Modulation of NMDA Receptor Current in Layer V Pyramidal Neurons of the Rat Prefrontal Cortex by P2Y Receptor Activation

Current responses to \(N\)-methyl-D-aspartate (NMDA) in layer V pyramidal neurons of the rat prefrontal cortex were potentiated by the P2 receptor agonists adenosine 5'-triphosphate (ATP) and uridine 5'-triphosphate (UTP). The failure of these nucleotides to induce inward current on fast local superfusion suggested the activation of P2Y rather than P2X receptors. The potentiation by ATP persisted in a \(Ca^{2+}\)-free superfusion medium but was abolished by 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis(acetoxymethyl) ester, cyclopiazonic acid, 7-nitroindazole, fluorooxacetic acid, bafilomycin, and tatanus toxin, indicating that an astrocytic signaling molecule may participate. Because the metabotropic glutamate receptor (mGluR) agonists (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid (ACPD) (group I/II) and (RS)-3,5-dihydroxyphenylglycine (group I) both imitated the effect of ATP and the group I mGluR antagonist 1-aminoindan-1,5-dicarboxylic acid or a combination of selective mGluR1 (7-hydroxymimo)-cyclopropa[b]benzene-1a-carboxylate) and mGluR5 (2-methyl-6-phenylethylpyridine) antagonists abolished the facilitation by ATP, it was concluded that the signaling molecule may be glutamate. Pharmacological tools known to interfere with the transduction cascade of type I mGluRs (guanosine 5'-O-(3-thiodiphosphate), U-73122, estoxospongic 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetracetic acid, calmodulin kinase II [CAMKKII] inhibitor peptide) depressed the actions of both ATP and ACPD. Characterization of the P2Y receptor by agonists (ATP and UTP), antagonists (suramin and pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid), and knockout mice (P2Y2/subtype) suggested that the nucleotides act at the P2Y\(_2\) subtype. In conclusion, we propose that exogenous and probably also endogenous ATP release vesicular glutamate from astrocytes by P2Y\(_2\) receptor activation. This gluta-mate then stimulates type I mGluRs of layer V pyramidal neurons and via the G\(_\text{o}\)/phospholipase C/inositol 1,4,5-trisphosphate/Ca\(^{2+}\)/CAMKII transduction pathway facilitates NMDA receptor currents.

Keywords: astrocyte, NMDA receptor, pyramidal neuron, P2 receptor, rat prefrontal cortex

Introduction

Layer V/VI pyramidal neurons in the prefrontal cortex (PFC) of primates and rodents are crucial for processing short-term working memory (Goldman-Rakic 1998). These neurons receive afferent innervation from the dopaminergic cell groups localized in the ventral tegmental area, being critical for the modulation of cognitive functions (Brozoski and others 1979). Dopamine may affect pyramidal neurons primarily through D1 receptor-mediated immediate excitability changes (Gulledge and Jaffe 2001; Dong and White 2003) or through an increased synaptic input from \(\gamma\)-aminobutyric acid (GABAergic) interneurons (Seamans and others 2001). Finally, dopamine appears to facilitate glutamatergic transmission onto PFC pyramidal neurons via a postsynaptic interaction between D1 and \(N\)-methyl-D-aspartate (NMDA) receptors (Cepeda and others 1992; Seamans and others 2001; Wirkner and others 2004) situated at the soma, dendritic shafts, and synaptic spines of these neurons themselves (Goldman-Rakic and others 1989). The glutamatergic input originates from the medialdorsal nucleus of the thalamus and from neighboring PFC pyramidal cells interconnected with each other (Groenewegen 1988).

Adenosine 5'-triphosphate (ATP) has been shown to be an extracellular signaling molecule in the central and peripheral nervous system, which acts as a neurotransmitter on its own or a cotransmitter with a multiplicity of other transmitters (Burnstock 1976; Zimmermann 1994). Especially the corelease of ATP and noradrenaline has been repeatedly demonstrated from postganglionic sympathetic neurons in the periphery (von Kügelgen and Starke 1991) and from locus coeruleus neurons of the central nervous system (CNS) (Poelchen and others 2001). Ligand-gated P2X receptors mediate a fraction of excitatory post-synaptic currents (EPSCs) of rat pyramidal neurons in the hippocampal cornu ammonis (CA1) layer (Pankratov, Lalo, and Krishtal 2002) and layer II/III of the somatosensory cortex (Pankratov, Lalo, Krishtal, and Verkhratsky 2002). G protein-coupled P2Y receptors were shown to positively interact with NMDA receptors situated at PFC layer V pyramidal neurons (Wirkner and others 2002). Thereby, the possible cotransmitters dopamine and ATP (Krügel and others 2001) may shape in an equal and possibly additive fashion the glutamatergic excitation in the prelimbic area.

