Jpn. J. Pharmacol. 78, 405–410 (1998)

REVIEW —*Current Perspective*—

ATP Receptors for the Protection of Hippocampal Functions

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Received October 9, 1998

ABSTRACT—The inhibitory effects of extracellular adenosine 5′-triphosphate (ATP) are reviewed in the present paper. ATP inhibits the release of the excitatory transmitter glutamate and stimulates the release of the inhibitory transmitter GABA from hippocampal neurons. Also, ATP activates potassium conductance directly through G protein, resulting in hyperpolarization of membrane potential. ATP activates microglia to secrete plasminogen that promotes the development of mesencephalic dopaminergic neurons and enhances neurite outgrowth from explants of neocortical tissue. Moreover, ATP may protect hippocampal neurons from excitotoxic cell death by preserving mitochondrial function. Thus, ATP may have a role in the protection of the function of hippocampus from over-stimulation by glutamate.

Keywords: ATP, Hippocampus, Neuron, Protection, Excitotoxicity

Introduction

Extracellular ATP produces responses in various tissues (1) including the central nervous system (CNS) (2–5) through ionotropic P2X ($P2X_{1-7}$) and G protein-coupled P2Y ($P2Y_{1,2,4,6,8,11}$) receptors (6–8). The P2Y has been reported to couple to PLC_β or adenylyl cyclase. The investigation of the molecular biology of P2 receptors has grown rapidly in recent years, resulting in significant amounts of new information.

The hippocampus is well known to be involved in memory and learning and to be particularly sensitive to ischemic insults. Glutamate, the major excitatory neurotransmitter in the hippocampus has been extensively studied in relation to neuronal cell death and long term potentiation (LTP). Though the activation of glutamate receptors is an event considered to be necessary for the induction of LTP in the hippocampus, many other factors such as arachidonic acid (9), nitric oxide (NO) and carbon monoxide (10) as well as ATP are thought to be involved in synaptic plasticity. ATP is released from the hippocampus (11) and several mRNAs for certain types of ATP receptors are present in this area (12).

There are many reports showing the excitatory action of ATP in the hippocampus. ATP induces fast synaptic currents in cultured hippocampal neurons (2) and long-lasting enhancement of the population spikes (11, 13, 14). ATP can produce glutamate release and an increase in $[Ca^{2+}]_i$ in cultured rat hippocampal neurons through the stimulation of excitatory P2 receptors in the postsynaptic neurons (2, 15). However, the subpopulation of ATP-responder cells is only 20% of all tested. In about 100% of all tested neurons, a decrease of $[Ca^{2+}]_i$ was detected after the ATP stimulation (16). Thus, the major action of ATP in hippocampal neurons may be inhibitory. Indeed, it has been shown by Ca^{2+} imaging (16), an electrophysiological method (K. Inoue and S. Ueno, unpublished) and direct detection of glutamate that ATP inhibits the glutamate release from cultured rat hippocampus. In addition, it is known that ATP stimulates the release of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter that inhibits the excitable function of glutamate.

ATP receptors can play reciprocal roles; i.e., both inhibitory and stimulatory in the functions of the hippocampus. Such reciprocal roles have been reported in other neurotransmitter systems in the CNS. Exogenously applied ATP inhibits the release of norepinephrine from rat brain cortex (17) and rat hippocampus (18), whereas ATP causes depolarization (4) and inward currents (5) in the noradrenergic neurons of the nucleus locus coeruleus. Also, ATP inhibits dopamine release from the neostriatum (19), whereas ATP increases the release of dopamine from the striatum (20, 21).

Besides these actions of ATP on the regulation of

neurotransmitters, ATP also activates microglia cells to release plasminogen, which has been reported to promote the development of mesencephalic dopaminergic neurons and to enhance neurite outgrowth from explants of neocortical tissue (22, 23). This paper will focus on the inhibitory actions of ATP.

