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ORIGINAL ARTICLE

# Modulation of ATP-induced LTP by cannabinoid receptors in rat hippocampus

**Olexandr Ievglevskyi · Oleg Palygin · Elena Kondratskaya · Sergei Grebenyuk · Oleg Krishtal**

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**Abstract** Cannabinoids exert powerful action on various forms of synaptic plasticity. These retrograde messengers modulate GABA and glutamate release from presynaptic terminals by acting on presynaptic CB1 receptors. In particular, they inhibit long-term potentiation (LTP) elicited by electrical stimulation of excitatory pathways in rat hippocampus. Recently, LTP of the field excitatory postsynaptic potential (fEPSP) induced by exogenous ATP has been thoroughly explored. The present study demonstrates that cannabinoids inhibit ATP-induced LTP in hippocampal slices of rat. Administration of 10 μM of ATP led to strong inhibition of fEPSPs in CA1/CA3 hippocampal synapses. Within 40 min after ATP removal from bath solution, robust LTP was observed (fEPSP amplitude comprised 130.1± 3.8% of control, $n$=10). This LTP never appeared when ATP was applied in addition to cannabinoid receptor agonist WIN55,212-2 (100 nM). Selective CB1 receptor antagonist, AM251 (500 nM), completely abolished this effect of WIN55,212-2. Our data indicate that like canonical LTP elicited by electrical stimulation, ATP-induced LTP is under control of CB1 receptors.

**Keywords** LTP · ATP · Cannabinoids · WIN · Adenosine

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O. Ievglevskyi · O. Palygin · E. Kondratskaya · S. Grebenyuk (✉) · O. Krishtal
Department of Cellular Membranology,
Bogomoletz Institute of Physiology,
Kiev, Ukraine
e-mail: sg@biph.kiev.ua

O. Krishtal
State Key Laboratory for Molecular and Cellular Biology,
Kiev, Ukraine

## Introduction

Apart from its role as "molecular unit of currency" of intracellular energy transfer, ATP is involved in intercellular signaling: when released, it acts as a neurotransmitter and powerful neuromodulator. Its role in neuronal plasticity mechanisms has been also disclosed [9–11].

To date, the sources of extracellular ATP are well determined. ATP is co-released with classical neurotransmitters at neuronal synapses, where it activates purinergic receptors and contributes to postsynaptic signals in these synapses [12–18]. Purinoreceptors, extracellular sensors of ATP, are abundantly expressed throughout the brain in glial cells and neurons [12, 19]. A number of studies indicate that the locus of modulatory action of ATP on synaptic transmission can be both post- and presynaptic [15, 20–23]. Although neurons have been traditionally considered as the sole source of ATP and adenosine in the CNS, glial cells have been recently shown to release ATP as well [24, 25].

Within hippocampal neuronal circuits, ATP is involved in numerous mechanisms of signal transduction, for example, in facilitation of excitatory transmission to *stratum radiatum* interneurons [22]. ATP mediates glutamatergic activity-dependent heterosynaptic suppression when released from astrocytes [26]. A significant part of ATP-evoked effects can be attributed to the hydrolysis products of this nucleotide.

ATP can act directly via P2X [27], P2Y [28] receptors and indirectly via its catabolites, ADP, AMP, cAMP, and adenosine produced by membrane-bound ecto-nucleotidases, ecto-protein kinases, and ecto-ATPases [17, 29, 30]. Adenosine is known to be a potent inhibitory neuromodulator, increasing postsynaptic K+ conductance and decreasing presynaptic Ca2+ conductance [18, 31–34].

In a series of studies [9, 10, 35–37], superfusion of hippocampal slices with ATP caused, after initial depression

of evoked potentials, rebound facilitation of field excitatory postsynaptic potentials (fEPSP). The latter effect is observed after drug removal and persists in in vitro preparations for a relatively prolonged period, up to 1 h. This phenomenon has been classified as "ATP-induced long-term potentiation (LTP)" [10] and thus can be compared to electrically evoked LTP, originally described in the hippocampus by Bliss and Lomo [38]

Long-lasting changes in synaptic efficiency caused by ATP were first defined by Wieraszko [10]. Extensive analysis of ATP-induced plasticity has been carried out by Fujii group [9]. The phenomenon has been found to depend on NMDA receptor activity [8]. In further studies, phosphorylation of extracellular membrane domains (possibly those of NMDA receptors) by ecto-protein kinases has been suggested as a part of underlying mechanism of ATP-induced LTP [9]. Recent studies demonstrate that ATP does not modulate activity-dependent homosynaptic long-term depression (LTD) in rat CA1 hippocampal region. These studies suggest that the effects of ATP on the synaptic plasticity are selective, triggering LTP but not interfering with LTD [39].

