Published in final edited form as:
J Pharmacol Exp Ther. 2008 October ; 327(1): 196–205. doi:10.1124/jpet.108.139766.

P2 Receptors at GABAergic Synapses on VTA Dopamine Neurons are Targets for Ethanol Action

Cheng Xiao, **Chunyi Zhou**, **Kaixun Li**, **Daryl L. Davies**, and **Jiang H. Ye***
Department of Anesthesiology, Pharmacology and Physiology, UMDNJ, New Jersey Medical School, Newark, NJ07103 (CX, CZ, JHY). Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles 90033, USA (KL, DLD)

Abstract

The current study investigated whether ethanol alters adenosine 5′-triphosphate (ATP) activation of purinergic receptors (P2Rs) in the ventral tegmental area (VTA). The VTA is a key region of the brain that has been implicated in the development of alcohol addiction. We investigated the effects of ATP and ethanol on spontaneous inhibitory postsynaptic currents (sIPSCs) and the spontaneous firings in the VTA dopaminergic neurons, obtained using an enzyme-free procedure. These neurons preserved some functional GABA-releasing terminals after isolation. We found that ATP (1–200 μM) either increased or decreased the frequency of sIPSCs and the activity of VTA dopaminergic neurons. The effects of ATP on sIPSC frequency inversely correlated with its effects on dopaminergic neuron activity. The ATP-induced changes in sIPSC frequency were blocked by tetrodotoxin, a sodium channel blocker and by suramin, a non-selective P2R antagonist. Furthermore, α, β-MeATP, a selective $P2X_1$ and $P2X_3$ receptor agonist, increased sIPSC frequency while ADP-βS, a preferential agonist of P2Y receptors, decreased sIPSC frequency. Testing the effects of ethanol (10 and 40 mM) on sIPSCs found that ethanol significantly attenuated ATP-induced increase and enhanced ATP-induced decrease in sIPSC frequency. Taken together, the results demonstrate that multiple subtypes of P2Rs exist on GABA-releasing terminals which make synapses on VTA dopaminergic neurons. It appears that ATP increases sIPSC frequency involving $P2X_1$ or/and $P2X_3$ receptors, and ATP decreases sIPSC frequency involving P2YRs. The findings are also consistent with the notion that P2Rs at GABA-releasing terminals on VTA dopaminergic neurons are important targets for ethanol action.

Introduction

The molecular mechanism underlying ethanol (EtOH) addiction is not completely understood. It is well documented that the mesolimbic dopaminergic (DA) system originating from the ventral tegmental area (VTA) plays a critical role (Imperato and Di Chiara, 1986; Nowak et al., 1998). The VTA DA neurons are under the tonic control of GABA neurons, and both DA and GABA neurons in the VTA are sensitive to EtOH (Brodie et al., 1990; Stobbs et al., 2004; Xiao et al., 2007).

Adenosine 5′- triphosphate (ATP) activates the P2 receptor (P2R) family, which includes the P2X superfamily of ligand-gated cationic channels and the P2Y superfamily of seven transmembrane domain G protein-coupled receptors. P2XRs are fast acting, cation-permeable ion channels gated by synaptically released extracellular ATP (Khakh, 2001; North, 2002). To

*Corresponding author: Jiang Hong Ye, Department of Anesthesiology, New Jersey Medical School (UMDNJ), 185 South Orange Avenue, Newark, NJ 07103-2714, USA, TEL # (973) 972-1866, FAX # (973) 972-4172, ye@umdnj.edu.

date, seven subtypes of the P2X family of ligand-gated cationic channels have been identified ($P2X_1$-$P2X_7$). ATP binding to P2Y receptors leads to a G-Protein cascade, including activation of phospholipase C and inhibition of adenylyl cyclase (Lazarowski et al., 2003). Presently eight members of the P2Y superfamily have been identified ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$). Messenger RNA or subunit proteins for all of the P2Rs have been found in the central nervous system (Khakh, 2001; Rubio and Soto, 2001; North, 2002; Franke et al., 2006). The function of P2XRs and P2YRs in the CNS is not well understood, but it is a rapidly growing area of research in the neuroscience community.

Results from previous studies support the notion that P2XRs play a role in mediating and/or modulating at least a subset of the cellular and behavioral effects of EtOH. In recombinant expression systems, EtOH inhibits or potentiates P2XR function depending on the particular P2XR subtype tested. EtOH inhibits $P2X_2R$ and $P2X_4R$ function but potentiates $P2X_3R$ function (Li et al., 2000; Davies et al., 2002; Fischer et al., 2003; Davies et al., 2005). Native P2XRs are sensitive to direct modulation by pharmacologically relevant EtOH concentrations (Li et al., 1998; Li et al., 2000). In addition, EtOH can modulate GABAergic transmission and change GABA receptor expression levels in the VTA (Charlton et al., 1997; Melis et al., 2002; Xiao and Ye, 2008). Moreover, presynaptic P2XRs can modulate the release of neurotransmitters such as GABA, glycine and glutamate (Mori et al., 2001; Papp et al., 2004), that are implicated in mediating behavioral effects of EtOH (Mihic et al., 1997; Woodward, 2000; Davies et al., 2003). Therefore, EtOH modulation of P2XRs may directly and indirectly affect neuronal activity leading to altered behavioral functions.

Microinjection of the ATP analogue 2-methylthio ATP into the VTA increases the activity of VTA DA neurons, the release of dopamine, and the locomotor activity of the animals in the open field (Krugel et al., 2001; Krugel et al., 2003). Furthermore, immunohistochemistry studies have demonstrated that P2Rs exist in the VTA (Heine et al., 2007). Nevertheless, P2R modulation of VTA DA neurons remains intricate in the anatomical and pharmacological scenario because of the following observations: (1) P2X and P2Y receptors exist on both the soma and the neurites of VTA neurons (Heine et al., 2007), (2) Activation of the P2X and the P2Y receptors respectively depolarizes and hyperpolarizes VTA neurons, (3) Functional P2X receptors exist on GABA-releasing terminals that are on VTA DA neurons and (4) EtOH regulates the function of the GABA-releasing terminals that are on the VTA DA neurons (Theile et al., 2008; Xiao and Ye, 2008).

The goal of the current study was to begin to determine the contribution of P2Rs to the activity of VTA neurons and the sensitivity of the P2Rs to EtOH. To accomplish this goal, we tested the following hypotheses: (1) ATP modulates GABA release onto VTA DA neurons through the activation of the P2Rs on the GABA-releasing terminals. The effect of ATP on GABA release negatively correlates with its effect on the activity of VTA DA neurons and (2) These P2Rs are sensitive to EtOH. We recorded spontaneous inhibitory postsynaptic currents (sIPSCs) and spontaneous firings in the VTA DA neurons, obtained using an enzyme-free procedure, which preserved some functional GABA-releasing terminals.

Methods

Brain Slice Preparation and Mechanical Dissociation of Neurons

Midbrain slices from Sprague Dawley rats (14- 19 postnatal days) were prepared as described previously (Xiao et al., 2007). In brief, rats were anesthetized and sacrificed by decapitation. The midbrain was isolated and transversely sliced at a thickness of 400 μm using a VF-200 Slicer (Precisionary Instruments, Greenville, NC, USA) in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95%O_2 and 5%CO_2 (Carbogen). The ACSF contained (in mM): 126 NaCl, 1.6 KCl, 1.25 NaH_2PO_4, 1.5 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, and 10 glucose. Midbrain

slices were then kept in the carbogenated ACSF at room temperature (22-24 °C) for at least 1 hr before use.

Neurons with functional presynaptic terminals attached were obtained by mechanical dissociation, as previously described (Akaike and Moorhouse, 2003), with some modifications (Ye et al., 2004). Briefly, a midbrain slice was transferred to a 35 mm culture dish (Falcon, Rutherford, NJ, USA) and held down with a flat U-shape-wire. The dish was filled with standard external solution containing (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 glucose, 320 mOsm, pH was adjusted to 7.3 with Tris base. Under an inverted microscope (DIAPHOT, Nikon, Tokyo, Japan), VTA was identified. A heavily fire polished pipette lightly touched the surface of the VTA and vibrated horizontally at 15 - 20 Hz for 2 - 5 min using a homemade device. Finally, the slice was removed. After 20 min, isolated neurons adhered to the bottom of the dish were used for electrophysiological recordings.

Electrophysiological Recordings

Whole-cell and cell-attached configurations were used to record the electrical activity using Axopatch 200B amplifier, Digidata 1320A analog-to-digital converter and Clampex 9 software (Molecular Devices Corp, Union City, CA, USA). Data were sampled at 5 kHz and filtered at 1 kHz. The patch electrodes had a resistance of 3-5 MΩ when filled with the following internal solution (in mM): 140 KCl, 2 $MgCl_2$, 5 EGTA, 0.5 $CaCl_2$, 10 HEPES, 2 MgATP and 0.1 GTP. pH was adjusted to 7.2 with KOH, and osmolarity was adjusted to 280-300 mOsm with sucrose. All recordings were collected at room temperature (22-24°C).

