ATP Inhibits Glutamate Synaptic Release by Acting at P2Y Receptors in Pyramidal Neurons of Hippocampal Slices

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ABSTRACT

It has been proposed that extracellular ATP inhibits synaptic release of glutamate from hippocampal CA1 synapses after its catabolism to adenosine. We investigated the possibility that at least part of this effect is mediated by ATP itself acting on P2Y receptors. ATP and various analogs decreased the amplitude and duration of glutamate-mediated excitatory postsynaptic potentials in all tested neurons. This effect was reversible and concentration-dependent and had the following rank order of agonist potency: AMP = ATP = adenosine-5′-O-(3-thio)triphosphate > adenosine-5′-triphosphate, GTP, and UTP induced only a partial response. The depolarization induced by exogenous glutamate was not affected by ATP, indicating that this nucleotide acts presynaptically to inhibit glutamate-mediated excitatory postsynaptic potentials. Neither inhibition of ectonucleotidase activity with α,β-methylene ADP, suramin, or pyridaxalphosphate-6-azophenyl-2′,4′-disulfonic acid 4-sodium nor removal of extracellular adenosine (with adenosine deaminase) altered ATP effects. 8-Cyclopentyltheophylline competitively inhibited ATP effects, whereas P2 receptor antagonists (pyridaxalphosphate-6-azophenyl-2′,4′-disulfonic acid 4-sodium, suramin, and reactive blue 2) were ineffective. ATP effects were by far more sensitive to pertussis toxin (PTX) than those of adenosine. After PTX, adenosine-5′-O-(3-thio)triphosphate induced only a partial response, and ATP concentration–response curve was biphasic. The second phase of this curve was blocked by adenosine deaminase, implying that it is mediated by adenosine as a result of ATP catabolism. Under control conditions, however, catabolism of ATP is not required to explain its actions. In conclusion, ATP inhibits synaptic release of glutamate by direct activation of P2Y receptors that are PTX- and 8-cyclopentyltheophylline-sensitive.

ATP is a neuromodulator and neurotransmitter in the peripheral (Burnstock, 1990; Evans et al., 1992; Silinsky and Gerzanich, 1993) and central (Edwards et al., 1992; Pankratov et al., 1998) nervous systems. Various actions have been described for the extracellular ATP in hippocampal neurons, including the inhibition of synaptic transmission (Dunwiddie et al., 1997; Cunha et al., 1998), opening of ligand-gated channels (Pankratov et al., 1998; Baljit et al., 1999), and the phosphorylation of ecto-protein kinase proteins involved in long-term potentiation (Chen et al., 1996b). Recent experimental evidence indicates that ATP might be playing a role as a fast neurotransmitter in the CA1 hippocampal region (Pankratov et al., 1998).

Two types of purine extracellular receptors are widely recognized (Abbracchio and Burnstock, 1994; Fredholm et al., 1994). P1 sites are adenosine receptors sensitive to theophylline derivatives and P2 sites are nucleotide receptors insensitive to theophylline derivatives. Nevertheless, nucleotide receptors sensitive to theophylline or its derivatives have been described in peripheral and central neurons (Silinsky and Ginsborg, 1983; Shinozuka et al., 1988; Forsyth et al., 1991; Cunha et al., 1994; Barajas-López et al., 1995). At this receptor, 1) P1 receptor agonists [adenosine or 2-chloroadenosine (CADO)] and ATP appear to be equipotent (but see Silinsky and Ginsborg, 1983; von Kügelgen et al., 1989; Barajas-López et al., 1995) at this pharmacological profile (Shinozuka et al., 1988; Forsyth et al., 1991; Dalziel and Westfall, 1994). ATP can directly activate two types of P2 receptors, P2X and P2Y, in many different types of cells, including neurons. P2X receptors are ligand-gated ion channels, whereas P2Y are coupled via GTP-binding proteins (Burnstock, 1990; Ab-

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ABBREVIATIONS: CADO, 2-chloroadenosine; 2-MeSATP, 2-methylthioadenosine 5′-triphosphate; ATPγS, adenosine-5′-O-(3-thio)triphosphate; gEPSP, glutamate-mediated excitatory postsynaptic potential; PTX, pertussis toxin; PPADS, pyridaxalphosphate-6-azophenyl-2′,4′-disulfonic acid 4-sodium; aCSF, artificial cerebrospinal fluid; NEM, N-ethylmaleimide; AD, adenosine deaminase; CPT, 8-cyclopentyltheophylline; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine.
brachio and Burnstock, 1994; Fredholm et al., 1994). ATP can also be hydrolyzed by nucleotidases extracellularly (Dunwiddie et al., 1997; Cunha et al., 1998), and products of its hydrolysis (ADP, AMP, and adenosine) can influence neurons by interacting with specific receptors (Burnstock, 1990).