The expression of cannabinoid receptors is relatively high in hippocampus, cerebellum, and basal ganglia [40]. Endogenous cannabinoids are intercellular signaling molecules. They are synthesized from postsynaptic membrane lipids in Ca2+-triggered manner and travel back through the postsynaptic membrane reaching presynaptic terminals [41]. As a result, these retrograde messengers bind to presynaptic CB1 receptors and depress synaptic transmission in hippocampus and cerebellum via the presynaptic mechanism. Typically, these relatively rapid changes in synaptic transmission involve depression of neurotransmitter release both in inhibitory and excitatory synapses [42]. Several studies indicate that tetanus-induced LTP at hippocampal excitatory synapses is impaired by cannabinoids [43–45]. Induction of LTP in CA1 area can trigger a group I mGluR-dependent LTD at inhibitory synapses (I-LTD) which is mediated by retrograde endocannabinoid signaling. I-LTD may, in turn, underlie changes of pyramidal cell excitability associated with LTP at excitatory synapses [46]. This is indicative of CB1 receptors playing a critical role for learning and memory formation in hippocampus (see also [1–7]). In this paper, we demonstrate that exogenous cannabinoids prevent ATP-induced LTP in CA1 area of rat hippocampus.

## Materials and methods

### Rat hippocampal slice preparation

All experiments were performed in accordance with the Guiding Principles for Care and Use of Animals in the Field of Physiological Sciences of the Physiological Society of Ukraine and approved by the local animal care Committee of the Bogomoletz Institute of Physiology. Wistar rats (19–20 days old) were anesthetized by diethyl ether and then decapitated. Hippocampi were gently removed and cut into transverse slices (400 μm) with a vibrating slice cutter (Campden Vibroslicer) in ice-cold artificial cerebrospinal fluid (ACSF). The cutting procedure was performed in ACSF that contained (in millimolar): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$ $7H_2O$, 1 $NaH_2PO_4$, 26.2 $NaHCO_3$, 2.5 $CaCl_2$, and 11 glucose. ACSF was equilibrated with 95% $O_2$/5% $CO_2$ (310 mOsm/L; pH 7.4 when saturated with the gas mixture).The slices were incubated in the standard ACSF for 40–60 min at room temperature before the experiments. Just before recording, a slice was transferred to the recording chamber that was continuously perfused with ACSF which was delivered with gravity-fed perfusion system at a flow rate of 2.5–3 ml/min. The CA1 pyramidal cell layer and Schaffer collateral pathway were visually identified with the infrared differential interference contrast video microscope (Olympus); IR-1000 (DAGE-MTI, Michigan City) and captured with CoolSNAP ES2 (CCD ICX285). Recordings were made at temperature 30–32°C in the recording chamber.

### Field postsynaptic potential recording (fEPSP) in the rat hippocampal slices

Standard electrophysiological techniques were used to record field potentials. Presynaptic stimulation was applied to the medial part of Shaffer collaterals using a bipolar tungsten electrode at 0.05 Hz frequency. fEPSPs were recorded in a pyramidal layer of CA1 area using a glass microelectrode. In all experiments, the amplitude of test fEPSP was adjusted to one third of a maximum. Recording electrodes were fabricated from borosilicate glass capillaries of 1.5 mm outer diameter (Model GD-1.5, Narishige Scientific Instruments Lab.) using programmable puller (P-97; Sutter Instruments).

### Data acquisition and analysis

Recordings and preprocessing of data were made by custom software written by Dr. Grebenyuk in Labview 8.0. Data analysis was performed with p-CLAMP (Molecular Devices, CA, USA) and Origin 7. Values are the mean±SE for $n$ slices. Two-tailed Student's $t$ test for equal sample size and Welch's $t$ test for unequal sample size were used for statistical comparison.