VTA DA neurons were identified according to the following criteria: a slow firing rate (< 5 Hz), wide action potentials with widths of ≥ 1.2 ms (measured from the beginning of inward current to the peak of outward current of the action potential) recorded in the cell-attached mode. These features are found in tyrosine hydroxylase (TH)+ dopamine cells in the VTA (Ford et al., 2006). In addition, the DA neurons also show the presence of prominent hyperpolarization-induced currents (I_h), inhibition by the D_2/D_3 receptor antagonist quinpirole, and disinhibition by Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol enkephalin (DAMGO, an agonist of μ-opioid receptor) (Johnson and North, 1992; Margolis et al., 2003).

Data Analysis

Spontaneous inhibitory postsynaptic currents (sIPSCs) and spontaneous firings were counted and analyzed using Clampfit 9.2. sIPSCs were screened automatically using an amplitude threshold of 5 pA and then visually accepted or rejected based upon rise and decay times. The average value of sIPSC frequency during the control period was calculated. The effect of a chemical was quantified as a percentage change in sIPSC frequency compared to the control value. Differences in absolute value or % change of amplitude and frequency were statistically tested using a Student's paired two-tailed t-test. Data were presented as the mean ± SEM. $p < 0.05$ was considered statistically significant.

Chemicals and Applications

Adenosine 5-triphosphate disodium salt (ATP), Adenosine 5′-[β-thio]diphosphate trilithium salt (ADP-βS), α,β-Methylene ATP lithium salt (α,β-MeATP), 7-Chloro-4-hydroxy-2-phenyl-1,8- naphthyridine (CPNT), DL-2-Amino-5-phosphonopentanoic acid (APV), 7-Dinitroquinoxaline-2,3(1H, 4H)-dione (DNQX), pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate (PPADS), suramin (8-[(4-methyl-3-{[3-({[3-({2-methyl 5- [(4,6,8-trisulfo-1-naphthyl) carbamoyl] phenyl}carbamoyl)phenyl]carbamoyl}amino) benzoyl] amino} benzoyl) amino]naphthalene- 1,3,5-trisulfonic acid) and tetrodotoxin (TTX), were purchased from Sigma (St. Louis, MO). EtOH (95%, prepared from grains) was purchased from Pharmco (Brookfield, CT). Solutions were prepared on the day of the experiment and applied to a

dissociated neuron using a 'Y- tube' perfusion system. A perfusion pipette with diameter of 50 μm was placed ~100 μm away from the neuron. Solutions in the vicinity of a neuron can be completely exchanged within 40 ms without damaging the seal (Zhou et al., 2006).

Results

ATP Effects on sIPSCs in VTA DA Neurons

Mechanically dissociated VTA DA neurons had oval or triangular soma with diameters between 20 and 35 μm. Such preparations had several advantages: good space clamp, preservation of some functioning synaptic terminals and better control of the surrounding solution (Akaike and Moorhouse, 2003; Ye et al., 2004; Zhu and Lovinger, 2005). As previously reported, some functional presynaptic nerve endings remained attached on the isolated neurons; therefore, spontaneous inhibitory postsynaptic currents (sIPSCs) could be recorded. The sIPSCs recorded in the presence of glutamate receptor antagonist (20 μM DNQX and 50 μM APV) were blocked by 10 μM bicuculline, a $GABA_A$ receptor antagonist, indicating that the measured sIPSCs were mediated by $GABA_A$ receptors (Fig. 1A).

We found that ATP (1 200 μM) showed multiple effects on sIPSC frequency, including decrease (Fig. 1B), increase (Fig. 1C), or no effect (< 10% change from baseline, data not shown). However, the effect of ATP on the same neurons was consistent and dependent on its concentration (Fig. $1B_3$, left panel, and Fig. $1C_3$, left panel). In contrast to its effect on sIPSC frequency, ATP at concentrations less than 200 μM had no significant effect on the mean amplitude of sIPSCs.

As illustrated in Fig. 1B, ATP reversibly decreased the sIPSC frequency in 54% (n = 24/44) of the VTA DA neurons tested (Fig. $1B_1$, B_2 and B_3, left panel). The decrease was dependent on ATP concentration: 1, 10, 100 and 200 μM ATP decreased sIPSC frequency by 23 ± 6% (n = 15, p > 0.05), 39 ± 3% (n = 14, p = 0.001), 61 ± 6% (n = 10, p < 0.01), and 63 ± 10% (n = 7, p < 0.01), respectively (Fig.$1B_3$, left panel). ATP (≤ 100 μM) did not significantly alter sIPSC amplitude: 1, 10 and 100 μM ATP altered sIPSC amplitude by -8 ± 7% (n = 15, p = 0.13), -2 ± 6% (n = 24, p = 0.12), and -11 ± 11% (n = 10, p = 0.17), respectively. ATP at 200 μM significantly reduced sIPSC amplitude (by 27 ± 10%, n = 7, p = 0.04, Fig. $1B_3$, right panel).

As shown in Fig. 1C, ATP reversibly increased sIPSC frequency in 32% (n = 14/44) of the VTA DA neurons tested (Fig. $1C_1$, C_2, and C_3, left panel). The increase in sIPSC frequency was dependent on the concentration of ATP: 1, 10 and 100 μM ATP increased sIPSC frequency by 20 ± 5% (n = 7, p = 0.003), 55 ± 11% (n = 14, p = 0.00005) and 46 ± 16% (n = 6, p = 0.02), respectively. However, ATP did not significantly alter sIPSC amplitude: 1, 10 and 100 μM ATP altered sIPSC amplitude by -13 ± 7 (n = 7, p = 0.13), 17 ± 7% (n = 14, p = 0.23), and 6 ± 7% (n = 6, p = 0.22), respectively (Fig. 1 C_3, right panel) which was not significantly different than control values. Finally, ATP (1- 200 μM) had no effect on the frequency and the amplitude of sIPSCs in 14% (6/44) of the VTA DA neurons examined.

We also found that 10 μM ATP induced a transient current (< 5 s, ranging from 20 to 400 pA, 137 ± 25 pA) in 28% (29/105) of the DA neurons (Fig. 2). This current was dramatically attenuated by suramin (100 μM) or PPADS (10 μM), and eliminated by the removal of extracellular calcium. We interpret these data to indicate the existence of functional P2XR on the postsynaptic membrane of some VTA DA neurons, which is consistent with previous morphological findings (Vulchanova et al., 1996;Heine et al., 2007).

To determine if P2Rs were involved in the action of ATP, we tested the effect of ATP on sIPSCs in the presence of suramin, a broad spectrum antagonist for P2Rs. Interestingly, suramin (100 μM) alone significantly reduced both the frequency and the amplitude of sIPSCs. In

neurons in which ATP decreased sIPSC frequency, 100 μM suramin reduced sIPSC frequency by 29 ± 6% (n = 6, p = 0.005), and amplitude by 39 ± 8% (n = 6, p = 0.003), respectively. In those neurons in which ATP enhanced sIPSC frequency, 100 μM suramin significantly reduced sIPSC frequency by 56 ± 8% (n = 6, p = 0.005), and sIPSC amplitude by 36 ± 9% (n = 6, p = 0.003), respectively. Suramin-induced reduction of sIPSC frequency might be resultant of its inhibition of postsynaptic $GABA_A$ receptors (Nakazawa et al., 1995), leading to a decrease in the sIPSC amplitude to below detectable levels. To test this possibility, we tested the effect of PPADS, another P2R antagonist. While PPADS (10 μM) significantly decreased sIPSC frequency (by 48 ± 5%, n = 5, p = 0.01), it did not significantly alter the amplitude of sIPSCs (by -9 ± 14 %, n = 5, p = 0.39) indicating that PPADS acts on the presynaptic site to reduce the release of GABA. Taken together, these data indicate that P2Rs exist on the GABA-releasing terminals (boutons), and they are tonically activated.

Suramin (100 μM) significantly attenuated either the decrease or the increase in sIPSC frequency induced by 10 μM ATP. Specifically, as illustrated in Fig. 3A, in those neurons, in which 10 μM ATP alone decreased sIPSC frequency (by 37 ± 8%, n = 5, p = 0.005), after 4 min preincubation with 100 μM suramin, 10 μM ATP had no significant effect on sIPSC frequency (by 0 ± 9%, n = 5, p = 0.5, Fig. $3A_3$). Similarly, in those neurons, in which 10 μM ATP alone increased sIPSC frequency (by 53 ± 11%, n = 5, p = 0.01), after 4 min preincubation with 100 μM suramin, 10 μM ATP had no significant effect on sIPSC frequency (by 17 ± 15%, n = 5, p = 0.17, Fig. $3B_3$).