P1 receptors are found in nerve terminals in many regions of the central and peripheral nervous systems. Their activation inhibits the release of various neurotransmitters, including glutamate (Dolphin and Prestwich, 1985; Cunha et al., 1998), acetylcholine (Fredholm et al., 1989; Barajas-López et al., 1991), norepinephrine (Fredholm et al., 1989; Barajas-López et al., 1991; Forsyth et al., 1991), and peptides (Broad et al., 1993). Presynaptic ATP receptor activation also modulates neurotransmitter release in peripheral (Silinskas and Ginsborg, 1983; Shinozuka et al., 1988; Silinsky et al., 1990; Forsyth et al., 1991; Barajas-López et al., 1995) and central (Fredholm and Dunwiddie, 1988; Dunwiddie and Fredholm, 1989; Cunha et al., 1994) neurons. In hippocampus, ATP inhibits the neural release of glutamate, but it is not clear whether such an action is mediated by the ATP metabolite adenosine acting on P1 receptors or by ATP activation of P2 receptors. In general, a recent study by Cunha et al. (1998) favors the first hypothesis, but at least two of their observations support the hypothesis that P2 receptors are present in nerve terminals and might mediate part of the inhibition. Specifically, ATP hydrolysis appears to be slower than its effects on glutamate release. Moreover, its analog, adenosine-5′-O-(3-thio)triphosphate (ATP-β-S), behaves as a full agonist despite its hydrolysis being only marginal. Based on these observations, we hypothesized that part of the ATP-induced presynaptic inhibition is mediated by P2 receptors. Therefore, the aim of the present study was to further characterize the receptors that mediate the inhibitory actions of ATP on the synaptic release of glutamate.

Materials and Methods

Slice Preparation. Rat hippocampal slices were prepared as previously described (Edward et al., 1989). After decapitation, the brain was quickly removed, and a block of tissue containing the hippocampus was prepared and placed in an ice-cold artificial cerebrospinal fluid (α-CSF) with the following composition: 124 mM NaCl, 5 mM KCl, 1.2 mM NaH2PO4, 1.3 mM MgSO4, 2.4 mM CaCl2, 26 mM NaHCO3, and 10 mM glucose. Coronal slices were cut with a vibratome (Campden Instruments, Ltd.) 400-μm thick and were incubated at room temperature in α-CSF bubbled with 95% O2, 5% CO2 mixture.

Intracellular Recordings. A single slice was then placed in the recording chamber and superfused continuously with heated (34–35°C) α-CSF at 1.5 to 2 ml/min. Intracellular recordings were made with glass microelectrodes filled with 2 to 3 M KCl (resistance 40–60 mΩ). Membrane potential was measured with an Axoclamp-2A preamplifier (Axon Instruments Inc., Foster City, CA). The output of this preamplifier was displayed on an oscilloscope (TDs 210; Tektronics) and recorded with a PC and Axotape or pClamp software (Axon Instruments). An intracellular impalement of a CA1 pyramidal cell was judged satisfactory if the membrane potential was ±55 mV and action potentials were ±60 mV in amplitude. Glutamate-mediated excitatory postsynaptic potentials (gEPSPs) were evoked by an electrical pulse (20–100 μs) applied at 0.1 Hz to the Schaffer collateral-commissural afferents, using a bipolar electrode made by twisting tungsten wires of a diameter of 20 μm (Teflon-coated). In the presence of 30 μM picrotoxin, five consecutive gEPSPs were averaged under each experimental condition, and the mean amplitude was calculated. To avoid changes in the amplitude of gEPSPs due to membrane potential changes and the generation of overriding actions potentials, the membrane potential was held constant near the potassium equilibrium potential (~100 mV).