### Drugs

The following drugs were purchased from Sigma (St Louis, MO, USA): adenosine 5′-triphosphate sodium salt (ATP),

WIN55,212-2 mesylate, ®-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt, DPCPX (8-cyclopentyl-1,3-dipropylxanthine), and adenosine. AM251 (*N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide) NF 279 (8,8′-[Carbonylbis(imino-4,1-phenylenecarbonylimino-4,1- phenylenecarbonylimino)]bis-1,3,5-naphthalenetrisulfonic acid hexasodium salt), A74003 (*N*-[1-[[(Cyanoamino)(5-quinolinylamino)methylene]amino]- 2,2-dimethylpropyl]-3,4-dimethoxybenzeneacetamide). Reactive Blue 2 and PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid tetrasodium salt) were all purchased from Tocris Cookson (Bristol, UK). WIN55,212-2 and AM251 were dissolved in DMSO, and the other drugs were dissolved in deionized water. The drugs were stored as concentrated stock solutions at −20°C and dissolved in the extracellular solution right before recordings.

## Results

### Effect of cannabinoids on ATP-mediated plasticity in hippocampus

Bath application of ATP (10 μM, 10 min) caused strong inhibition of both population spike and fEPSP amplitude (up to 40% of control amplitude). After washout of ATP, prolonged enhancement of fEPSP (up to 140% for at least 40 min, see Fig.1a) was observed. These results are in concert with earlier observations by other groups [8, 10, 20, 35]. Interestingly, short application of ATP (10 μM, 5 min, Supplementary Fig. S1a, $n$=5) as well as prolonged application (10 μM, 30 min, Supplementary Fig. S1b, $n$=4) was not followed by potentiation.

To test the hypothesis that cannabinoids affect ATP-mediated LTP processes in hippocampus, we applied 10 μM of ATP in addition to cannabinoid receptor agonist, WIN55,212-2 (WIN-2, below), 100 nM. In line with other study, Fig. 1b demonstrates a rundown of fEPSP amplitude in continuous presence of WIN-2 (100 nM, solid circles, $n$=4). In another set of experiments, application of ATP (10 μM, 10 min, Fig. 1b, open circles, $n$=4) during continuous WIN-2 application reproduced short-term drop in fEPSP amplitude with no sign of subsequent potentiation. We have succeeded in separation of inhibitory action of WIN-2 on synaptic transmission from its specific effect on the synaptic plasticity. Application of 100 nM WIN-2 for 20 min did not result in the rundown of synaptic transmission (Fig.1c, $n$=5).

Application of 10 μM ATP in addition to WIN-2 produced inhibition of fEPSP amplitude. However, in contrast to ATP-only treated slices, the inhibition was not followed by fEPSP potentiation after drug washout. The final amplitude of fEPSP after the washout of both drugs comprised 97.4±4.5% of control fEPSP amplitude (Fig.1d, $n$=4). When applied after establishment of LTP, WIN-2 (100 nM, 20 min) did not alter the amplitude of fEPSP (Fig. S2, $n$=2).

We verified whether the action of WIN-2 is mediated by CB1 receptors. Selective antagonist of CB1 receptors, AM251 (500 nM), enabled ATP-induced LTP in the presence of WIN-2. Application of the cocktail comprising 10 μM ATP, 500 nM AM251, and 2 μM WIN-2 resulted in transient depression and slow augmentation of fEPSP above control level upon cocktail washout (126.6±4.2% of control fEPSP amplitude, $n$=6, Fig.2a). AM251 itself did not affect synaptic transmission (data not shown). Application of ATP in addition to AM251 (Fig. 2b) resulted in significantly stronger LTP as compared to a result of sole ATP application (149.5±5.2%, $n$=5 vs. 130.1±3.8%, $n$=10, $p$<0.01). This observation may indicate that CB1 receptors mediate tonic inhibition of ATP-mediated plasticity. The summary of data on the induction of LTP in various conditions is presented in Fig. 3.

### Effects of ATP analogs

ATP is known to degrade very rapidly to adenosine and other products of catabolism [29]. In order to check the possibility that observed phenomena result from the activity of ATP hydrolysis products, we used non-hydrolysable ATP analog, α,β,methylene-ATP, attempting to reproduce the effects of ATP.