ATP may also activate A1 adenosine receptors via a conversion of ATP to adenosine by an enzymatic process. The resultant available adenosine could conceivably cause its own change in sIPSC frequency. To investigate this possibility, we next tested the effect of 7-Chloro-4-hydroxy-2-phenyl-1,8-naphthyridine (CPNT), an A_1 adenosine receptor antagonist. CPNT (10 μM) had no significant effect on ATP inhibition of sIPSC frequency (ATP alone, by 35 ± 6%, n = 4, p = 0.0007; ATP + CPNT: by 28 ± 6%, n = 4, p = 0.002; paired t-test, p = 0.27; ATP verses ATP + CPNT, data not illustrated) suggesting that the change in sIPSC frequency cannot be attributed to adenosine receptors. Taken together, these data indicate that ATP regulation of GABA release is mediated predominately by P2Rs.

ATP Modulation of sIPSCs and the Subtypes of P2Rs

As briefly discussed in the Introduction, activation of P2X receptors at nerve terminals increases neurotransmitter release by facilitating calcium entry, whereas activation of P2Y receptors decreases neurotransmitter release through a G protein-coupled second messenger pathway. To determine which subtype of P2Rs are involved (i.e., P2X or P2Y) in ATP-induced increase or decrease of GABA release, we next tested the effect of α,β-MeATP (a potent and selective agonist for $P2X_1$ and $P2X_3$ receptors) on sIPSCs. As presented in Fig. 4A and 4B respectively 10 μM α,β-MeATP increased sIPSC frequency by 68 ± 14% (n = 9, p = 0.001) in neurons (Group 1) in which 10 μM ATP decreased sIPSC frequency (by 47 ± 5%, n = 9, p = 0.0002, Fig. 4A), and by 103 ± 24% (n = 7, p = 0.007) in neurons (Group 2) in which 10 μM ATP increased sIPSC frequency (by 53 ± 9%, n = 7, p = 0.002, Fig. 4B). However, 10 μM α, β-MeATP had no effect on sIPSC amplitude in both groups (by 1 ± 14%, n = 9, p = 0.25 in Group 1 and by 1 ± 12%, n = 7, p = 0.31 in Group 2). In 4 other neurons in Group 1, the increase in sIPSC frequency induced by 10 μM ATP was blunted in the absence of extracellular Ca^{2+} (2 mM Ca^{2+}: by 33 ± 5%, n = 4, p = 0.004; 0 mM Ca^{2+}: by -2 ± 11%, n = 4, p = 0.44; paired t-test, p = 0.02, data not illustrated). These data suggest that $P2X_1$ and/or $P2X_3$ receptors exist at the GABA-releasing terminals, which make synapses on VTA DA neurons. The activation of the P2XRs enhances GABA release, which is dependent on extracellular calcium.

In another set of experiments, we examined the effect of ADP-βS, a preferential agonist to P2Y receptors, on sIPSCs. The application of ADP-βS inhibited sIPSC frequency of neurons in both

Group 1 and Group 2 (Fig. 5). In Group 1 neurons, on which 10 μM ATP enhanced sIPSC frequency by 50 ± 15% (n = 4, p = 0.03), 10 μM ADP-βS inhibited sIPSC frequency by 32 ± 12% (n = 4, p = 0.04) (Fig. 5A_{1-2}). In Group 2 neurons, on which 10 μM ATP inhibited sIPSC frequency by 46 ± 3% (n = 4, p = 0.0004), 10 μM ADP-βS inhibited sIPSC frequency by 55 ± 8% (n = 4, p = 0.003) (Fig. 5B_{1-2}). These findings suggest the existence of P2Y receptors on GABA-releasing terminals which make synapses onto VTA DA neurons. The activation of these P2Y receptors inhibits GABA release. Taken together, the data is consistent with the notion that ATP changes in sIPSC frequency is dependent on which class of P2Rs (P2X or P2Y) dominates in the individual neuron tested.

ATP Modulation of Spontaneous Firing of VTA DA Neurons

We also tested the effect of ATP on the spontaneous firing of VTA DA neurons. ATP (10 μM) reversibly increased the firing rate by 69 ± 16% in 59% (10/17) of the neurons (n = 10, p = 0.0001, Fig. 6A, C), decreased by 32 ± 14% in 29% (5/17) of the neurons (Fig. 6B, C) and had no effect in 12% (2/17) of the neurons. On some DA neurons, we first tested the effect of 10 μM ATP on the firing, recorded in the cell-attached mode, and then the sIPSCs, recorded in whole-cell mode. In 3 neurons, 10 μM ATP inhibited the firing rate by 40 ± 11% (p = 0.03), while enhanced sIPSC frequency by 86 ± 12% (p = 0.009). In other 4 neurons, 10 μM ATP enhanced the firing rate by 71 ± 15% (p = 0.009), while inhibited sIPSC frequency by 50 ± 7% (p = 0.003). There is a significant inverse correlation between ATP regulation of the firing rate and the sIPSC frequency (r = -0.88, n = 7, p = 0.01, Pearson correlation). These data suggest a possible relationship between ATP regulation of sIPSCs and the activity of the DA neurons.

Dependence of ATP Modulation of sIPSCs on TTX-Sensitive Sodium Channels

We tested the effect of ATP on mIPSCs in the presence of both DNQX (20 μM) and TTX (0.5 μM). In this set of experiments, we first reexamined the effects of ATP on sIPSCs. Consistent with the results in Fig. 1, in some neurons, 10 μM ATP increased sIPSC frequency (Fig. 7A_1, left column), by 77 ± 24% (n = 6, p = 0.003, Fig. 7A_2, left panel). In another group of neurons, 10 μM ATP reduced sIPSC frequency (Fig. 7 B_1, left column) by 46 ± 7% (n = 6, p = 0.0008, Fig. 7B_2, left panel). After washout of ATP, TTX was applied to the neurons. As illustrated in the upper panels in Fig. 7A_1 and B_1, 0.5 μM TTX significantly reduced the frequency of sIPSCs (by 64 ± 7%, n = 10, p = 0.003), but did not significantly change their mean amplitude (by 5 ± 17%, n = 10, p = 0.4). After a new baseline (in the presence of TTX) was established, 10 μM ATP was applied again. As illustrated in the right columns of Fig. 7A_1 and B_1, as well as the right panel of Fig. 7A_2 and B_2, in the presence of TTX, 10 μM ATP had no significant effect on sIPSC frequency in either one of these two groups: Enhancement: by 8 ± 21% (n = 6, p = 0.37, Fig. 7A_1 and A_2; Inhibition: by -14 ± 8%, n = 6, (p = 0.17, Fig. 7B_1 and B_2). These results are significantly different from those obtained in the absence of TTX (p < 0.01 in either groups), indicating the involvement of sodium channels in ATP modulation.

EtOH Effects on ATP Modulation of sIPSCs

EtOH inhibits P2X receptors in a variety of preparations, including bullfrog dorsal root ganglion neurons, hippocampal neurons, and Xenopus (Li et al., 1998; Li et al., 2000; Davies et al., 2002; Davies et al., 2005), and HEK293 expression systems. To determine whether the P2Rs at the GABA-releasing terminals on VTA DA neurons are sensitive to EtOH, we tested the effects of 10 μM ATP on sIPSC frequency in the absence and the presence of EtOH (10 or 40 mM).

In the neurons in which 10 μM ATP alone decreased sIPSC frequency (by -43 ± 9%, n = 5, p = 0.004, Fig. 8A_1 left column and A_2), after 4 min preincubation with 40 mM EtOH, 10 μM ATP (plus 40 mM EtOH) decreased sIPSC frequency by -66 ± 5% (n = 5, p = 0.0001, Fig.

$8A_1$ right column and A_2). These two values are significantly different (n = 5, p = 0.04, paired t-test, Fig. $8A_2$). However, 4 min preincubation with 10 mM EtOH did not significantly affect the inhibitory effect of 10 μM ATP on sIPSC frequency: ATP alone, by -43 ± 5% (n = 7, p = 0.0002), ATP plus 10 mM EtOH, by -48 ± 11% (n = 7, p = 0.003). These two values have no statistically significant difference (p = 0.33, paired t-test, Fig. $8A_2$).

In those neurons in which 10 μM ATP alone increased sIPSC frequency (by 45 ± 13%, n = 5, p = 0.02, Fig. $8B_1$, left column and B_2), after 4 min preincubation with 40 mM EtOH, 10 μM ATP (plus 40 mM EtOH) did not significantly change sIPSC frequency (by -17 ± 19%, n = 5, p = 0.12, Fig. $8B_1$, right column and B_2). Thus, the increase in sIPSC frequency induced by 10 μM ATP was eliminated by 40 mM EtOH (p = 0.03, paired t-test, Fig. $8B_2$). Similarly, 10 mM EtOH also eliminated the increase in sIPSC frequency induced by 10 μM ATP (43 ± 13%, n = 7, p = 0.01 in ACSF, p = 0.007 verses -22 ± 17%, n = 7, p = 0.17 in EtOH, paired t-test, Fig. $8B_2$).

Overall, the findings from the EtOH studies suggest that the P2Rs at GABA releasing terminals are sensitive to EtOH, and the P2Rs that mediate ATP-induced increase in sIPSC frequency are more sensitive to EtOH than those that mediate ATP-induced decrease in sIPSC frequency.

