ATP- and Adenosine-Mediated Signaling in the Central Nervous System: The Role of Extracellular ATP in Hippocampal Long-Term Potentiation

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Abstract. Application of 10 μM ATP for 10 min transiently depressed, then slowly augmented, synaptic transmission in CA1 neurons, leading to long-term potentiation (LTP) (ATP-induced LTP). This ATP-induced LTP was blocked by addition of an N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, d,l-2-amino-5-phosphonovalerate (5 μM). For ATP-induced LTP, delivery of test synaptic inputs once every 20 s to CA1 neurons could be substituted by application of 100 nM NMDA during ATP perfusion. In addition, ATP-induced LTP was blocked by co-application of an ecto-protein kinase inhibitor, K-252b (40 nM), whereas a P2X purinoreceptor antagonist, pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid 4-sodium (50 μM), or a P2Y purinoreceptor antagonist, basilen blue (10 μM), had no effect. These results, therefore, indicate that the mechanisms of ATP-induced LTP involve the modulation of NMDA receptors/Ca2+ channels and the phosphorylation of extracellular domains of synaptic membrane proteins, one of which could be the NMDA receptor/Ca2+ channel.

Keywords: long-term depression, NMDA receptor, extracellular phosphorylation, synaptic plasticity

Adenosine 5'-triphosphate (ATP), a common constituent of synaptic vesicles which is released mainly as a co-transmitter together with noradrenaline, acetylcholine, or other substances (1), enters hippocampal CA1 neuron synapses following electrical stimulation of Schaffer collaterals (2). In CA1 neurons, long-term potentiation (LTP) can be induced by extracellular application of ATP (ATP-induced LTP) during test electrical stimulation delivered at 0.05 Hz (3). However, the mechanism of ATP-induced LTP remains unclear. Thus, the role of ATP in the mechanism involved in ATP-induced LTP was studied. Some of the results were presented elsewhere (3, 4).

Perfusion for 10 min with 10 μM ATP caused a transient reduction in the response, which, after removal of ATP, was followed by a gradual increase to a potentiated plateau, which was maintained for at least 30 min (ATP-induced LTP, filled circles in Fig. 1). Ecto-protein kinase, reported to be present in neuronal cells as a plasma membrane-associated protein kinase with an extracellular catalytic site (5), may use extracellular ATP as an orthophosphate donor for membrane protein phosphorylation (5). Thus, one possible mechanism could be that ATP acts via ecto-protein kinases (6). K-252b is a derivative of K-252a, a potent ecto-protein kinase inhibitor that is effective on several types of kinase (7, 8).

In order to determine whether the mechanisms of ATP-induced LTP in hippocampal CA1 neurons involved the activation of ecto-protein kinases, we examined the effects of K-252b, an ecto-protein kinase inhibitor. After co-perfusion of slices for 10 min with 10 μM ATP and 40 nM K-252b followed by drug removal, no potentiation of the slope of the excitatory postsynaptic potential (S-EPSP) was seen for at least 40 min, the S-EPSP 40 – 50 min after the end of perfusion being significantly lower than that measured 40 – 50 min after application of ATP alone (P<0.01, Fig. 1). This result shows that K-252b blocked ATP-induced LTP by preventing the activation of ecto-protein kinases, which phosphorylate the extracellular domains of synaptic membrane proteins, one of which could be the NMDA receptor/Ca2+ channel.

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Another possible mechanism could be an effect on P2 purinoceptors that has been reported to cause increased release of glutamate at presynaptic terminals and increased excitability of postsynaptic neurons in rat hippocampal neurons (9, 10). P2 purinoceptors fall into two primary subclasses (11), the P2X subclass, which consists of ligand-gated calcium channels functionally related to glutamate and nicotinic acetylcholine receptors, and the P2Y subclass, which consists of metabotropic receptors that initiate biological processes by G-protein-dependent activation of phospholipase C and a subsequent increase in intracellular Ca\(^{2+}\) levels (12). In this study, we therefore examined whether co-application of pyridoxal phosphate 6-azophenyl-2',4'-disulfonic acid 4-sodium (PPADS), a P2X purinoceptor antagonist (13), or basilen blue, a P2Y purinoceptor antagonist (14), affected the induction of LTP by ATP in these cells.

It was possible that the ATP hydrolysis product adenosine 5'-diphosphate (ADP) plays a role in LTP induction which could involve ATP binding to P1 purinoceptors (1). ADP at 10 \(\mu\)M caused a transient response; however, in contrast to the result with 10 \(\mu\)M ATP, following removal of ADP, no potentiation was seen (Fig. 2). Therefore, P1 receptors do not seem to be involved in the mechanism of ATP-induced LTP at CA1 synapses.

During test electrical stimulation applied to the Schaffer collaterals, glutamate is released at presynaptic terminals and can bind to postsynaptic NMDA receptors. Since ATP-induced LTP was blocked by co-application of 5 \(\mu\)M D,L-2-amino-5-phosphonovalerate (AP5) (Fig. 1), a combination of extracellular ATP and NMDA during test electrical stimulation might be
required for the induction of ATP-induced LTP in CA1 neurons. We therefore studied whether application of 100 nM NMDA could replace electrical stimuli in inducing LTP during ATP perfusion. When 100 nM NMDA and 10 μM ATP or 100 μM L-glutamate and 10 μM ATP were co-applied to slices in the absence of test stimulation during drug application and wash-out, test synaptic inputs after this period showed a gradual increase to a potentiated plateau, which was maintained for at least 30 min (empty circles or filled triangles in Fig. 3). This result shows that application of 100 nM NMDA or 100 μM L-glutamate during ATP perfusion can replace test electrical stimuli of Schaffer collaterals in inducing LTP at CA1 synapses. Since application of 100 nM NMDA alone did not induce LTP (empty triangles in Fig. 3), but the combined application of ATP and NMDA did induce LTP in the same cells, it is possible that extracellular ATP enhances NMDA receptor activation during NMDA application and allows NMDA receptors to trigger the mechanism for LTP induction at CA1 synapses.

In ATP-induced LTP, the delivery of test stimulation at 0.05 Hz could be replaced by perfusion with 100 nM NMDA or 100 μM L-glutamate (Figs. 1 and 3), suggesting that the postsynaptic NMDA receptors activated during test inputs could cooperate with ATP in triggering LTP induction. Since a previous study on the hippocampal CA1 region has shown that postsynaptic NMDA-receptor activation occurs even at 0.05 Hz (15), it is possible that increase in extracellular ATP augments the activation of NMDA receptors during ATP application and results in LTP production at CA1 synapses.

In our previous reports (3, 4), we have shown that in ATP-induced LTP in CA1 neurons, extracellularly applied ATP provides enough substrate for ecto-protein kinase to induce LTP via the extracellular phosphorylation of synaptic membrane proteins. The results shown in Figs. 1 and 3 suggest that one such protein substrate for ecto-protein kinases in ATP-induced LTP could be the NMDA receptor/Ca²⁺ channels. Since LTP induction in CA1 neurons involves intracellular second messenger systems that are triggered by a large Ca²⁺ influx into the cell through NMDA receptors/channels (16) and since activation of NMDA receptors/channels is a necessary step in producing ATP-induced LTP (Fig. 4), phosphorylation of NMDA receptors/channels allows a large Ca²⁺ influx into the cell to activate intracellular second messenger systems to produce ATP-induced LTP.
cellular phosphorylation of NMDA receptors/channels is involved in the mechanism of activity-dependent LTP in hippocampal CA1 neurons.

References