α,β,methylene-ATP failed to reproduce either inhibition of fEPSP during the drug superfusion or potentiation of fEPSP upon drug removal (Fig. 3). Complete lack of α,β, methylene-ATP effect indicates that ATP hydrolysis is critical for induction of ATP-induced LTP. Thus, we confirm previous data, suggesting critical dependence of ATP-induced LTP on ATP dephosphorylation by ecto-protein kinases and concomitant phosphorylation of membrane proteins [9].

Rapid breakdown of ATP resulting from ecto-protein kinase and ecto-ATPase activity leads to local accumulation of adenosine in extracellular space. This can be a primary cause of fEPSP depression accompanying application of ATP.

In order to examine the involvement of adenosine receptors in the observed effects, we used selective A1 receptor antagonist, DPCPX (200 nM). ATP was applied in addition to the A1 antagonist after 10 min of preincubation with the latter. DPCPX alone induced potentiation of fEPSP amplitude (up to 150–170%, Fig.4a). When applied in addition to DPCPX, ATP produced much smaller inhibition of fEPSC than in experiments given in Fig.1a (11.8±2.7% vs. 41.6±3.9%, Fig.4b) and did not result in LTP after washout.

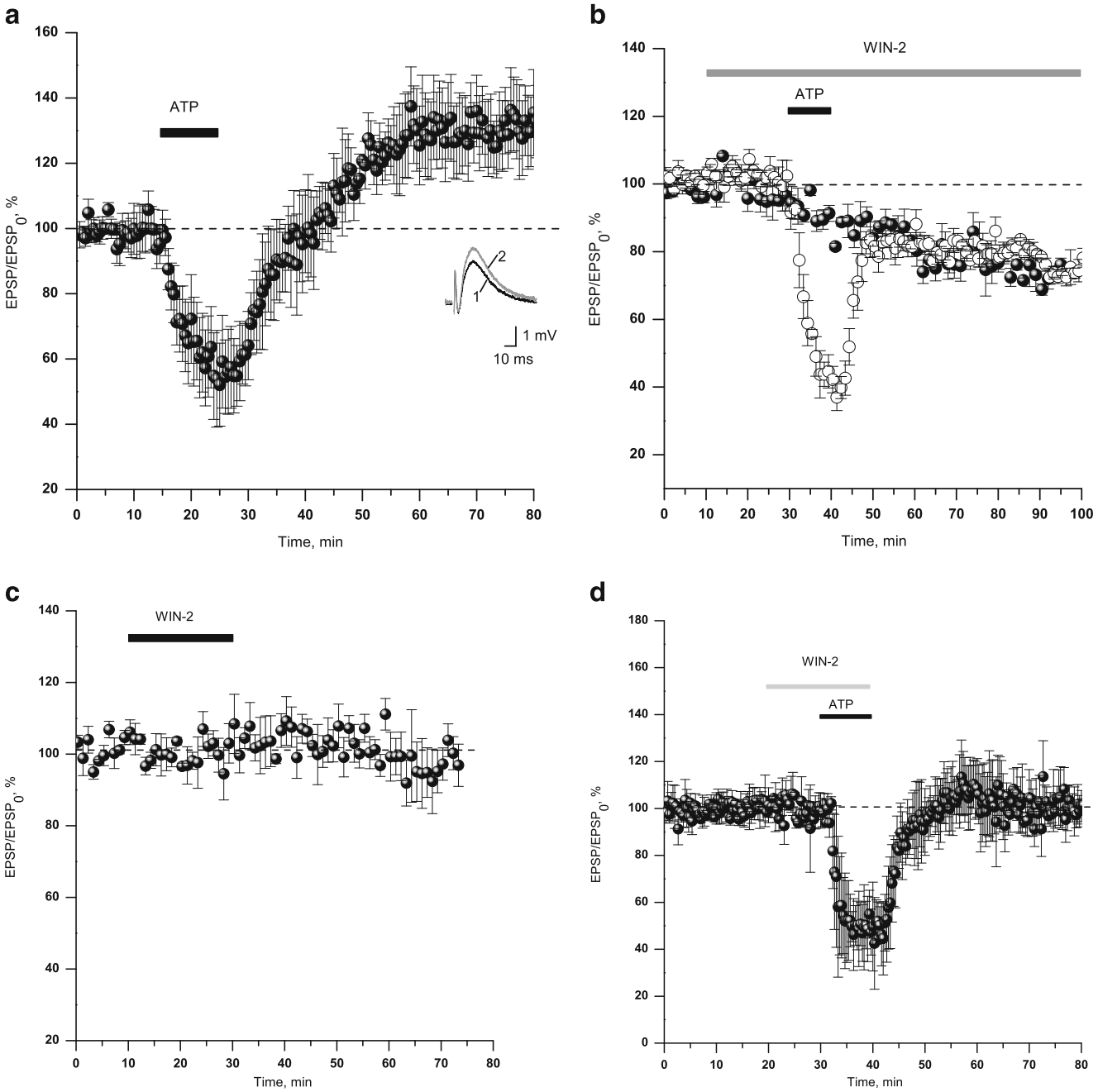