ATP inhibits glutamate release from hippocampal neurons

Ca^{2+}-imaging study: Kudo's group found Ca^{2+} oscillations in the cultured hippocampus (24). Removal of extracellular Ca^{2+}; the application of tetrodotoxin (TTX, 3 μM); D-2-amino-phosphonovalerate (APV, 100 μM), an *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist; or 6-cyano-7-nitro quinoxalline-2,3-dione (CNQX, 30 μM), a non-NMDA-receptor antagonist inhibited the Ca^{2+} oscillations. Thus the Ca^{2+} oscillations can be used as an indication of glutamate release from hippocampus. This means that we can examine the effect of ATP on the glutamate release by the measurement of Ca^{2+} oscillations. Koizumi and Inoue (1997) have examined the action of ATP using rat cultured hippocampal synapses and found that ATP inhibits the Ca^{2+} oscillations in a concentration-dependent manner from 10 nM to 10 μM (16). The inhibition by ATP is independent from adenosine because 100 μM aminophylline completely blocked the inhibitory action of adenosine, but aminophylline did not affect ATP-induced inhibition. Various ATP analogues, ATPγS and α,β-methylene ATP ($\alpha\beta$-MeATP), which are non-hydrolyzable ATP analogues, also mimicked the inhibitory action. Moreover, UTP, which is not an adenosine derivative, mimicked the inhibition. The potency rank order of inhibition is as follows: 2MeSATP $>$ATP $>$ATPγS $>$UTP $>\alpha\beta$-MeATP. Since the Ca^{2+} responses to glutamate were almost the same with or without ATP treatments in the presence of TTX, ATP (10 μM) did not inhibit the sensitivity of postsynaptic glutamate receptors. This suggests that the site of action of ATP is not postsynaptic.

Another inhibitory neurotransmitter, GABA, may affect the Ca^{2+} oscillations. Indeed, Ca^{2+} oscillations were inhibited by GABA and were amplified by bicuculline (an antagonist to $GABA_A$ receptors) in some neurons (S. Koizumi and K. Inoue, unpublished). This indicates the continuous inhibition by GABA of the glutamatergic system in the hippocampus. The inhibition was dependent on the number of culture days because the amplification by bicuculline was greater in neurons cultured for more than 10 days than those cultured for less than 7 days. The data is in agreement with the electrophysiological data mentioned below and previous reports; i.e., the inhibition by GABA of Ca^{2+} oscillations in various brain regions including the hippocampus was observed only in mature neurons (25), and a GABA-mediated inhibition was found to be developmentally dependent in the hippocampus (26). The inhibition by ATP of glutamate release is not through the GABA system because ATP-evoked inhibition was detected in the presence of the $GABA_A$-blocker bicuculline in an electrophysiological experiment (K. Inoue and S. Ueno, unpublished).

An electrophysiological study and a direct measurement: The hippocampal pyramidal neurons are innervated by glutamatergic neurons and GABAergic interneurons. In the cultured rat hippocampal neurons, we can detect spontaneous synaptic currents that are blocked by TTX (3 μM) or the extracellular-Ca^{2+}-free condition. These currents are composed of currents through glutamate receptor/channels (I_{glu}) and those through $GABA_A$ receptor/channels (I_{GABA}). According to the Nernst equation, ionic equilibrium potentials vary linearly with the absolute temperature and logarithmically with the ionic concentration ratio. Since I_{GABA} is equal to the Cl^- current and I_{glu} is a non-selective cation current, we can adjust the equilibrium potential of I_{GABA} to around -30 mV and that of I_{glu} to around 0 mV by setting the concentration of Cl^- or cations of the extracellular buffer and internal pipette solution. At 0 mV of holding potential, only outward currents, which is theoretically I_{Cl^-} (I_{GABA}), appeared and these currents were blocked by bicuculline (10 μM). At -30 mV of holding potential in the Mg^{2+}-free extracellular buffer, only inward currents, which is theoretically I_{glu}, appeared and these currents were blocked by APV (100 μM) and CNQX (30 μM). The amplitude and frequency of both currents increased with the age of the culture. I_{glu} was detected in all neurons cultured for more than 10 days but not less than 7 days in a physiological buffer (containing Mg^{2+}). ATP (30 μM) strongly inhibited the current. The inhibitory action of ATP was not blocked by PPADS, a blocker of several subtypes of ATP receptors. Since ATP (30 μM) did not affect the glutamate-evoked current in the postsynaptic neurons (K. Inoue and S. Ueno, unpublished data), the inhibition of I_{glu} by ATP is thought to be via presynaptic PPADS-insensitive ATP receptor(s) by the decrease of the glutamate release.