Recently, a wealth of data indicate that astrocytes are an integral element of the circuitry for synaptic plasticity (Araque and others 2001). In addition to the neuronal release of excitatory amino acids, glutamate may be secreted from astrocytes not only by exocytotic processes but also by connexin hemichannels, providing the substrate for gap junction formation, glutamate transporters operating in the reverse mode, and a subtype of P2X receptors (P2X5), establishing a link between the release of ATP and glutamate (Illes and Ribeiro 2004). In consequence, astrocytic glutamate is a possible factor modulating excitatory neurotransmission in neuronal networks (Nedergaard and others 2002; Newman 2003). ATP secretion from astrocytes has been suggested to occur by 4 alternative pathways, such as an exocytotic vesicular release, ATP cassette proteins, connexin hemichannels, and osmolytic transporters linked to anion channels (Illes and Ribeiro 2004).
The aim of the present study was 2-fold. First, we tried to find out whether P2Y receptors are localized at astrocytes rather than neurons to modulate NMDA receptors of PFC layer V pyramidal cells. Second, the P2Y receptor subtype involved in this effect was characterized, and its second messenger mechanisms were investigated. It is suggested that ATP may act at an astrocytic P2Y<sub>2</sub> receptor to exocytotically release vesicular glutamate onto neighboring pyramidal neurons. This glutamate stimulates type I metabotropic glutamate receptors (mGlurS) that positively interact with NMDA receptors via the G<sub>q</sub>/phospholipase C/inositol 1,4,5-trisphosphate (IP<sub>3</sub>)/Ca<sup>2+</sup>/calmodulin kinase II transduction pathway.

Materials and Methods

**Experimental Animals**

Wistar rats (own breed), P2Y<sub>2</sub> mice bred to 129Sv background (Beverly H. Koller, University of North Carolina, Chapel Hill, NC; Homolya and others 1999), and the respective wild-type mice (Harlan-Winkelmann, Borchsen, Germany) were used. All experiments were in accordance with the German guidelines for the use of animals in biomedical research.

**Brain Slice Preparation**

Wistar rats (12–15 days old) or mice (10–15 days old) were decapitated. The brains were quickly removed and were placed in ice-cold, oxygenated (95% O<sub>2</sub> plus 5% CO<sub>2</sub>) artificial cerebrospinal fluid (ACSF, pH 7.4) at room temperature (20–22 °C) for superfusion (3 ml/min) with oxygenated ACSF containing (in mM) NaCl 126, KCl 2.5, NaH<sub>2</sub>PO<sub>4</sub> 1.2, CaCl<sub>2</sub> 2.4, MgCl<sub>2</sub> 1.3, NaHCO<sub>3</sub> 25, and glucose 11. Coronal slices (200 μm thick) were cut from a block of tissue containing the prelimbic portion of the medial PFC in cold ACSF using Vibratome 1000 (Piano, Marburg, Germany). After being stored in a holding chamber for at least 1 h at a temperature of 35–36 °C, a single slice was transferred to a recording chamber and was superfused (3 ml/min) with oxygenated ACSF at room temperature (20–22 °C). Only one cell was measured from each brain slice. The composition of the ACSF used for superfusion was the same as the composition of the ACSF used for incubation, except when the effect of a nominally Ca<sup>2+</sup>-free medium was investigated. In this case, CaCl<sub>2</sub> was omitted from the ACSF without altering the concentration of all residual constituents.