Drugs. Adenosine, picrotoxin, pyridalxalphosphate-6-azophenyl-2,4-disulfonic acid 4-sodium (PPADS), CADO, 8-cyclopentylethylpholine (CPT), suramin, and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) were obtained from Research Biochemicals Inc. (Natick, MA). AMP, ADP, ATP, α-β-methylene ATP, β-γ-methylene ATP, β-γ-methylene ATP lithium salt (α-β-methylene ATP), ATP-bis(2-methoxyadenosine 5′-triphosphate disodium salt (2-MeSATP), UTP, GTP, glutamate, PTX, N-ethylmaleimide (NEM), adenosine deaminase (AD), and Basilen Blue E-3G (reactive blue 2) were obtained from Sigma Chemical Co. (St. Louis, MO). Stock solutions of 0.1 to 10 mM were made in either ethanol (CPT and DPCPX) or water (all other substances) before being dissolved in the physiological saline solution. ATP, ADP, and AMP stock solutions were prepared the day of the experiment and kept at −4°C. Stock solutions of suramin and adenosine were kept at −40°C. All other stock solutions were kept at −20°C.

Statistical Analysis. Results are expressed as mean ± S.E. The unpaired Student’s t test was used to evaluate differences between mean values obtained in the same cell, whereas the unpaired Student’s t test was used for the data collected from different cells; two-tailed P values of ≤0.05 were considered statistically significant.

Results

General Observations. Results were obtained from 150 CA1 pyramidal cells. Electrophysiological properties of these neurons were similar to those published previously using patch-clamp and intracellular recordings (Malinow and Miller, 1986; Edwards et al., 1989). The mean membrane potential was −66 ± 1.6 mV (range, −55 to −76 mV), mean membrane input resistance was 46 ± 4.2 MΩ (range, 25–75 MΩ), and mean time constant was 11 ± 0.6 ms (range, 8.2–16.2 ms). The amplitude of action potentials recorded in pyramidal neurons in the CA1 region of rat hippocampus was 75 ± 1.7 mV (range, 60–80 mV) measured at resting membrane potential.

Unless otherwise stated, 30 μM concentration of the γ-aminobutyric acidA receptor antagonist (picrotoxin) was used to block the inhibitory postsynaptic potentials. These potentials were reversal (depolarizing) under our experimental conditions due to the high concentration of chloride ions inside the recording electrodes (2 M KCl). Picrotoxin superfusion dimin-
ished the amplitude of the fast synaptic potentials to an average of 42 ± 1.0% from its control values.

**Effect of ATP and Several Analogs on gEPSPs.** Superfusion of ATP and various of its analogs decreased the amplitude and duration of orthodromic gEPSPs in all of the pyramidal neurons tested, and this effect was concentration-dependent (1–300 μM; Fig. 1). The following IC50 values were calculated for several of these compounds that behaved as full agonists: 4.1 ± 0.2 μM for AMP, 5.4 ± 0.8 μM for ATPγS, 6.3 ± 0.9 μM for ATP, 13 ± 0.7 μM for adenosine, and 15.4 ± 1.2 μM for ADP. β-γ-Methylene ATP, α-β-methylene ATP, 2-MeSATP, GTP, and UTP induced only a partial response. A maximum concentration caused 30 to 50% of the maximum inhibition observed with ATP. The IC50 values for these compounds were 17.3 ± 0.3, 7.1 ± 1.2, 8.9 ± 1.2, 12.4 ± 1.5, and 8.3 ± 1.0 μM, respectively (Fig. 1). To avoid cell-to-cell variation, the effects of ATP (1–100 μM) and adenosine (1–300 μM) were compared in the same pyramidal neurons (n = 6). In such experiments, IC50 values for these agonists were 6.3 ± 0.9 and 13.0 ± 0.7 μM, respectively. The concentration-response curves for these experiments are not shown. The simplest explanation for these observations is that adenosine and ATP inhibit gEPSPs via different receptor sites.

The time dependence of ATP- and adenosine-induced inhibition of gEPSPs is shown in Fig. 2. This inhibitory effect of ATP and its analogs had the following kinetics: it was observed within 60 s after the arrival of the agonist to the recording chamber, reached maximum within 4 min, and completely reversed within 8 min after discontinuation of the application. The inhibitory response was not modified during repeated or prolonged applications (10–15 min) of maximal concentrations. Furthermore, similar responses were obtained by their cumulative and noncumulative applications. Thus, no evidence of desensitization was observed.