The release of glutamate may be under the continuous inhibitory regulation by GABA through $GABA_A$ receptors because bicuculline (10 μM) strongly enhanced I_{glu} for a long period. A similar effect was observed in the Ca^{2+}-imaging study mentioned above. The enhanced I_{glu} was completely blocked by CNQX (10 μM). The idea that the inhibitory action of ATP to glutamate release may be due to the enhancement of the GABA release is not likely because ATP (30 μM) was able to inhibit the enhanced I_{glu} under the application of bicuculline (10 μM). Recently, it has been reported that GABAergic interneurons are

the major postsynaptic targets of mossy fibers in the rat hippocampus. Therefore, CNQX can block the input by glutamate to interneurons, resulting in the inhibition of GABA release. Indeed, CNQX (30 μM) strongly inhibited the frequency of I_{GABA}.

The effect of ATP on the release of glutamate from more than one million cells of primary cultured rat hippocampus was examined using a biochemical analytical method. ATP inhibited the spontaneous and high K^+-evoked release of glutamate from these cells.

ATP activates a potassium conductance

It has been reported that the stimulation of P2 receptor(s) activates a potassium conductance via a pertussis toxin (PTX)-insensitive G protein in rat hippocampal neurons (27, 28). The currents were not affected by selective protein kinase C- or A-inhibitors. The currents may be regulated directly by the $\beta\gamma$-subunits of a G protein. Repetitive application of ATP potentiated the currents by Ca^{2+}/calmodulin kinase (29). The similar responses were obtained in other sites of the brain including cerebral cortical neurons (30). ATP also increased $[Ca^{2+}]_i$, but the currents may not be a Ca^{2+}-dependent potassium current for several reasons. The currents had conductances of 85–95 pS, whereas one Ca^{2+}-dependent potassium current, voltage-dependent BK, has a conductance of 100–250 pS and another Ca^{2+}-dependent potassium current, the voltage-independent SK, has one of 4–14 pS. ATP caused the currents under condition of zero intracellular Ca^{2+}.

ATP stimulates plasminogen release from microglia

Recently, it has been suggested that microglia, a type of glial cell, is activated in response to pathological changes in the CNS and can play a variety of important roles in the CNS (31). Activated microglia are mainly scavenger cells but also perform various other functions in tissue repair and neural regeneration. These cells are able to release several cytotoxic substances in vitro such as NO as well as arachidonic-acid derivatives, excitatory amino acids, quinolinic acid and cytokines (32, 33). Activated microglia may also play a protective role. They produce the urokinase-type plasminogen activator and plasminogen, which promotes the development of mesencephalic dopaminergic neurons and enhances neurite outgrowth from explants of neocortical tissue (22, 23). These properties of microglia can be modulated by cytokines and neurotransmitters acting through receptors for CNS signalling molecules including ATP.