**Fig. 1** Cannabinoids prevent the induction of ATP-induced LTP in CA1 region of hippocampus. **a** ATP-induced long-term potentiation (130.1±3.8%, $n=10$). Time course plot represents averaged amplitudes of fEPSPs recorded in CA1 neurons in response to test stimuli delivered to Schaffer collaterals/commissural afferents at 0.05 Hz. *Horizontal bar* represents application protocol: ATP (10 μM, here and *below*). *Inset*: Examples of field fEPSP in control (*1*) and after LTP induction (*2*). **b** fEPSP amplitude declines in the continuous presence of WIN55,212-2 (WIN-2, *below*), 100nM (*solid spheres*); WIN-2 disables induction of ATP-induced LTP ($n=4$, *open circles*). **c** Short application of WIN-2 (100 nM) did not affect fEPSP amplitude. **d** ATP does not induce LTP after short application of 100 nM of WIN-2

## Purinoreceptors

The data concerning the involvement of purinoreceptors in ATP-induced modulation of synaptic transmission are rather controversial [12, 16, 47]. We pursued this issue performing experiments with selective antagonists to various subtypes of P2X and P2Y receptors: PPADS (10 μM, nonselective P2X antagonist), NF279 (2 μM, $P2X_1$ selective antagonist), A74003 (1 μM, $P2X_7$-selective antagonist), and Reactive Blue 2 (10 μM, nonselective P2Y antagonist). Each of these antagonists was applied 10 min before ATP application; all the drugs were removed simultaneously with ATP. In all