Contrary to its effects on gEPSPs, ATP (100 μM) did not alter the depolarization induced by the local application of exogenous glutamate (5 mM; Fig. 3). Thus, the glutamate-induced depolarization was 14.8 ± 1.2 and 14.6 ± 1.2 mV before and in the presence of 100 μM ATP, respectively (n = 5). In the same neurons, this concentration of ATP completely inhibited the electrically evoked gEPSPs, which had a control value of 14.3 ± 1.6 mV. These results show that ATP inhibits gEPSPs by acting at a presynaptic site.

**Effects of Purine Receptor Antagonists on ATP-Mediated Inhibition of gEPSPs.** To further characterize the receptors mediating the ATP-induced inhibition of gEPSPs, we investigated the effects of suramin (100 μM) and PPADS (30 μM) on ATP-mediated inhibition of gEPSPs. Both substances antagonize effects of ATP in several tissues (Silinsky et al., 1990; Evans et al., 1992; Silinsky and Gerzanich, 1993;
Fredholm et al., 1994). In the hippocampus, however, these substances neither modified the gEPSPs nor altered the inhibitory effects of ATP on the gEPSPs (Fig. 4). Reactive blue 2 (30 μM), another P2 receptor antagonist (Fredholm et al., 1994), also failed to alter the inhibitory effects of 1 μM ATP. In these experiments, a 19 ± 1% (n = 4) inhibition of the gEPSPs was induced by ATP before and in the presence of reactive blue 2.

We also examined the effects of CPT, which blocks the presynaptic inhibition induced by either adenosine or ATP in enteric neurons (Barajas-López et al., 1995). When administered alone, CPT did not alter the gEPSPs. However, ATP shifted the concentration-response curves to ATP to the right in a parallel fashion without altering the efficacy of this nucleotide (Fig. 5, A and B). This effect of CPT was concentration-dependent. Schild plot analysis of these results (Fig. 5C) yielded an estimated dissociation equilibrium constant (K_D) value of 4 nM and a slope (0.82 ± 0.16) not significantly different from unity, indicating that CPT was acting as a competitive antagonist of the effects of ATP. DPCPX (30 nM), another theophylline derivative, also blocked the inhibitory effects of ATP (10 μM). ATP inhibitory effects were 67 ± 3.5 and 3 ± 1.5% before and in the presence of DPCPX (n = 4; P ≤ .01). Similar DPCPX effects were previously reported by Cunha et al. (1998). As expected, 10 nM CPT also shifted the CADO (adenosine receptor agonist) concentration-response curve to the right (Fig. 5, D and E). The IC_{50} values for CADO were 478 and 1595 nM before and in the presence of 10 nM CPT, respectively. Therefore, assuming a competitive antagonism, we can predict a K_D value of 4.3 nM CPT on CADO-activated receptors.

Is ATP Catabolism Required for ATP Presynaptic Actions? Figure 6A shows concentration-response curves for ATP inhibitory effects on gEPSPs in the same pyramidal neurons (n = 6) in the absence and presence of AD, which converts adenosine to its inactive metabolite inosine. Superfusion of this enzyme (2 U/ml) was initiated 30 min before the addition of ATP and was maintained as long as the ATP superfusion. These experiments were performed with an ATP stock solution (10 mM concentration) that had been preincubated with 10 U/ml AD for 1 to 6 h. We found that AD superfusion by itself increased the gEPSP amplitude by 18 ± 1% of its control values. AD, however, did not alter the ATP-mediated inhibition of these gEPSPs recorded from the same neuron in the absence and then the presence of three different concentrations of CPT. B, concentration-response curves for ATP effects obtained in the absence and then the presence of five different concentrations of CPT. Symbols are mean ± S.E. values of data obtained from five neurons. Lines were fitted by least-squares to sigmoidal curves. C, Schild plot derived from the curves shown in B. The slope of the Schild plot (fitted by linear regression) was 0.82 ± 0.16. D, concentration-response curves for CADO effects obtained in the absence (IC_{50} = 478 nM) and then the presence of 10 nM CPT (IC_{50} = 1595 nM). Symbols are mean ± S.E. of data obtained from six neurons. E, effects of CADO on gEPSPs recorded from the same neuron in the absence and then in the presence of 10 nM CPT. Notice that in A and E, the Control and Wash traces are superimposed.