It has been reported that ATP is released from hippocampal slices by electrical stimulation of Schaffer collateral-comissural afferents (11); that microglia express receptors for ATP, G-protein coupled-type ATP receptors such as P2Y and ionotropic ATP receptors such as $P2X_7$ (34, 35); and that microglia respond to ischemia in the CA1 area of the hippocampus (36). ATP induces the release of interleukin (IL)-1β from mouse brain microglia through P_{2Z} (37), produces currents in rat brain (38) or forebrain microglia (39), and stimulates an increase in intracellular Ca^{2+} in rat brain (38). These data suggest that ATP mediates signals from neurons to microglia, resulting in the stimulation of the release of plasminogen. Inoue et al. have reported that ATP stimulated the release of plasminogen in a concentration-dependent manner (the estimated EC_{50} is approximately 15 μM), whereas glutamate (100 μM) did not induce the release of plasminogen at 10 min after the stimulation (40). NO is released from various cells following the cascade of Ca^{2+} influx, activation of Ca^{2+}/calmoduline-dependent protein kinase and activation of NO synthase. It is also known that ATP stimulates NO release from endothelial cells, presumably through an increase in $[Ca^{2+}]_i$ (41). However, ATP (10 to 100 μM) did not release NO, but lipopolysaccharide (LPS, 0.5 μg/ml), as a positive control, did produce NO from the microglia (40).

It has been revealed using BAPTA-AM that ATP-evoked plasminogen release is dependent on the increase in $[Ca^{2+}]_i$. The next question is how ATP increased the $[Ca^{2+}]_i$. ATP induced an increase in $[Ca^{2+}]_i$ in a concentration-dependent manner from 1 to 100 μM with the estimated EC_{50} of 12 μM, and this was dependent on extracellular Ca^{2+} (12 out of 14 cells). These data indicate that the ionotropic ATP receptor, presumably $P2X_7$, is mainly responsible for the increase in $[Ca^{2+}]_i$ in microglia. This was supported by the data that BzATP, a selective agonist of $P2X_7$ (35), produced a long-lasting increase in $[Ca^{2+}]_i$ even at 1 μM, a concentration at which ATP did not evoke the increase; and oxidized ATP, a selective antagonist of $P2X_7$ (35), completely blocked the increase by ATP (10 μM). These data, taken together with the fact that the concentration-response curve for the increase in $[Ca^{2+}]_i$ by ATP was shifted to the left in the Mg^{2+}-free condition, indicate that the ATP-induced $[Ca^{2+}]_i$ increase is mainly due to the activation of $P2X_7$ receptors.

It is possible that ATP released from nerve endings transmits information to microglia and stimulates them to release plasminogen in the hippocampus. As mentioned above, the released plasminogen may modulate the function of the neurons (42). Thus, ATP from purinergic neurons can transmit information to glutamate-related neurons via microglia. In other words, a triangular connection of neuron-microglia-neuron may be completed by ATP and plasminogen in the hippocampus. ATP also exists in the cytosol at a concentration of several mM and is able to leak out of cells damaged by ischemia or trauma.

ATP leaking from damaged cells as well as ATP from nerve endings may be able to activate hippocampal microglia.

ATP intracellular-calcium independent inhibiton of glutamate-evoked excitotoxicity in hippocampal neurons

Recently, Nishiyama et al. have reported that ATP and related agents protects cultured rat hippocampus from glutamate-induced neuronal death (43) independently from $[Ca^{2+}]_i$. Cultured rat hippocampal neurons were exposed to 10 μM glutamate for 24 hr with or without purinergic agents. ATP, $\alpha\beta$-Me ATP, UTP and ADP, but not adenosine, protected neurons concentration-dependently (0.03–3 mM). These protective effects were independent of the intracellular Ca^{2+} concentration. $\alpha\beta$MeATP (1 mM) delayed glutamate (100 μM)-induced depletion of intracellular ATP content estimated by the luciferine-luciferase method. Confocal microscopic analysis with hydroethidine revealed that incubation with 100 μM glutamate for 1 hr resulted in twofold elevation of the intracellular content of superoxide. Coapplication of purine nucleotides (1 mM) almost completely prevented the increase in superoxide, but delayed administration of ATP did not reduce the elevated level of ethidium flouresence, which indicates that ATP blocked glutamate-induced generation of reactive oxygen species. In addition, ATP (1 mM) completely antagonized the neurotoxicity of mitochondria toxins (rotenone, 0.1 μM; 3-nitropropionic acid, 1 mM). These results indicate that ATP protected hippocampal neurons from excitotoxic cell death by preserving mitochondrial function.