Discussion

In the present study, we demonstrate that P2XR and P2YR existed at the GABA-releasing terminals which make synapses on the VTA DA neuron. By acting on these receptors, ATP either increased or decreased GABA release, and the net effect in a particular cell depended on which P2R predominates. The ATP-induced increase or decrease in GABA release probably resulted from the activation of the P2XRs or of the P2YRs, respectively. Further, ATP modulation of DA neuron activity may be correlated with its effect on GABA release. We also demonstrate that EtOH altered the effect of ATP on GABA release through the P2Rs at the GABA-releasing terminal. Our results indicate that both P2XR and P2YR are sensitive to EtOH at clinically relevant concentrations (≤ 40 mM). We postulate that the presynaptic P2Rs in the VTA are important targets for EtOH.

ATP Effect Depends on P2Rs at the GABA-Releasing Terminals on the VTA DA Neurons

Activation of presynaptic P2Rs modulates the release of a number of neurotransmitters including acetylcholine, noradrenaline, dopamine, glutamate, serotonin, GABA and glycine in various regions of the CNS. We showed that in the mechanically dissociated VTA DA neurons, ATP (at concentrations below 200 μM) modulated sIPSC frequency in a concentration-dependent manner without affecting sIPSC amplitude. This suggests that ATP works at the presynaptic site. Furthermore, the action of ATP was abolished by TTX, indicating that it depends on TTX-sensitive sodium channels. While the underlying mechanism is unclear, a simple explanation is that ATP may act on the upstream of GABA-releasing terminals that require action potential propagation to conduct the signal to the GABA- releasing site.

ATP modulation of GABA release was significantly suppressed by suramin (a nonselective P2R antagonist). Furthermore, suramin or PPADS alone profoundly suppressed GABA release. These suppressions indicate that: (1) P2Rs exist at the GABA-releasing terminal, and (2) These P2Rs tonically modulate the release of GABA. These results are consistent with previous reports that show spontaneous ATP release in brain tissues and the existence of functional P2Rs at GABA-releasing terminals. The sources of ATP in the isolated neurons remain to be identified. ATP could be released from nerve terminals (Burnstock, 1997), from the glia cells (Abbracchio and Ceruti, 2006), and/or from the soma of a neuron (Zhang et al., 2007). In the VTA, suramin affected not only the frequency but also the amplitude of sIPSCs. The reduction

in sIPSC amplitude could be resultant of an inhibition of postsynaptic $GABA_A$ receptors or of a reduction in the number of quanta in each release.

The dualistic effect of ATP may be resultant of the interplay of different types of P2Rs. We attempted to dissect the possible contribution of P2XR and of P2YR. In contrast to the variable effects of ATP, α,β-MeATP (a P2XR agonist) consistently increased sIPSC frequency. This observation is consistent with previous studies that show that activation of P2XR facilitates neurotransmitter release (see review (Illes and Ribeiro, 2004). In our experiments, α,β-MeATP increased sIPSCs more potently in those neurons in which ATP had a facilitating effect. It has been shown that α,β-MeATP activates many subunits of P2XR, including $P2X_1$, $P2X_3$, $P2X_{2/3}$, and $P2X_{1/5}$. The desensitization of heteromultimeric $P2X_{2/3}$ receptors in response to the agonist was slower (>10 s) than that of the other subtypes of P2XRs. Our results showed that the increase in sIPSC frequency induced by both ATP and α,β-MeATP had a similar slow desensitization (> 30 s) (Fig. 1B and Fig. 3). These data suggest that $P2X_{2/3}$ receptors exist at the GABA-releasing terminals on VTA DA neurons. This is supported by previous findings that show $P2X_3$ subunit expression in rat midbrain synaptosomes and high levels of $P2X_2$ subunit expression in the VTA (Krugel et al., 2001; Heine et al., 2001).

Unlike ATP and α,β-MeATP, ADP-βS (a preferential agonist for P2YR) consistently reduced sIPSC frequency. This suggests that P2YRs exist at GABA-releasing terminals, which mediate ATP inhibition of sIPSCs. Taken together, our data demonstrate that both P2XR and P2YR exist at the GABA-releasing terminal and that the contributions of these two P2Rs in different neurons are not equal. The interplay between these P2Rs may account for the complex effects of ATP. That is, in neurons in which P2XR contributes more, ATP will increase sIPSCs, whereas in neurons where P2YR predominates, ATP will reduce sIPSC frequency. This hypothesis is supported by a previous study that shows that both P2XRs and P2YRs are expressed in VTA non-dopaminergic neurons (Heine et al., 2007).

ATP altered sIPSC frequency in a dose-dependent manner. The ATP concentration at which ATP-induced increase in sIPSC frequency reached its maximum was 10 μM, whereas the concentration at which ATP-induced decrease in sIPSC frequency reached its maximum was much higher (100 μM). Although we are not able to quantify the EC_{50} or IC_{50} of ATP in each case, we speculate that the P2XR in our preparation was more sensitive to ATP than the P2YR. We also showed that 10 μM ATP increased or decreased the spontaneous firing rate in 59% and 29% of the VTA DA neurons, respectively. These percentages are close to the incidence of the ATP-induced decrease (54%) and increase (32%) in GABA release (Fig.1). Furthermore, ATP modulation of the firing rate inversely correlates with its effects on sIPSC frequency. These data support the possibility that through affecting GABA transmission, ATP plays a significant role in the regulation of DA neuronal activity. The complex effects of ATP on GABA release and firing rate of the VTA DA neurons seem not to be compliant with the previous observation that 2MeSATP, an ATP analogue, increases neuronal activity (Krugel et al., 2003) and somatodendritic release of dopamine (Krugel et al., 2001) in the VTA. However, the higher incidence of the excitatory effect of ATP suggests that in the VTA the activation of P2YR apparently overcomes that of P2XR, thus decreasing GABA release and increasing DA neuron activity.

We recorded the ATP-induced transient current in 28% (29/105) of the VTA DA neurons tested. This current was sensitive to suramin and PPADS and depended on extracellular calcium. This suggests the existence of functional P2XR on the soma, consistent with previous morphological studies (Vulchanova et al., 1996; Heine et al., 2007). In the current study, ATP effects on the firing rate of VTA DA neuron are as steady as its effects on sIPSCs during application; meanwhile, the incidence (59%) of ATP-induced excitation of VTA DA neuron is much higher than that (28%) of ATP-induced transient current. This suggests that the

presynaptic P2YRs, but not the pre- and post-synaptic P2XRs, are the primary mediators for the excitatory effect of ATP.

Presynaptic P2Rs in the VTA are Sensitive to EtOH

We have demonstrated that the ATP-induced increase in GABA release was abolished by 10 and 40 mM EtOH. This is consistent with previous studies showing that EtOH inhibits P2XRs. Previous studies have shown that the action of EtOH on P2XR is subunit-dependent; for example, homomeric $P2X_4$ receptor is more sensitive to the inhibitory effects of EtOH than homomeric $P2X_2$ receptors (Davies et al., 2002). In $P2X_3$ receptor-expressing Xenopus oocytes, the ATP-induced current was potentiated by EtOH at the concentration that has been shown to cause motor control deficits (Davies et al., 2005). However, in $P2X_3$ receptor-expressing HEK 293 cells, the ATP-induced current was not significantly altered by EtOH at concentrations as high as 100 mM (Fischer et al., 2003). We could not explain why EtOH shows different effect(s) in different expression systems at this moment, but the different expression systems are one of the possibilities. Our recent work on chimeric $P2X_2$ and $P2X_3$ receptors showed that the subunit of the P2XR could alter the responses to EtOH significantly (Personal communication, Dr. Liana Asatryan, University of Southern California). Therefore, it is possible that heteromeric $P2X_{2/3}$ receptor demonstrates different EtOH sensitivity from that of homomeric $P2X_2$ and $P2X_3$ receptors. In >50% of the VTA DA neurons tested ATP decreased GABA release, and this effect was enhanced by 40 mM EtOH. Since ATP activates both the P2XR and the P2YR, the potentiation by 40 mM EtOH of ATP-induced inhibition of GABA release could be superimposing effects of inhibition of P2XR and activation of P2YR. The net effect is to inhibit GABA release.

We previously reported that in brain slice preparations, EtOH increases or decreases GABA release onto VTA DA neurons in the presence or the absence of DAMGO, a μ-opioid agonist, which silences the GABA-releasing neurons (Xiao and Ye, 2008). This implies that multiple EtOH targets exist at GABA-releasing terminals. EtOH inhibition of P2XR and/or potentiation of P2YR may enhance ATP-induced disinhibition of VTA DA neurons, which may lead to increased dopamine release. Revealing mechanisms of the multiple effects of ATP and EtOH on GABA release onto the VTA DA neurons will provide new therapeutic avenues in the treatment of drug abuse and of alcoholism.

Acknowledgments

We thank Dr. Olga Ostrovskaya and Ms. Kimberly Sokol for helpful comments and suggestions.

This work is supported by NIH AA-11989, AA-016964, AT001182 and AA013922.