ATP inhibitory effects on gEPSPs in the same pyramidal neurons (n = 6) in the absence and presence of the enzyme AD, which converts adenosine to its inactive metabolite inosine. Superfusion of this enzyme (2 U/ml) was initiated 30 min before the addition of ATP and was maintained as long as the ATP superfusion. These experiments were performed with an ATP stock solution (10 mM concentration) that had been preincubated with 10 U/ml AD for 1 to 6 h. We found that AD superfusion by itself increased the gEPSP amplitude by 18 ± 1% of its control values. AD, however, did not alter the ATP-mediated inhibition of these gEPSPs (Fig. 6A). When control experiments were performed with adenosine instead of ATP, AD completely abolished the adenosine-mediated inhibition of gEPSPs (n = 3; not shown).

Figure 6B shows the inhibitory effects of ATP on the gEPSPs in the absence and presence of 100 μM α,β-methylene ADP, a potent (K_I = 6 nM) ecto-5′-nucleotidase inhibitor (Naito and Lowenstein, 1985). Superfusion of this inhibitor was initiated 30 min before ATP and maintained as long as the superfusion of ATP. α,β-Methylene ADP by itself caused...
a small (9 ± 1.0%) but significant (P < .01; n = 5) inhibition of gEPSPs but did not alter ATP-induced inhibition of these synaptic potentials. Despite the fact that suramin, PPADS, and reactive blue 2 are better known as P2 receptor antagonists, they also inhibit ectonucleotidase activity in various tissues (Ziganshin et al., 1995; Chen et al., 1996a), and as described earlier, they did not modify the ATP effects. These results are in agreement with those described in the previous paragraph, and both suggest that the ATP-mediated inhibition of gEPSPs is not due to catabolism of ATP.

Role of G Proteins in ATP Actions. Previous studies found that presynaptic actions of adenosine in the hippocampus are quite resistant to PTX (Fredholm et al., 1989; Stratton et al., 1989). The effects of PTX on presynaptic actions of ATP are unknown in the hippocampus. In rat adrenal chromaffin cells (Kim et al., 1997), however, this toxin prevents ATP inhibitory effects on exocytosis and calcium currents. Therefore, we investigated the possibility that PTX could have a differential effect on adenosine and ATP pre-synaptic actions in the hippocampus.

Slices of the rat hippocampus were incubated for 12 to 20 h in aCSF (incubation control) or in aCSF plus PTX (500 ng/ml and 5 μg/ml). The long incubation period per se did not significantly affect the membrane potential, action potentials, gEPSP amplitude, or inhibitory effect of ATP and adenosine (Fig. 7, A and B, and Table 1). In the slices pretreated with PTX, the concentration-response curves of ATP and adenosine were both significantly shifted to the right. This effect was greater for ATP than for adenosine such that ATP was less potent than adenosine. After treating the slices with 5 μg PTX, adenosine was a full agonist and the ATP concentration-response was biphasic, with a quasiflat portion between 10 and 30 μM. At this point, we investigated the hypotheses that the first portion of this curve was mediated by ATP itself and the second portion was mediated by the metabolism of ATP to adenosine. In support of these hypotheses and consistent with the observation that ATP effects are more sensitive to PTX than those induced by adenosine, we found that 1) ATP-sS induced only a partial response, producing a maximal effect similar to that induced by 10 to 30 μM ATP, and 2) the second portion of the ATP concentration-response curve, observed at concentrations of ≥100 μM, was completely inhibited by the addition of AD (2 U/ml; Fig. 7A).

We also examined the effects of NEM, which can uncouple G proteins from several receptors (Smith and Harden, 1984; Wu et al., 1992; Shapiro et al., 1994). The inhibitory effects of both ATP and adenosine were significantly (P < .001) diminished (Fig. 8) by NEM treatment (100 μM, 1 h). The effect of NEM, however, was less dramatic than that produced by PTX.
induced by NEM was not reversible and did not distinguish between actions mediated by ATP and adenosine.

**Discussion**

Our main finding is that nucleotides can directly inhibit glutamate synaptic release in CA1 axon terminals of hippocampus. Evidence for ATP effects mediated through its metabolite adenosine were seen only at high concentrations of ATP and only after PTX treatment. This effect is likely mediated by P2Y receptors (nucleotide G protein-coupled receptors), which have similar pharmacological properties as those described in peripheral neurons and have previously been called P3 purinoceptors (see the Introduction).