**Acute Isolation of Cortical Neurons**

Brain slices containing the prelimbic portion of the medial PFC were prepared as described above, with the exception that the slices had a thickness of 450 μm instead of 200 μm. The tissue was stored (1 h, 35–36 °C) in oxygenated Ca<sup>2+</sup>-free (N- (2-hydroxyethyl)piperazine-N- (2-ethanesulfonic acid) (HEPES)-buffered solution containing (in mM) NaCl 150, KCl 5, MgSO<sub>4</sub> 2, sodium pyruvate 1, glucose 10, and HEPES 10, pH 7.4 adjusted with NaOH. The slices were then transferred to a pronase-supplemented (11.2 U/ml, 25 min) piperazine-N,N-bis-(2-ethanesulfonic acid) (PIPES)-buffered solution containing (in mM) NaCl 126, KCl 2.5, MgCl<sub>2</sub> 2, CaCl<sub>2</sub> 10, glucose 10, NaH<sub>2</sub>PO<sub>4</sub> 1.25, PIPES 26, and glycine 0.01 (O<sub>2</sub> aeration), washed, and stored in Ca<sup>2+</sup>-free solution at room temperature (20–22 °C). Cell isolation was performed mechanically using Pasteur pipettes as described previously (Weber and others 2001).

**Whole-Cell Patch-Clamp Recordings in Brain Slices**

Membrane currents of layer V pyramidal cells in the PFC were recorded as in previous experiments (Wirkner and others 2002). Pyramidal cells were visualized with an upright interference contrast microscope and a ×40 water immersion objective (Axioskop FS, Carl Zeiss, Oberko-ichen, Germany). The microscope was connected to a video camera sensitive to infrared light (Newvicon C.2000-07-C, Hamamatsu, Herrsching, Germany). Patch pipettes were produced by a vertical micropipette puller (L-M-3P-A, List-Medical, Darmstadt, Germany) from borosilicate glass capillaries. They were filled with intracellular solution of the following composition (mM): K-glutonate 140, NaCl 10, MgCl<sub>2</sub> 1, HEPES 10, ethylene glycol-bis-(β-aminoethyl ether)-N,N',N'-tetraacetic acid (EGTA) 11, adenosine 5'-triphosphate magnesium salt (Mg-ATP) 1.5, and guanosine 5'-triphosphate (GTP) 0.3; pH 7.3 adjusted with KOH. Pipette resistances were in the range of 5–7 MΩ. Calculation of the liquid junction potential (V<sub>ij</sub>) between the bath and pipette solutions at 22 °C according to Barry (1994) yielded a value of 15.2 mV. All membrane potential values given in this study were corrected for V<sub>ij</sub>. The membrane potential of pyramidal cells was measured in the current-clamp mode of the patch-clamp amplifier (Axopatch 200B, Axon Instruments, Union City, CA) immediately after establishing whole-cell access. The membrane potential values of rat and mice PFC neurons were comparable (−60 to −80 mV). Then, the system was left for 5–10 min to allow for the settling of diffusion equilibria between the patch pipette and the cell interior, before current responses to NMDA were recorded in the voltage-clamp mode at a holding potential of −70 mV. Whole-cell recordings of 40–50 min duration could be routinely achieved with stable membrane properties of the cells throughout. Data were filtered at 2 kHz with the inbuilt filter of Axopatch 200B, digitized at 5 kHz, and stored on a laboratory computer using a Digidata 1200 interface and pClamp 8.0 software (Axon Instruments).