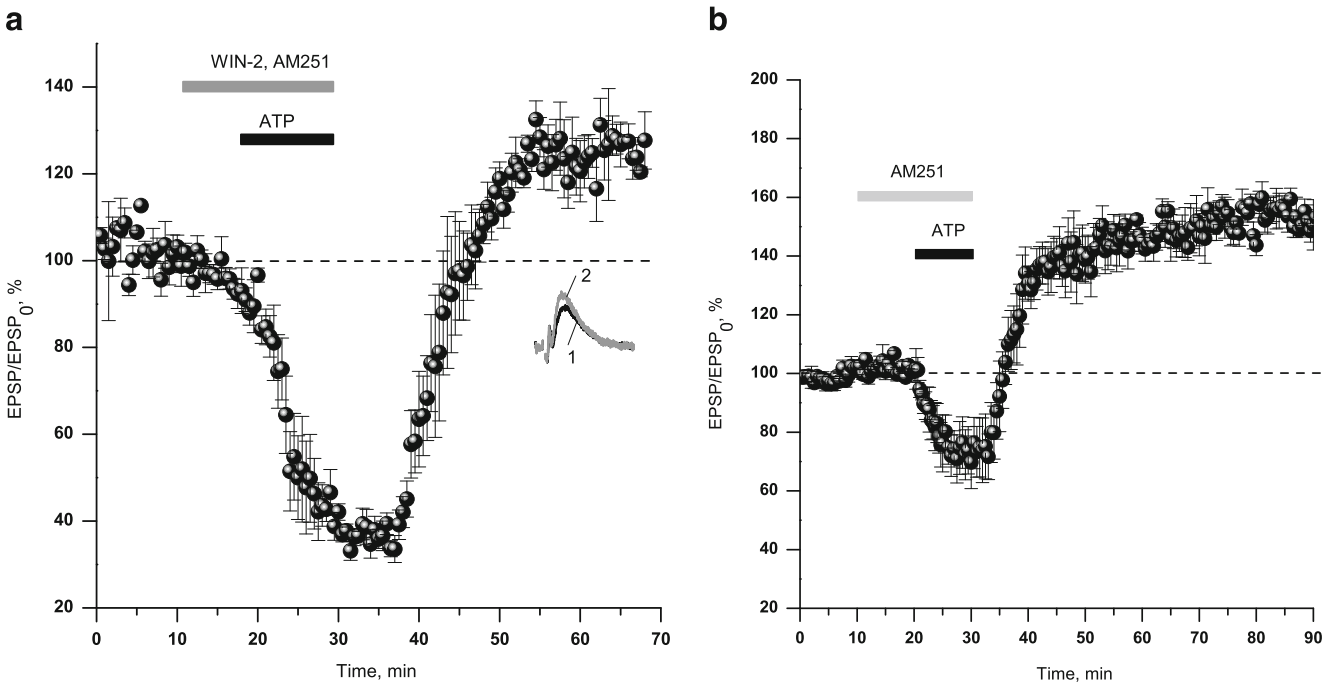


**Fig. 2** Cannabinoid receptors are responsible for the suppression of ATP-induced LTP. **a** Selective CB1 receptor antagonist, AM251 (500 nM), allows the induction of ATP-induced LTP in the presence of high concentration of WIN-2 (2 μM, 126.6±4.2%, $n$=6). **b** AM251 (500 nM) does not alter synaptic transmission, but yields a stronger ATP-induced LTP as compared to control LTP (149.5±5.2%, $n$=5)

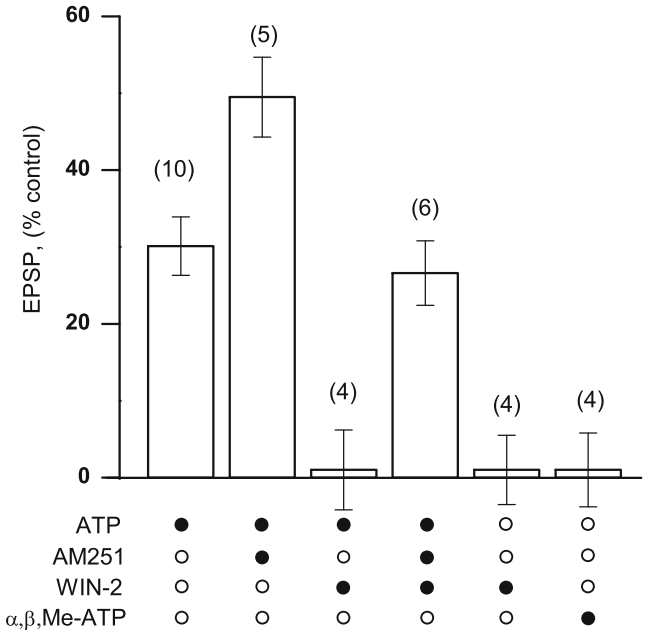


**Fig. 3** The summary of data on the induction of LTP in different combinations of ATP and cannabinoid receptor agonist/antagonist. *Columns* represent mean relative values of fEPSP amplitude after drug washout. The mean amplitude of fEPSP in the 10-min period before the application of any drug was defined as 100%. *Solid circles* denote the presence of corresponding drug in the experiment, whereas their absence in the application mixture is indicated by *open circles*

cases, ATP-induced depression of fEPSP was not markedly altered.

However, all these antagonists abolished or strongly inhibited LTP induction (Fig.5). Tentatively, we conclude that the inhibition of fEPSP during ATP application cannot be attributed to the activation of P2 receptors; however, these receptors may play a certain role in the initiation of ATP-induced LTP (see "Discussion" section).

## Discussion

Cannabinoid receptors have been shown to impair induction of tetanus-induced LTP in hippocampus [45, 48] and LTD in cerebellum [49]. We have tested a possibility of functional involvement of cannabinoid receptors into a novel type of synaptic plasticity in hippocampus, ATP-induced LTP. Our data demonstrate the ability of cannabinoid receptors to control the induction of this form of LTP in CA1 area of rat hippocampus: WIN-2 used in concentration of 100 nM completely abolished ATP-induced LTP.