**ATP Catabolism Is Not Required for ATP-Induced Presynaptic Inhibition.** We found that ATP hydrolysis to adenosine is not required for the ATP-induced inhibition of gEPSPs in the CA1 hippocampal region. First, ATP, AMP, and ATPγS are more potent than adenosine. Second, the concentration-response curves for ATP and ATPγS were not significantly different, despite the fact that ATPγS is known to be hydrolyzed more slowly than ATP in hippocampal slices (Cunha et al., 1998). Third, the ectonucleotidase inhibitors α,β-methylene ADP (Naito and Lowenstein, 1985), suramin, PPADS, and reactive blue 2 (Ziganshin et al., 1995; Chen et al., 1996a) did not alter the effect of ATP. Finally, AD abolished the effect of adenosine as expected but did not alter the effects of ATP. Altogether, these results indicate that ATP hydrolysis to adenosine is not required for ATP inhibitory effects on the gEPSPs and that the nucleotides themselves are responsible for these effects. Our results support the idea that ATP acts through its own receptor.

ATP degradation to adenosine does occur in our slices, as previously reported by Cunha et al. (1998). This, however, was evident in the PTX-pretreated slices only when the ATP concentration-response curve was biphasic, with the second phase of this curve (observed with concentrations of ≥100 μM) being completely blocked by AD. After PTX treatment, ATP induced a maximum gEPSP inhibition of only ~40% in the presence of this enzyme, supporting the idea that the second phase of this curve appears to be mediated by the production of adenosine. In agreement with this interpretation, the concentration-response curve for ATPγS (a slow degradable ATP analog) lacks the second phase. The prevention by AD of the effect of exogenous adenosine and the second phase of the ATP concentration-response curve (after PTX treatment) demonstrates the activity and diffusion of this enzyme in our preparation. Altogether, these observations and those discussed in the previous paragraph suggest that ATP metabolism to adenosine occurred in the study brain slices but that this is not normally required for the inhibitory actions of ATP on the gEPSPs.

The lack of effects of AD and α,β-methylene ADP on the ATP actions disagrees with a report by Cunha et al. (1998), who found that these two substances prevented the ATP effects on the gEPSPs. These discrepancies may result from different experimental protocols. Thus, they recorded at 30°C, whereas we recorded at 35–36°C. The temperature might affect the availability of adenosine outside the cells by modifying not only its production but also its membrane transport. Another difference is that we recorded intracellularly, whereas Cunha et al. (1998) recorded extracellularly. We are sampling neurons with characteristics of a standard CA1 cell (i.e., a membrane potential larger than −55 mV and an action potential amplitude of ≥60 mV). Extracellular recordings, in contrast, measures average neuronal activity. To avoid voltage-dependent changes in the synaptic activity, we always measured the gEPSPs at the same membrane potential, around the potassium equilibrium potential. Changes in the unclamped resting membrane potential due to postsynaptic actions of any experimental maneuver could indirectly affect synaptic transmission, which is a concern during extracellular recordings. Finally, unlike Cunha et al., (1998), we also performed our recordings in the presence of picROTOxin to prevent the synaptic transmission mediated by γ-aminobutyric acidA receptors.

Ross et al. (1998) reported experimental evidence in favor of AMP mediating the inhibitory actions of ATP on epileptiform activity in the CA3 region of the rat hippocampus. Here, we showed that AMP and ADP also inhibit the gEPSPs, perhaps indicating that these metabolites could also contribute to ATP action.

**ATP-Induced Presynaptic Inhibition Is Mediated by G Protein-Coupled Receptors That Are Different Than Those Activated by Adenosine.** Various observations indicate that nucleotide actions on gEPSPs are mediated by receptors coupled to G proteins. The first observation is the relative slow kinetics and lack of desensitization of this nucleotide response. Second is the lack of effects of suramin and PPADS. These antagonists are known to block P2X (ionotropic) receptors in hippocampal neurons (Pankratov et al., 1998; Baljit et al., 1999). Some homomeric P2X channels formed by either P2X4 or P2X6 are not sensitive to suramin or PPADS, but recent evidence suggests that P2X4 subunits are not the primary subtype of P2X receptor in hippocampal neurons (Baljit et al., 1999). Third, the ATP presynaptic effects were inhibited by PTX and NEM, substances that can alter responses mediated by various G protein-linked receptors (Smith and Harden, 1984; Wu et al., 1992; Shapiro et al., 1994; Barajas-López et al., 1996). Nucleotide receptors coupled with G proteins are P2Y receptors, according to the current nomenclature (Abbraccio and Burnstock, 1994; Fredholm et al., 1994).
ATP and the ATPγS presynaptic effects were more affected by PTX than those induced by adenosine. Indeed, adenosine was more potent than these nucleotides after the PTX treatment. This indicates that the G proteins mediating ATP actions are different from those mediating adenosine presynaptic actions. These observations would also imply that these nucleotides are activating a different pool of receptors than adenosine.