Abbreviations

ATP	Adenosine 5′- triphosphate
α,β-MeATP	α,β- Methylene ATP
APV	DL-2-Amino-5-phosphonopentanoic acid
DA neuron	dopaminergic neuron
DNQX	6,7-dinitroquinoxaline-2, 3-dione
GABA	γ-aminobutyric acid
mIPSCs	miniature inhibitory postsynaptic currents
P2 receptor	purinergic type 2 receptor

sIPSCs	spontaneous inhibitory postsynaptic currents
TTX	tetrodotoxin
VTA	ventral tegmental area

References

Abbracchio MP, Ceruti S. Roles of P2 receptors in glial cells: focus on astrocytes. Purinergic Signal 2006;2:595–604. [PubMed: 18404462]

Akaike N, Moorhouse AJ. Techniques: applications of the nerve-bouton preparation in neuropharmacology. Trends Pharmacol Sci 2003;24:44–47. [PubMed: 12498731]

Brodie MS, Shefner SA, Dunwiddie TV. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res 1990;508:65–69. [PubMed: 2337793]

Burnstock G. The past, present and future of purine nucleotides as signalling molecules. Neuropharmacology 1997;36:1127–1139. [PubMed: 9364468]

Charlton ME, Sweetnam PM, Fitzgerald LW, Terwilliger RZ, Nestler EJ, Duman RS. Chronic ethanol administration regulates the expression of GABAA receptor alpha 1 and alpha 5 subunits in the ventral tegmental area and hippocampus. J Neurochem 1997;68:121–127. [PubMed: 8978717]

Davies DL, Kochegarov AA, Kuo ST, Kulkarni AA, Woodward JJ, King BF, Alkana RL. Ethanol differentially affects ATP-gated P2X(3) and P2X(4) receptor subtypes expressed in Xenopus oocytes. Neuropharmacology 2005;49:243–253. [PubMed: 15993446]

Davies DL, Machu TK, Guo Y, Alkana RL. Ethanol sensitivity in ATP-gated P2X receptors is subunit dependent. Alcohol Clin Exp Res 2002;26:773–778. [PubMed: 12068244]

Davies DL, Trudell JR, Mihic SJ, Crawford DK, Alkana RL. Ethanol potentiation of glycine receptors expressed in Xenopus oocytes antagonized by increased atmospheric pressure. Alcohol Clin Exp Res 2003;27:743–755. [PubMed: 12766618]

Fischer W, Wirkner K, Weber M, Eberts C, Koles L, Reinhardt R, Franke H, Allgaier C, Gillen C, Illes P. Characterization of P2X3, P2Y1 and P2Y4 receptors in cultured HEK293-hP2X3 cells and their inhibition by ethanol and trichloroethanol. J Neurochem 2003;85:779–790. [PubMed: 12694404]

Ford CP, Mark GP, Williams JT. Properties and opioid inhibition of mesolimbic dopamine neurons vary according to target location. J Neurosci 2006;26:2788–2797. [PubMed: 16525058]

Franke H, Krugel U, Illes P. P2 receptors and neuronal injury. Pflugers Arch 2006;452:622–644. [PubMed: 16645849]

Heine C, Wegner A, Grosche J, Allgaier C, Illes P, Franke H. P2 receptor expression in the dopaminergic system of the rat brain during development. Neuroscience 2007;149:165–181. [PubMed: 17869006]

Illes P, Ribeiro JA. Neuronal P2 receptors of the central nervous system. Curr Top Med Chem 2004;4:831–838. [PubMed: 15078214]

Imperato A, Di Chiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 1986;239:219–228. [PubMed: 3761194]

Johnson SW, North RA. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol 1992;450:455–468. [PubMed: 1331427]

Khakh BS. Molecular physiology of P2X receptors and ATP signalling at synapses. Nat Rev Neurosci 2001;2:165–174. [PubMed: 11256077]

Krugel U, Kittner H, Franke H, Illes P. Stimulation of P2 receptors in the ventral tegmental area enhances dopaminergic mechanisms in vivo. Neuropharmacology 2001;40:1084–1093. [PubMed: 11406200]

Krugel U, Kittner H, Franke H, Illes P. Purinergic modulation of neuronal activity in the mesolimbic dopaminergic system in vivo. Synapse 2003;47:134–142. [PubMed: 12454951]

Lazarowski ER, Boucher RC, Harden TK. Mechanisms of release of nucleotides and integration of their action as P2X- and P2Y-receptor activating molecules. Mol Pharmacol 2003;64:785–795. [PubMed: 14500734]

Li C, Peoples RW, Weight FF. Ethanol-induced inhibition of a neuronal P2X purinoceptor by an allosteric mechanism. Br J Pharmacol 1998;123:1–3. [PubMed: 9484847]

Li C, Xiong K, Weight FF. Ethanol inhibition of adenosine 5′-triphosphate-activated current in freshly isolated adult rat hippocampal CA1 neurons. Neurosci Lett 2000;295:77–80. [PubMed: 11090978]

Margolis EB, Hjelmstad GO, Bonci A, Fields HL. Kappa-opioid agonists directly inhibit midbrain dopaminergic neurons. J Neurosci 2003;23:9981–9986. [PubMed: 14602811]

Melis M, Camarini R, Ungless MA, Bonci A. Long-lasting potentiation of GABAergic synapses in dopamine neurons after a single in vivo ethanol exposure. J Neurosci 2002;22:2074–2082. [PubMed: 11896147]

Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL. Sites of alcohol and volatile anaesthetic action on GABA(A) and glycine receptors. Nature 1997;389:385–389. [PubMed: 9311780]

Mori M, Heuss C, Gahwiler BH, Gerber U. Fast synaptic transmission mediated by P2X receptors in CA3 pyramidal cells of rat hippocampal slice cultures. J Physiol 2001;535:115–123. [PubMed: 11507162]

Nakazawa K, Inoue K, Ito K, Koizumi S, Inoue K. Inhibition by suramin and reactive blue 2 of GABA and glutamate receptor channels in rat hippocampal neurons. Naunyn Schmiedebergs Arch Pharmacol 1995;351:202–208. [PubMed: 7770102]

North RA. Molecular physiology of P2X receptors. Physiol Rev 2002;82:1013–1067. [PubMed: 12270951]

Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM. Blocking GABA(A) receptors in the anterior ventral tegmental area attenuates ethanol intake of the alcohol-preferring P rat. Psychopharmacology (Berl) 1998;139:108–116. [PubMed: 9768548]

Papp L, Vizi ES, Sperlagh B. Lack of ATP-evoked GABA and glutamate release in the hippocampus of P2X7 receptor-/- mice. Neuroreport 2004;15:2387–2391. [PubMed: 15640761]

Rubio ME, Soto F. Distinct Localization of P2X receptors at excitatory postsynaptic specializations. J Neurosci 2001;21:641–653. [PubMed: 11160443]

Stobbs SH, Ohran AJ, Lassen MB, Allison DW, Brown JE, Steffensen SC. Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves N-methyl-D-aspartate receptors. J Pharmacol Exp Ther 2004;311:282–289. [PubMed: 15169831]

Theile JW, Morikawa H, Gonzales RA, Morrisett RA. Ethanol enhances GABAergic transmission onto dopamine neurons in the ventral tegmental area of the rat. Alcohol Clin Exp Res 2008;32:1040–1048. [PubMed: 18422836]

Vulchanova L, Arvidsson U, Riedl M, Wang J, Buell G, Surprenant A, North RA, Elde R. Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. Proc Natl Acad Sci U S A 1996;93:8063–8067. [PubMed: 8755603]

Woodward JJ. Ethanol and NMDA receptor signaling. Crit Rev Neurobiol 2000;14:69–89. [PubMed: 11253956]

Xiao C, Ye JH. Ethanol dually modulates GABAergic synaptic transmission onto dopaminergic neurons in ventral tegmental area: Role of mu-opioid receptors. Neuroscience. 2008

Xiao C, Zhang J, Krnjevic K, Ye JH. Effects of ethanol on midbrain neurons: role of opioid receptors. Alcohol Clin Exp Res 2007;31:1106–1113. [PubMed: 17577392]

Ye JH, Wang F, Krnjevic K, Wang W, Xiong ZG, Zhang J. Presynaptic glycine receptors on GABAergic terminals facilitate discharge of dopaminergic neurons in ventral tegmental area. J Neurosci 2004;24:8961–8974. [PubMed: 15483115]

Zhang X, Chen Y, Wang C, Huang LY. Neuronal somatic ATP release triggers neuron-satellite glial cell communication in dorsal root ganglia. Proc Natl Acad Sci U S A 2007;104:9864–9869. [PubMed: 17525149]

Zhou C, Xiao C, McArdle JJ, Ye JH. Mefloquine enhances nigral gamma-aminobutyric acid release via inhibition of cholinesterase. J Pharmacol Exp Ther 2006;317:1155–1160. [PubMed: 16501066]

Zhu PJ, Lovinger DM. Retrograde endocannabinoid signaling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. J Neurosci 2005;25:6199–6207. [PubMed: 15987949]