There is diversity of reports in regard to the effects exhibited by cannabinoids on the synaptic transmission. Along with the cases where WIN-2 acted to inhibit synaptic transmission [50], cannabinoid-induced astrocyte-mediated potentiation of synaptic transmission has been shown [51]. The effect of cannabinoids was also principally dependent

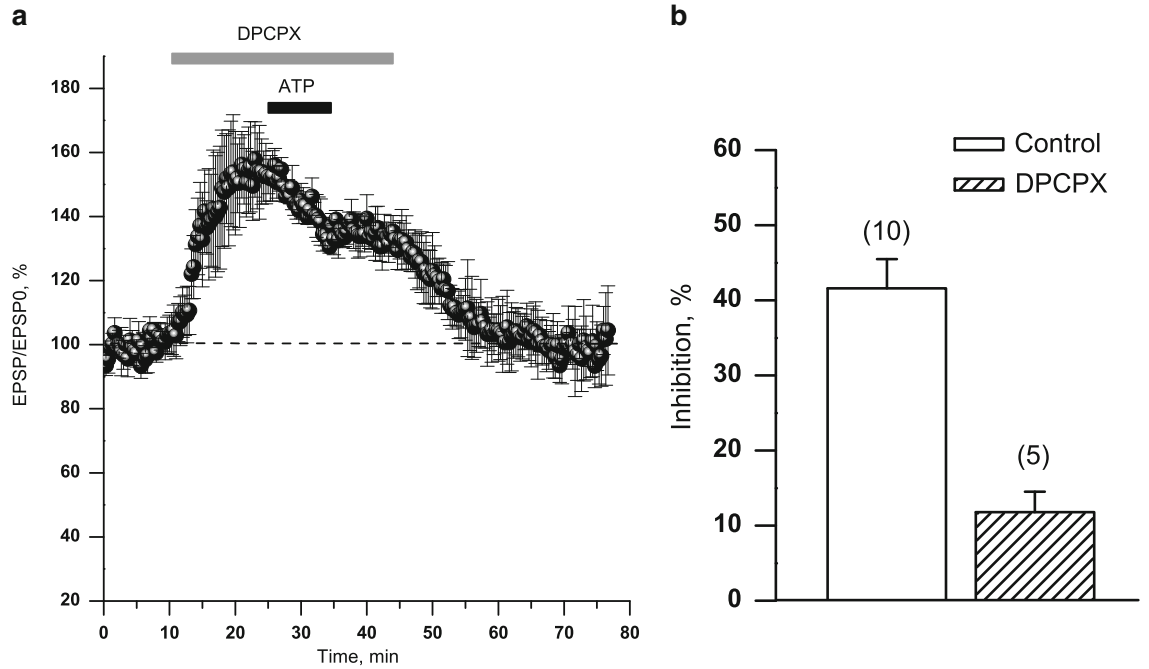


**Fig. 4** DPCPX, selective A1 receptor antagonist prevents induction of ATP-induced LTP. **a** fEPSPs recorded from the slices perfused with 10 μM ATP in addition to 200 nM DPCPX. fEPSP is back to control after removal of both drugs. **b** *Open column*: Magnitude of fEPSP inhibition by ATP in control (obtained in the experiments demonstrated in Fig. 1a). *Solid column*: a drop in fEPSP amplitude measured as the steady-state after ATP application **a**

on the age of animals: in neonatal rats (10–13 days) WIN-2 inhibited synaptic transmission, but did not alter single fEPSPs in young adult rats (4–6 weeks) [52]. Even for a given neuron, cannabinoids inhibited vesicular release to a lesser extent at dendritic synapses as compared to perisomatic ones [53]. We have found that, when applied at 100-nM concentration (minimal concentration at which reproducible effects could be observed) for 20 min, WIN-2 did not alter fEPSP amplitude, but completely prevented ATP-induced LTP. When applied after induction of LTP by ATP, WIN did not affect fEPSP indicating that cannabinoid receptors affect induction rather than maintenance of ATP-induced LTP.