Adenosine presynaptic actions in hippocampal neurons are thought to be mediated by the activation of A1 receptors (Dunwiddie and Fredholm, 1989; Fredholm et al., 1989; Fredholm et al., 1990; Scholz and Miller, 1991), which are G protein-coupled receptors (Abbracchio and Burnstock, 1994; Fredholm et al., 1994). Some adenosine effects mediated by these receptors are PTX-sensitive (Dolphin and Prestwich, 1985; Silinsky et al., 1990; Scholz and Miller, 1992; Barajas-López et al., 1996) but not in all preparations (Fredholm et al., 1989; Broad et al., 1993; Barajas-López et al., 1996). We found that adenosine presynaptic effects were only slightly decreased despite the large concentrations (5 μg/ml) and the relative long applications (16 h) of PTX used here. Consistent with our findings, previous studies found that presynaptic actions of this nucleoside are highly resistant to PTX (Fredholm et al., 1989; Stratton et al., 1989). Indeed, intrahippocampal injections of PTX can prevent adenosine effects in hippocampal slices only when ≥1 μg is injected and only when it is applied 3 to 8 days before adenosine effects are measured (Stratton et al., 1989). In culture preparations of hippocampal neurons, 250 ng/ml PTX can readily prevent presynaptic effects of A1 receptor agonists (Scholz and Miller, 1991), which suggests that PTX diffusion to the subcellular region where adenosine receptors are located might be limited in the slice preparation or during intracerebral injections. Our observation that ATP and ATPγS effects are more affected by PTX than adenosine actions suggests that nucleotide and adenosine receptors in the hippocampus are located in different subcellular regions. Another alternative explanation is that adenosine receptor pool in the slice and the intact brain is larger than the nucleotide receptor pool and therefore less sensitive to PTX.

**ATP Presynaptic Receptors Are Sensitive to Theophylline Analogs.** The nucleotide receptors present in the CA1 collateral fibers (present study) have similar pharmacological properties as P3-purinoreceptors (see the Introduction). Thus, CPT was a potent (K_D = 4 nM) and competitive antagonist at the receptors that mediate the ATP effects on gEPSPs. This K_D value is virtually the same as that of CPT at A1 adenosine receptors (Dunwiddie and Fredholm, 1989; Fredholm et al., 1994). Because of the reasons discussed earlier, it is unlikely that ATP is acting via adenosine receptors, and therefore, we favor the alternative explanation that CPT cannot distinguish between presynaptic receptors activated by ATP and adenosine in hippocampal synapses, as previously noted in submucosal neurons (Barajas-López et al., 1995). Because the receptors described here are activated by nucleotides and appear to be G protein-linked (because they are PTX and NEM sensitive) and because they are sensitive to theophylline derivatives, we proposed to classify them as P2Y-theophylline-sensitive.

PTX sensitivity also suggests that ATP could be inhibiting the gEPSPs by decreasing calcium currents and/or adenyl cyclase activity. This hypothesis is based on the fact that PTX is known to specifically target G and G protein, which activation inhibits adenyl cyclase activity and calcium channels, respectively (see Hille, 1992). In agreement with this hypothesis, ATP receptors can inhibit calcium currents, excytosis (Lim et al., 1997), and adenyl cyclase activity. The presence of P2 receptors insensitive to suramin and negatively coupled to adenylyl cyclase has also been reported in hepatocytes (Tomura et al., 1992) and PC12 cells (Murayama et al., 1998).

At these P2Y-theophylline-sensitive receptors of hippocampal nerve terminals, α,β-methylene ATP, γ,β-methylene ATP, UTP, GTP, and 2-MeSATP appear to be less effective than ATP. Full agonists have the following rank order of potency: AMP = ATP > ATPγS > adenosine = ADP. Suramin and PPADS and reactive blue 2 (P2 receptor antagonists) were inactive on these receptors. Similar agonist profile was described in submucosal neurons (Barajas-López et al., 1995).

In conclusion, presynaptic fibers in the CA1 hippocampal region express ATP receptors whose activation inhibits synaptic release of glutamate. These receptors appear to be P2Y (G protein-coupled), which, however, are sensitive to theophylline derivatives.

**References**