In most experiments, drugs were applied by changing the superfusion medium by means of 3-way taps. At the constant flow rate of 3 ml/min, about 20 s were required until the drug reached the bath. NMDA (50 μM) was applied 3 times for 1.5 min each (T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>) being separated by superfusion periods of 10 min with drug-free ACSF. Because the currents considerably decreased from T<sub>1</sub> to T<sub>2</sub> and at higher agonist concentrations even from T<sub>2</sub> to T<sub>3</sub>, an agonist concentration (30 μM) was chosen in all further experiments, which evoked reproducible inward currents (see also Wirkner and others 2002). ATP (30 and 300 μM), uridine 5'-diphosphate (UDP, 100 μM), uridine 5'-triphosphate (UTP, 100 μM), (1S,3R)-1-aminoacyclopentane-1,3-dicarboxylic acid (ACPD, 50 and 100 μM), or (RS)-3,5-dihydroxyphenylglycine (DHPG, 50 μM) was present in the bath 5 min before and during the third application of NMDA (50 μM) at T<sub>3</sub>. Suramin (30 μM), pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS, 30 μM), N6-(2'-(3-thiodiphosphate) (GDP-β-S, 30 μM), or 2-methyl-6-(phenylethyl)pyridyn (MPEP, 30 μM), 1-[6-[[1(7)]-3-methoxysteara-1,3,5[10]-trien-17-yl]amino[hexyl]-1H-pyrorol-2,5-dione (U-73122, 10 μM), 1-[6-[[1(7)]-3-methoxysteara-1,3,5[10]-trien-17-yl]amino[hexyl]-2,5-pyrrolininedione (U-73124, 10 μM), genistein (30 μM), 1,2-bis(2-amino-5-fluorophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA, 30 μM), 1-(1,1-dimethylethyl)-1-(4-pyridyl)pyrazol-3,4-dipirimidin-1-amine (PP1, 10 μM), or 7-nitroindazole (100 μM) was added to the pipette solution. Single brain slices were incubated at room temperature in ACSF containing bafilomycin (1 μM for 1 h) or tetrodotoxin (1 μg/ml for 2 h) prior to the experiment. In addition, 20 s before and during the patch-clamp recording, the slice was continuously perfused with bafilomycin-containing (1 μM) or tetrodotoxin-containing (1 μg/ml) ACSF, respectively. Other brain slices were incubated with fluorocitic acid (100 μM), bafilomycin (100 μM), or 7-nitroindazole (100 μM) medium for 2 h. Because the amplitudes of NMDA-induced currents showed great variabilities, the effects of all drugs applied before and during T<sub>3</sub> were expressed as percentage increase of the respective currents measured at T<sub>2</sub>.

In the residual experiments, NMDA (100 μM) was applied by pressure locally onto single cells 7 times for 2 s each with a time interval of 60 s between 2 applications (T<sub>1</sub>–T<sub>6</sub>) using a drug application device 12 (DAD12) superfusion system (Adams and List, Westbury, NY). At first, 2
control NMDA currents were recorded; then, superfusion with UTP (30, 100, and 300 μM) or 2-methylthioadenosine 5′-triphosphate (2-MeSATP, 300 μM) started 30 s after the second NMDA application (T2) and lasted for a total of 2.5 min with subsequent washout. The amplitudes of the NMDA-induced currents at T2 were expressed as a percentage of the second control current (the last current before UTP or 2-MeSATP application). Antagonists (PPADS, 8-cyclopentyl-1,3-dipropylxanthine [DPCPX]) were present in the medium at least 15 min before the start of the experiment.

Our intention was to work under near physiological conditions, and therefore, in agreement with a previous study (Wirkner and others 2002), we used an Mg2+-containing ACSF solution and did not apply the coagonistic glycine. Although NMDA (30 μM) caused much larger current responses in the absence (635.0 ± 117.2 pA, n = 6) than in the presence of Mg2+ (311.2 ± 47.6 pA, n = 8, P < 0.05) at T2, the ATP-induced (300 μM) facilitation at T2 was the same irrespective of the absence (57.9 ± 28.6%, n = 6) or presence of Mg2+ (51.0 ± 28.9%, n = 8). A number of publications have documented that in brain slice preparations, saturating concentrations of endogenous ligands (such as D-serine or taurine) are able to fully support the operation of NMDA receptor channels (Flint and others 1998, Mothet and others 2000).

Whole Cell Patch-Clamp Recordings in Acutely Isolated Neurons

Recordings using acutely isolated neurons were made according to Weber and others (2001). In brief, NMDA-induced currents were recorded in the whole-cell configuration of the patch-clamp amplifier at a holding potential of ~80 mV (EPC9, List-Medical). Currents were filtered at 3 kHz and sampled at 10 kHz. The pipette solution contained (in mM) KC1 90, KF 40, MgCl2 2, HEPES 10, and Na2ATP 3 (pH 7.3). The standard bath solution consisted of (in mM) NaCl 150, KCl 5, CaCl2 3, t-glucose 10, and HEPES 10 and contained routinely glycine (10 μM) and TTX (0.5 μM).