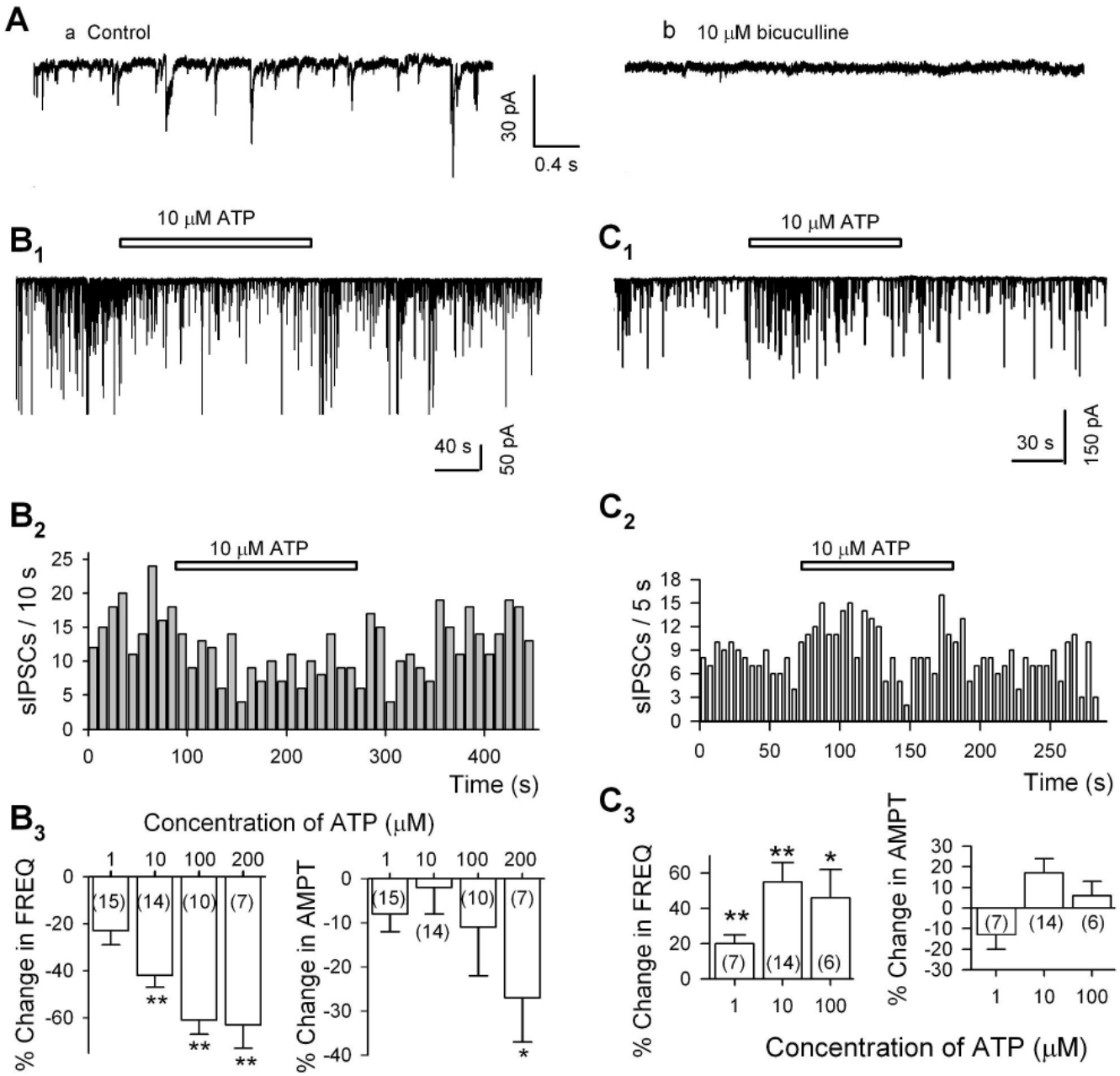

Fig.1.

ATP has multiple effects on GABA release in VTA DA neurons. A, The spontaneous IPSCs were blocked by 10 μM bicuculline. B_1, sIPSCs recorded from a DA neuron of a P16 rat, before, during and after the application of 10 μM ATP. B_2, Time course of ATP inhibition of sIPSC frequency. B_3, Summary of the effect of ATP (1-200 μM) on the frequency (FREQ, left panel) and amplitude (AMPT, right panel) of sIPSCs. The number in brackets is the number of neurons examined. C_1, sIPSCs recorded from a DA neuron of a P14 rat, before, during and after the application of 10 μM ATP. C_2, Time course of ATP facilitation of sIPSC frequency. C_3, Summary of the effect of ATP (1-100 μM) on the frequency (left panel) and amplitude (right panel) of sIPSCs. * $p < 0.05$; ** $p < 0.01$, paired t test, ATP verses pre-ATP conditions.

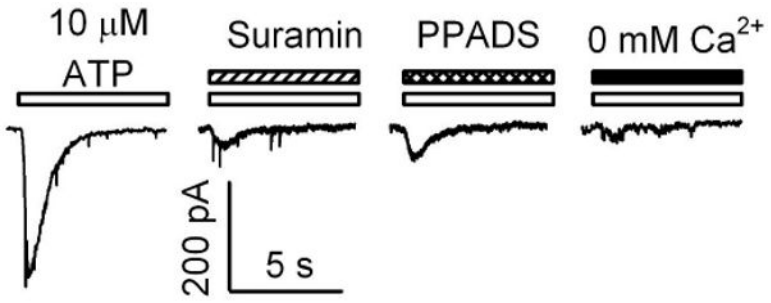

Fig. 2.

ATP induces transient current in VTA DA neurons. Representative traces of ATP (10 μM)-induced currents, which was dramatically inhibited by 100 μM Suramin and 10 μM PPADS, and eliminated in the absence of extracellular calcium.

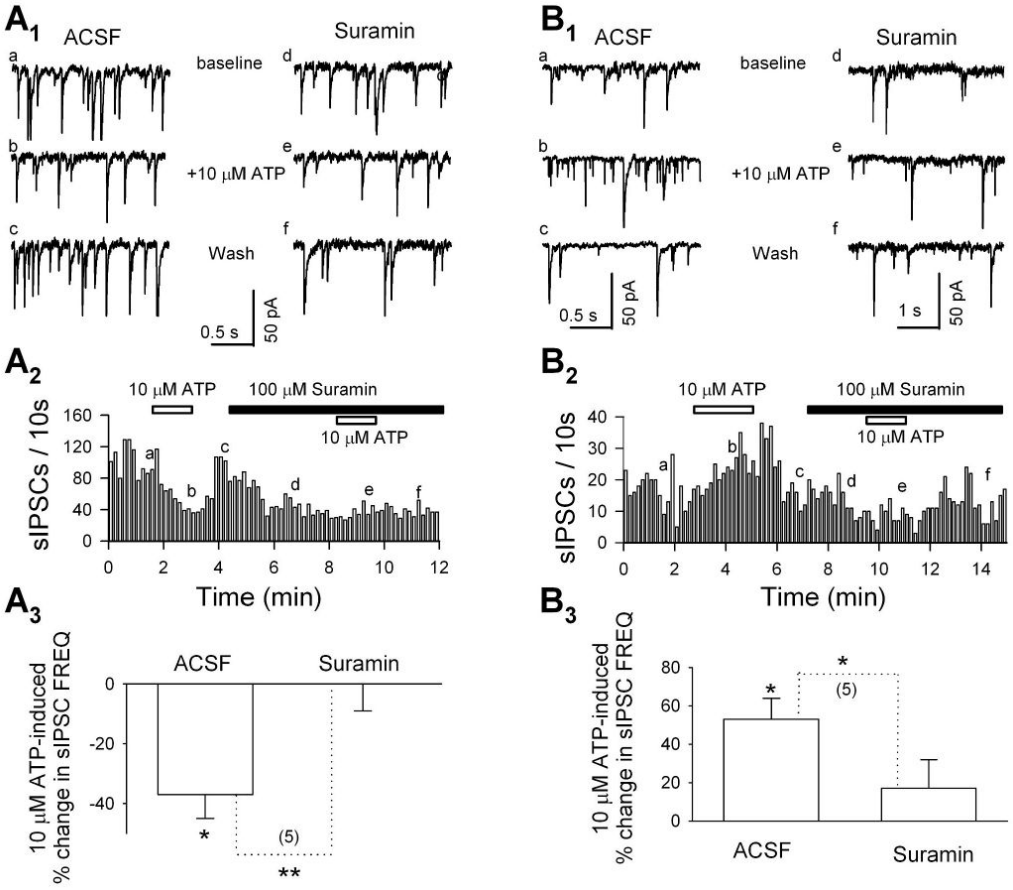

Fig.3.

