dependent Ca^{2+} channels (VDCCs) in DRG neurons (Gerevich et al., 2004), suggesting that $P2Y_1$ receptors can inhibit primary afferent synaptic transmission in spinal dorsal horn neurons. Furthermore, since ATP hydrolyzing enzymes are found within the DRG and spinal dorsal horn (Zylka et al., 2008; Sowa et al., 2010), ATP via the activation of P1 receptors might affect nociceptive transmission in the spinal cord. While TG neurons and their afferents also express a number of purinoceptors (Chen et al., 1995; Xiang et al., 1998; Ruan and Burnstock, 2003), much less is known about the functional role of ATP in trigeminal nociceptive transmission from peripheral tissues. In the present study, therefore, we directly addressed the effect of ATP on primary afferent-evoked glutamatergic transmission using horizontal brain stem slices.

2. Materials and methods

2.1. Preparation

All experiments complied with the guiding principles for the care and use of animals approved by the Council of the Physiological Society of Korea and the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and every effort was made to minimize both the number of animals used and their suffering.

Sprague Dawley rats (12–16 d old, either sex) were decapitated under ketamine anesthesia (100 mg/kg, i. p.). The brain stem was dissected and horizontally sliced at a thickness of 400 μm by use of a microslicer (VT1000S; Leica, Nussloch, Germany) in a cold artificial cerebrospinal fluid (ACSF; 120 NaCl, 2 KCl, 1 KH_2PO_4, 26 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$ and 10 glucose, saturated with 95% O_2 and 5% CO_2). Slices were kept in an ACSF saturated with 95% O_2 and 5% CO_2 at room temperature (22–25 °C) for at least 1 h before electrophysiological recording. Thereafter, the slices were transferred into a recording chamber, and both the medullary dorsal horn region and trigeminal roots were identified under an upright microscope (E600FN, Nikon, Tokyo, Japan) with a water-immersion objective (×40). The ACSF routinely contained 10 μM SR95531, 1 μM strychnine, 50 μM APV to block $GABA_A$, glycine and NMDA receptors, respectively. The bath was perfused with ACSF at 2 ml/min by the use of a peristaltic pump (MP-1000, EYELA, Tokyo, Japan).

2.2. Electrical measurements

All electrical measurements were performed by use of a computer-controlled patch clamp amplifier (MultiClamp 700B; Molecular Devices; Union City, CA, USA). For whole-cell recording, patch pipettes were made from borosilicate capillary glass (1.5 mm outer diameter, 0.9 mm inner diameter; G-1.5; Narishige, Tokyo, Japan) by use of a pipette puller (P-97; Sutter Instrument Co., Novato, CA, USA). The resistance of the recording pipettes filled with internal solution (in mM; 140 $CsMeHSO_3$, 5 TEA-Cl, 5 CsCl, 2 EGTA, 2 Mg-ATP and 10 Hepes, pH 7.2 with Tris-base) was 4–6 MΩ. Membrane currents were filtered at 2 kHz (MultiClamp Commander; Molecular Devices), digitized at 10 kHz (Digidata 1322A, Molecular Devices), and stored on a computer equipped with pCLAMP 10.0 (Molecular Devices). In whole-cell recordings, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically delivered to monitor the access resistance, and recordings were discontinued if access resistance changed by more than 15%. All electrophysiological experiments were performed at room temperature (22–25 °C). To record action potential-dependent glutamatergic excitatory postsynaptic currents (EPSCs), a glass stimulation pipette (~10 μm diameter) filled with a bath solution, was positioned around the trigeminal root (see Choi et al., 2011, 2012). Brief paired pulses (500 μs, 1–5 V, 20 Hz) were applied by the stimulation pipette at a frequency of 0.1 Hz using a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan) equipped with an isolator unit (SS-701J, Nihon Kohden).

2.3. Data analysis

The amplitudes of action potential-dependent glutamatergic EPSCs were calculated by subtracting the baseline from the peak amplitude. The conduction velocity of primary afferents innervating medullary dorsal horn neurons was calculated by dividing the distance between stimulation and recording sites by the latency of EPSCs (see Choi et al., 2012). The effect of drugs on EPSCs was quantified as a percentage change in EPSC amplitude compared to the control values. Miniature EPSCs (mEPSCs) were counted and analyzed using the MiniAnalysis program (Synaptosoft, Inc., Decatur, GA, USA) as described previously (Jang et al., 2002). The average values of both the frequency and amplitude of mEPSCs during the control period (10–20 min) were calculated for each recording, and the frequency and amplitude of all the events during the ATP or ADP application (5 min) were normalized to these values. The effects of these different conditions were quantified as a percentage increase in mEPSC frequency compared to the control values. The inter-event intervals and amplitudes of a large number of synaptic events obtained from the same neuron were examined by constructing cumulative probability distributions and compared using the Kolmogorov–Smirnov (K–S) test with Stat View software (SAS Institute, Inc., Cary, NC, USA). The continuous curves for the concentration–inhibition relationship were fitted using a least-squares fit to the following equation:

$$I = 1 - [C^n/(C^n + EC_{50}^n)],$$

where I is the ATP- or ADP-induced inhibition of EPSC amplitude, C is the concentration of ATP or ADP, EC_{50} is the concentration for the half-effective response and n is the Hill coefficient. Numerical values are provided as the mean and standard error of the mean (SEM) using values normalized to the control. Significant differences in the mean amplitude and frequency were tested using the Student's two-tailed paired t-test, using absolute values rather than normalized ones. Values of $p < 0.05$ were considered significant.

2.4. Drugs

The drugs used in the present study were ATP, ATPγS, ADP, ADPβS, UTP, UDP, αβ-me-ATP, Bz-ATP, adenosine, DPCPX, suramin, PPADS, BBG, strychnine, APV, CNQX, ARL67156, MRS2179, MRS2395, *S*-(4-nitrobenzyl)-6-thioinosine (NBMPR) (from Sigma, St. Louis, MO, USA) and SR95531, MRS2211, TTX, POM-1, baclofen (from Tocris, Bristol, UK), and 2,5-dimethoxy-N-(quinolin-3-yl)benzenesulfonamide (TNAP inhibitor, TNAP-I) (from Merck Milipore, Darmstadt, Germany), adenosine deaminase (from Worthington, Lakewood, NJ, USA). All drugs were applied by bath application. In a subset of experiments, ATP was applied using the 'Y–tube system' for rapid solution exchange (Murase et al., 1989).

3. Results

3.1. ATP acts presynaptically to inhibit glutamate release in medullary dorsal horn neurons

Action potential-dependent EPSCs were recorded from medullary dorsal horn neurons at a V_H of −60 mV by electrical stimulation through a glass pipette placed to the spinal trigeminal tract. These EPSCs were mediated by AMPA/KA receptors because 10 μM CNQX, a selective AMPA/KA receptor antagonist, completely blocked all synaptic currents (data not shown, see also Choi et al., 2011). Under these conditions, the effect of ATP on glutamatergic EPSCs evoked by paired stimulation at an interval of 50 ms (20 Hz) was observed. As shown in Fig. 1A and B, bath applied ATP (100 μM), an endogenous P2 receptor agonist, reversibly decreased the first EPSC ($EPSC_1$)