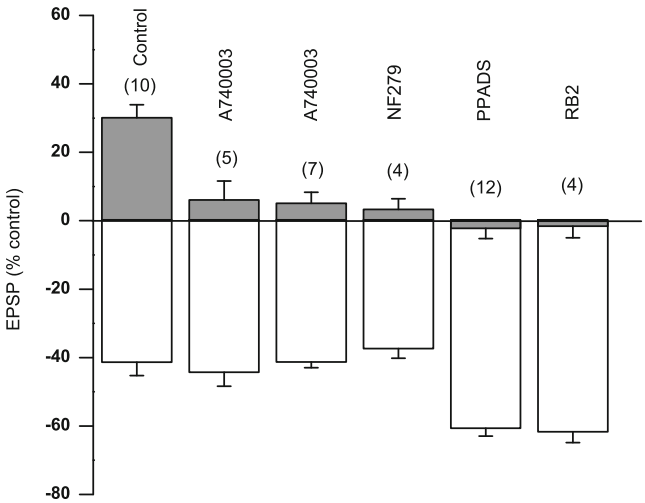


**Fig. 5** P2 receptor antagonists prevent ATP-induced LTP. The figure summarizes effects of various P2 antagonists on fEPSP amplitude. Each drug was applied 10 min before ATP and washed out simultaneously with ATP. *Open columns* represent maximal inhibition of fEPSP amplitude by ATP. *Solid columns* represent fEPSP amplitudes at the steady-state after drug washout. *Numbers in brackets* indicate the number of experiments

Recent data show the presence of postsynaptic CB2 cannabinoid receptors in hippocampus [54]. Although WIN-2 activates both CB1 and CB2 receptors, the effect of WIN was completely abolished by AM251, selective antagonist of CB1 receptors, which is indicative of CB1 receptors playing a regulatory role in ATP-induced LTP. Presynaptic localization of CB1 receptors prompts that the blockade of LTP by cannabinoid agonist results from a CB1 receptor-mediated decrease in the probability of glutamate release [55, 56]. However, used in our experiments in minimal concentration, WIN-2 did not result in apparent changes in the synaptic transmission while leading to the complete inhibition of ATP-induced LTP. These observations indicate the existence of a specific mechanism by which cannabinoids affect ATP-induced synaptic plasticity. The nature of such mechanism remains to be addressed.

The phenomenon of ATP-induced LTP has been studied by several groups [9–11], but exact mechanisms of the phenomenon remain obscure. According to Dunwiddie et al., ATP is rapidly converted to adenosine by membrane ecto-ATPases and ecto-protein kinases. The half-life of

ATP in extracellular space comprises approximately 200 ms [29]. This fact suggests that some manifestations of ATP as a signaling agent can be related to adenosine. Indeed, our experiments indicate that A1 adenosine receptors are necessary for the development of ATP-induced LTP (Fig.4). On the other hand, experiments with non-hydrolysable analog of ATP, α,β,methylene-ATP, demonstrate that the hydrolysis of ATP is necessary for LTP to occur. Fujii and co-authors suggested NMDA receptors as the target for the phosphorylation [9] during ATP hydrolysis. In our study, blockade of almost any type of purinoreceptors led to failure in LTP induction (Fig. 5). In view of this observation, the role of P2X and P2Y receptors in this phenomenon seems highly probable. It should be noted though that there is a strong evidence on the nonspecific actions of P2 receptor antagonists [57, 58]. Thus, it has been shown that all P2X and P2Y antagonists also inhibit ecto-nucleotidases and ecto-ATPases in concentrations similar to those used to block purinoreceptors. One can hypothesize that ecto-protein kinases supposed to form a principal link in ATP-induced LTP [8, 9, 35, 36] may be also inhibited by P2-receptor antagonists, thus preventing the development of ATP-induced LTP.

Glial cells are established modulators of neuronal activity and memory processes [59]. Being a potent source of extracellular ATP, adenosine, and neurotransmitters in CNS, glial cells may play a role in ATP-induced LTP. Extracellular ATP directly targets astroglial and neuronal P2X receptors [60] stimulating ATP and glutamate release from astrocytes and modulating the function of postsynaptic receptors [61–64]. In addition, extracellular adenosine has been shown to induce release of glutamate and NO [65, 66] from glial cells. NO, in its turn, enhances vesicular release by stimulating cGMP synthesis at the presynaptic site and upregulates trafficking of AMPA receptors at the postsynaptic site [67]. Thereby, it may contribute to the induction of ATP-induced LTP like it has been demonstrated for tetanus-induced LTP [68–72]. The present study indicates that long-term synaptic plasticity induced by ATP is cannabinoid-dependent: ATP-induced LTP is inhibited by the activation CB1 receptors.

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