Suramin abolishes ATP modulation of sIPSC frequency in VTA DA neurons. Representative traces (A_1) showing that the inhibition of sIPSC frequency by 10 μM ATP (left column) is abolished in the presence of suramin (100 μM) (right column). A_2, The corresponding time course of A_1. B_1, Representative traces showing that the enhancement of sIPSC frequency by 10 μM ATP (left column) is abolished in the presence of suramin (100 μM) (right column). B_2, The corresponding time course of B_1. A3, and B3, Summary of the effect of ATP (10 μM) on sIPSC frequency in the absence (ACSF) and presence of suramin. * $p < 0.05$; ** $p < 0.01$, paired t test, ATP verses pre-ATP conditions, or as indicated.

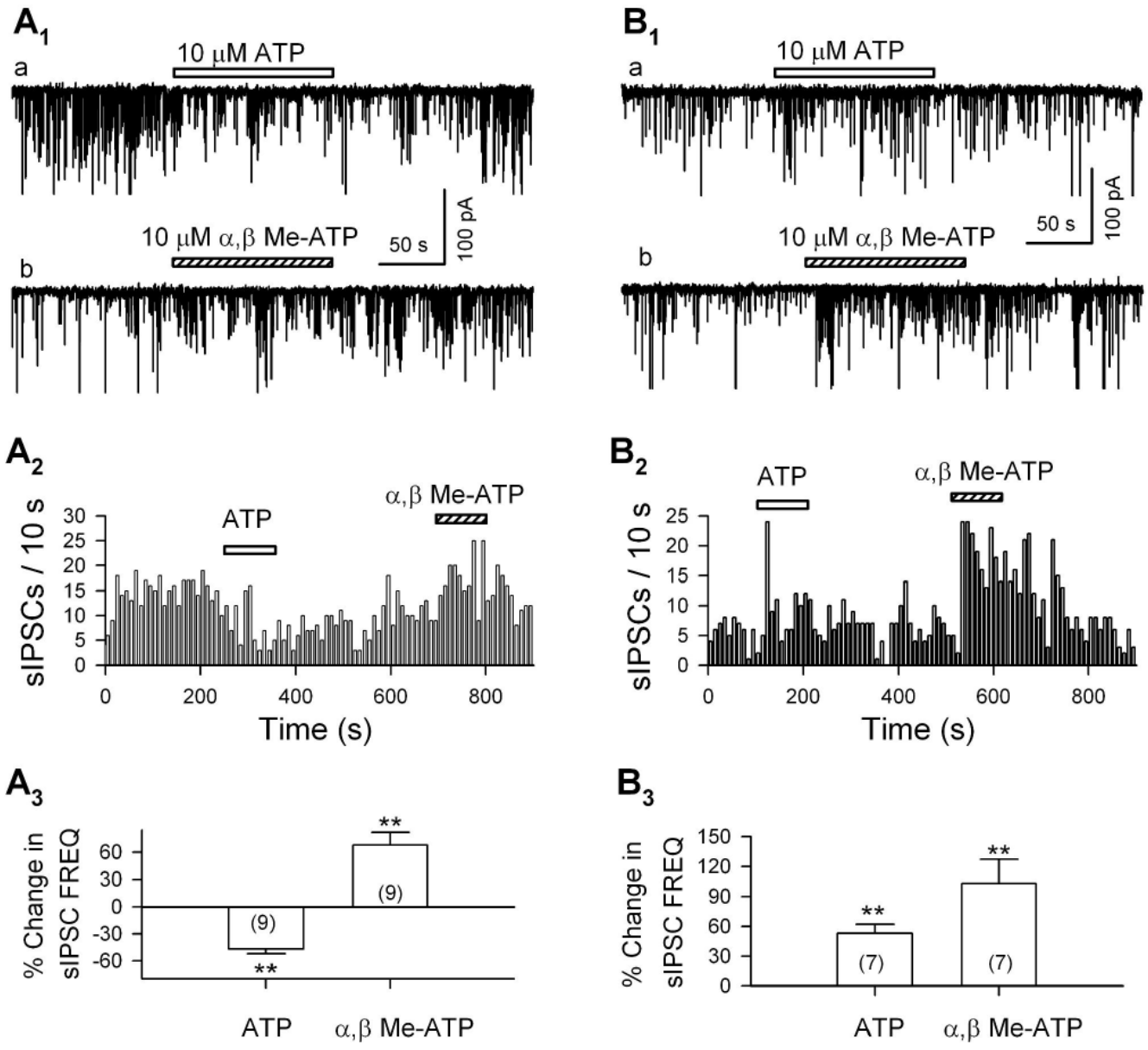

Fig.4.

α,β-MeATP enhances sIPSC frequency in VTA DA neurons. $A_{1\text{-}3}$, 10 μM α,β-MeATP increased sIPSC frequency (Representative traces (A_1, b), corresponding time course (A_2), and summary (A_3)) on VTA DA neurons, on which 10 μM ATP decreases sIPSC frequency (Representative traces (A_1, a), corresponding time course (A_2), and summary (A_3)). $B_{1\text{-}3}$, 10 μM α,β-MeATP increases sIPSC frequency (typical traces (B_1, b), corresponding time course (B_2), and summary (B_3) on VTA DA neurons, in which 10 μM ATP decreases sIPSC frequency (Representative traces (B_1, a), corresponding time course (B_2), and summary (B_3)). The number in brackets is the number of neurons examined. ** $p < 0.01$, paired t test of the effects of ATP or α,β-MeATP verses pre ATP or α,β-MeATP conditions.

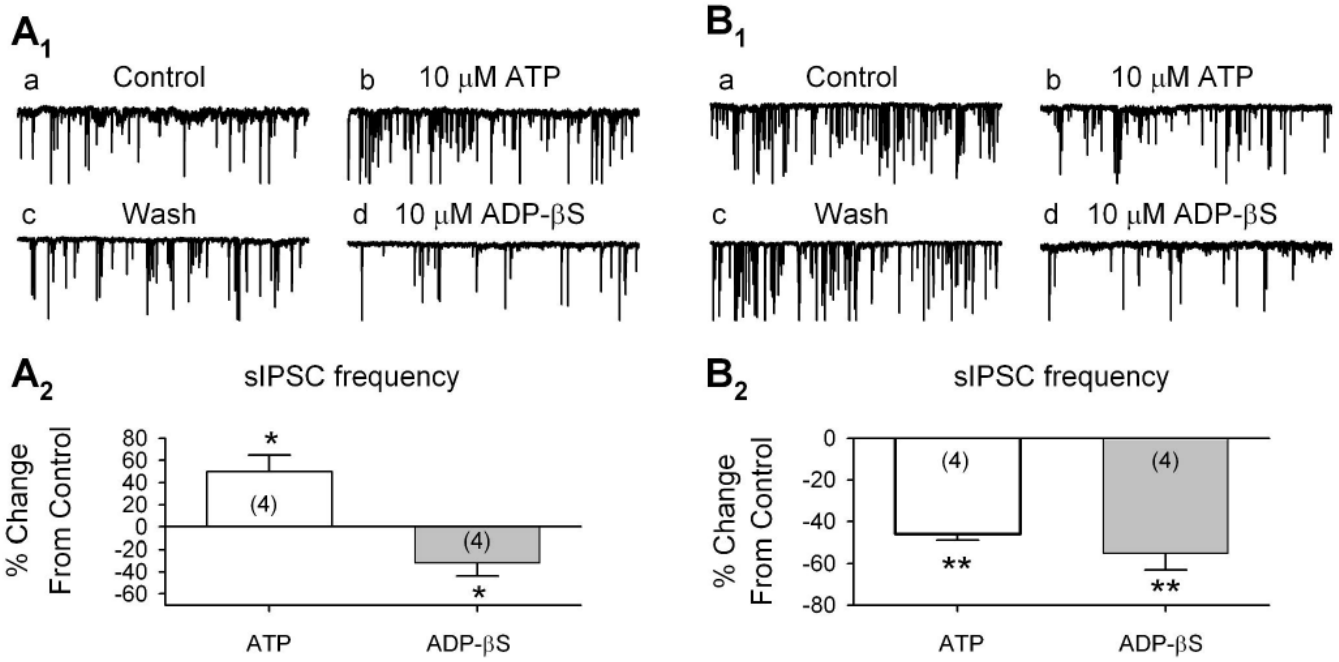

Fig. 5.

ADP-βS inhibits sIPSC frequency in VTA DA neurons. $A_{1\text{-}2}$, 10 μM ADP-βS decreased sIPSC frequency (Representative traces (A_1, c, d) and summary (A_2)) on VTA DA neurons, on which 10 μM ATP increases sIPSC frequency (Representative traces (A_1, a, b) and summary (A_2)). $B_{1\text{-}2}$, 10 μM ADP-βS decreases sIPSC frequency (typical traces (B_1, c, d) and summary (B_2) on VTA DA neurons, in which 10 μM ATP decreases sIPSC frequency (Representative traces (B_1, a, b) and summary (B_2)). The number in brackets is the number of neurons examined. ** $p < 0.01$, paired t test of the effects of ATP or ADP-βS verses pre-ATP or pre-ADP-βS conditions.