Conclusion

ATP inhibits presynaptically the release of glutamate, an excitatory neurotransmitter from the hippocampus. Meanwhile, ATP stimulates the release of GABA and glycine, an inhibitory neurotransmitter from the hippocampus (K. Inoue and S. Ueno, unpublished data) and interneurons of the dorsal horn (N. Akaike, personal communication, 1998), respectively. The inhibition of glutamate release and the stimulation of GABA and glycine release have the same effect as a negative feedback loop; i.e., turning-down the activity of neurons stimulated by glutamate. ATP also activates K^+ conductance in the hippocampal neurons. This leads to hyperpolarization that causes the excitable cells to exist in a stable condition. Moreover, ATP activates microglia to release plasminogen, a type of neurotrofic factor, that promotes the development of mesencephalic dopaminergic neurons and enhances neurite outgrowth from explants of neocortical tissue. ATP may thus stimulate the reconstruction of synaptic networks through activating microglia. There also are many reports on the trophic action of ATP itself in the CNS (34). Moreover, ATP may protect against glutamate-evoked excitotoxicity in an intracellular-independent manner. Thus, ATP may have a role in protecting the function of hippocampus from overstimulation by glutamate (Fig. 1).

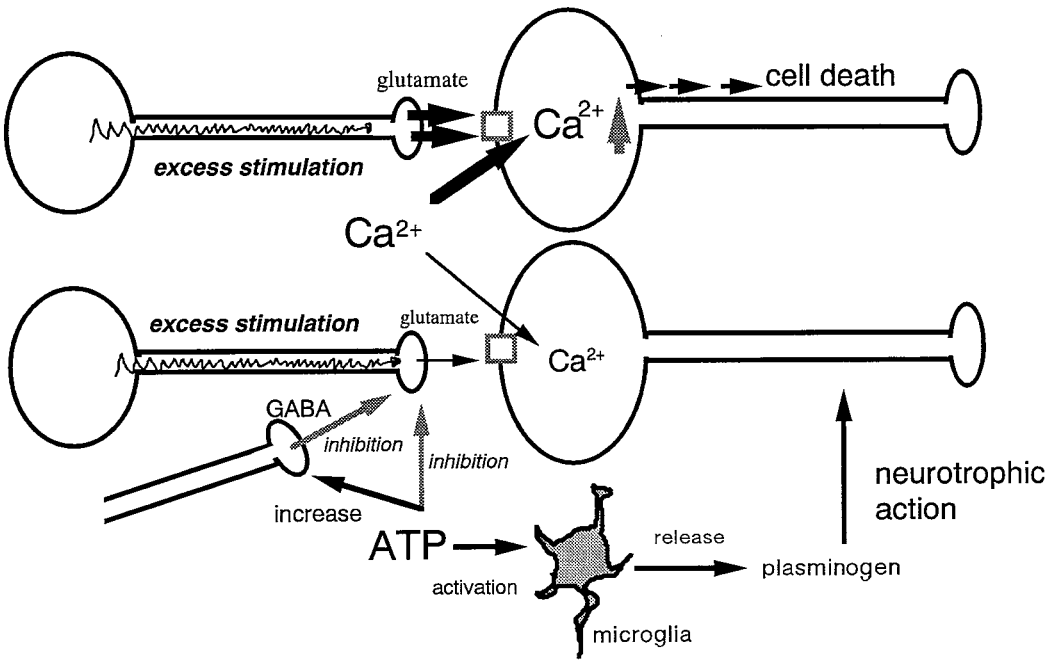

Fig. 1. Schema of the protection of hippocampal neurons by extracellular ATP.

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