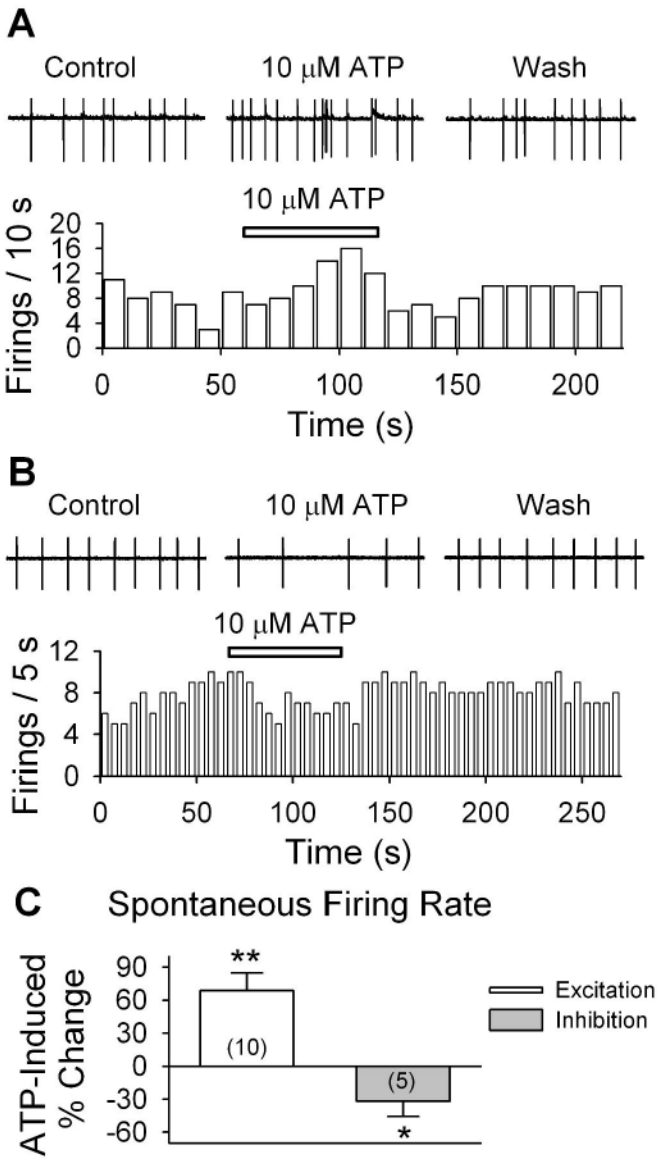

Fig. 6.

ATP modulates the activity of VTA DA neurons. A, 10 s traces (upper panels) and time course (lower panel) showing that 10 μM ATP reversibly increases spontaneous firing in a VTA DA neuron. B, 10 s traces (upper panels) and time course (lower panel) showing that 10 μM ATP reversibly decreases spontaneous firing in VTA DA neurons. C, Summary of ATP-induced increase (open square) or decrease (filled square) in the spontaneous firing rates in VTA DA neurons. The number in brackets is the number of neurons examined.* $p < 0.05$, ** $p < 0.01$, ATP verses pre-ATP conditions.

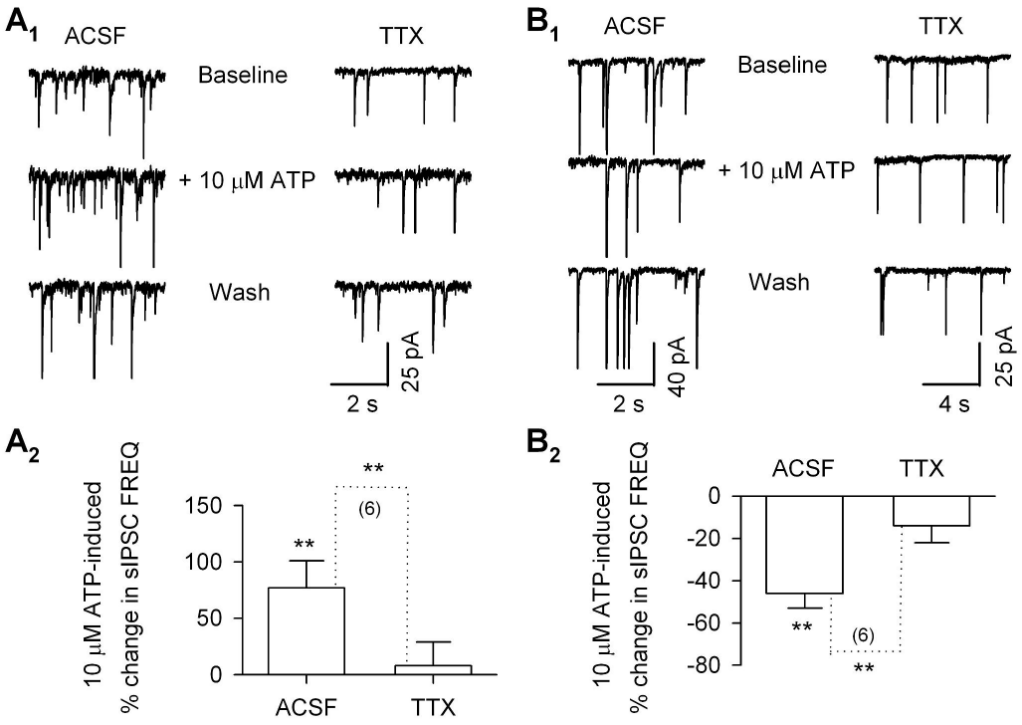

Fig. 7.

The effects of ATP on sIPSCs depend on TTX-sensitive sodium channels. Miniature IPSCs (mIPSCs) are recorded in the presence of TTX (0.5 μM). A_1, Representative traces showing that in a VTA DA neuron, 10 μM ATP increases the frequency of spontaneous IPSCs in the absence of TTX (left panels, ACSF), but has no effect on the frequency of mIPSCs in the presence of TTX (right panels, TTX). B_1, Representative traces showing that in another VTA DA neuron, 10 μM ATP reduces sIPSC frequency in the absence of TTX (left panels, ACSF), but has no effect in the presence of TTX (right panels, TTX). A_2-B_2, Summary of the effect of 10 μM ATP on the frequency of sIPSCs and mIPSCs. The number in brackets is the number of neurons examined. ** $p < 0.01$, paired t test, ATP verses pre ATP conditions.

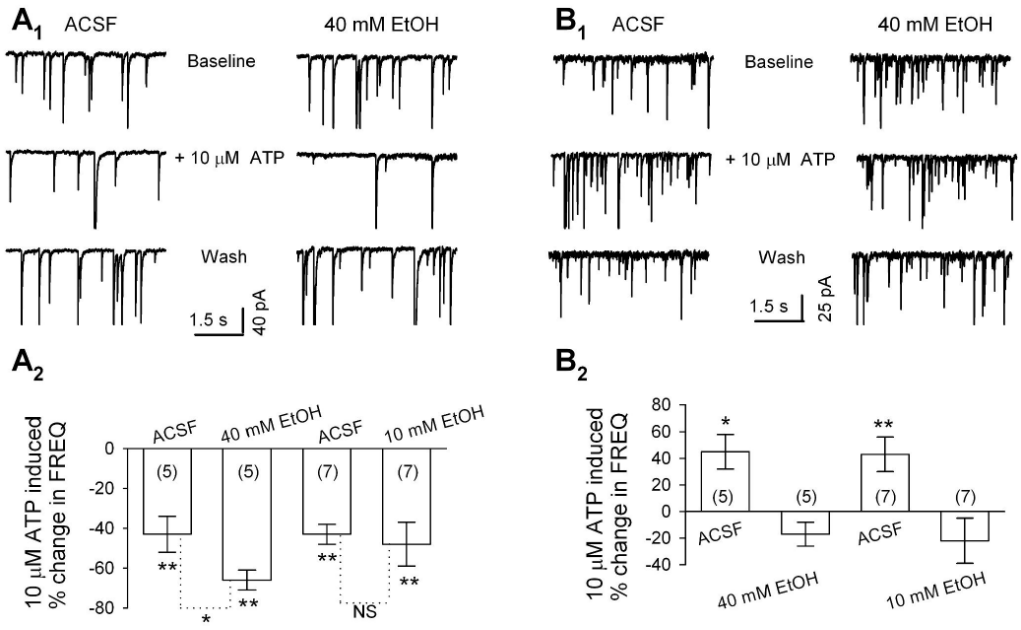

Fig. 8.

EtOH enhances the decrease, but reduces the increase of sIPSC frequency induced by 10 μM ATP. A_1, EtOH at 40 mM, but not 10 mM increases 10 μM ATP- induced decrease of sIPSC frequency. B_1, 40 mM EtOH reduces10 μM ATP –induced increase in sIPSC frequency. A_2, B_2, Summary of the effect of 10 μM ATP on sIPSC frequency in the absence or the presence of 10 or 40 mM EtOH. The number in brackets is the number of neurons examined.* $p < 0.05$, ** $p < 0.01$, paired t test, ATP verses pre ATP conditions.