NMDA (1 mM) was locally applied by means of a fast superfusion system (DAD12, Adams and List) 6 times for 2 s each every 60 s (T1–T6) onto individual pyramidal-shaped neurons. Two NMDA currents were recorded before, during, and after the application of ATP (30 and 300 μM) for 2 min. Superfusion with ATP started immediately after the second ejection of NMDA (T2). The amplitudes of NMDA-induced currents at T2 were expressed as a percentage of the second control current (the last current before ATP application). PPADS (30 μM) was present in the medium at least 15 min before the start of the experiment and then throughout.

Materials

The following drugs and chemicals were used: ACPD, AIDA, (S)-2-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 7-(hydroxyimino)cyclopenta[b]chromen-1a-carboxylate ethyl ester (CPCCOEt), DHPG, genistein, N-[2-[[3-[(4-chlorophenyl)-2-propenyl]methylamino]-methyl]phenyl]-N-(2-hydroxyethyl)-4-methoxybenzenesulfonamide (KN-93), 2-MeSATP tetrasodium salt, MPEP hydrochloride, PPADS tetrasodium salt, PP1 (RBI, Natick, MA); ATP disodium salt, Mg-ATP, bafilomycin 93), 2-MeSATP tetrasodium salt, MPEP hydrochloride, PPADS tetrasodium salt, suramin hydrochloride, xestospongin C, DPCPX) and dimethyl sulfoxide (DMSO) (CPCCOEt, xestospongin C, DPCPX) and were dissolved further in medium. Further dilutions were made daily with distilled water or in dimethyl sulfoxide (DMSO) (CPCCOEt, xestospongin C, DPCPX) and were dissolved further in medium. Further dilutions were made daily with the extra- or intracellular solution as appropriate. The final concentration of NMDA (30 μM) was active in the presence of both suramin and PPADS, when compared with recordings in the absence of these antagonists, allowing no definite conclusion about the operation of an endogenous ATPergic tone probably facilitating NMDA receptor function; however, the action of NMDA was reproducible both at T2 and T5 (Wirkner and others 2002). ATP (300 μM) was active in the overwhelming majority of layer V pyramidal cells of the rat PFC (46 out of a total of 52 cells). The fraction of pyramidal neurons responding to UDP or UTP applied by slow superfusion (100 μM each, 5 out of 10 and 14 out of 22 cells, respectively) appears to

Facilitation by ATP and UTP of the NMDA Current Amplitude in Layer V Pyramidal Neurons of the PFC

In the present experiments, we used PFC slices of young rats (12–15 days) and mice (10–15 days) for recording; under these conditions, all cells belonged to the “regular spiking” type (Zhang 2004). The repetitive superfusion of a submaximal concentration of NMDA (30 μM; Wirkner and others 2002) every 10 min caused reproducible responses during its second and third application (T2 and T5). When ATP (30 and 300 μM) was present 5 min before and during T5, a concentration-dependent potentiation of the response to NMDA occurred in comparison with that measured at T2 (Fig. 1Ad,B,C). In accordance with previous results (Wirkner and others 2002), partial synaptic isolation of pyramidal neurons by either a nominally Ca2+-free medium or TTX (0.5 μM) did not alter the ATP-induced potentiation of NMDA currents (Fig. 1B). It is noteworthy that when in experiments analogous to those discussed above, a submaximal concentration of AMPA (3 μM) was used instead of NMDA (30 μM), ATP (300 μM) had no effect. The current responses caused by AMPA (3 μM, T2 = 324.6 ± 104.3 pA, n = 8) and NMDA (30 μM, T2 = −310.2 ± 38.7 pA, n = 8, P > 0.05) were similar in amplitude. These results, in conjunction with the previously observed failure of ATP to modulate the nonNMDA component of the excitatory synaptic potential and the depolarization induced by a rapid local application of AMPA (Wirkner and others 2002), suggest that the facilitatory effect of ATP is restricted to the NMDA class of ionotropic, excitatory amino acid receptors. Further, ATP (300 μM) caused facilitation of NMDA (30 μM) currents in layer V but not in layer III pyramidal neurons of the PFC (−10.6 ± 6.3%, n = 7), indicating selectivity for a certain type of pyramidal cells. Finally, we never observed a rapid inward current after the application of ATP/ UTP, either under these conditions or under the conditions of a rapid local superfusion disfavoring receptor desensitization (see below). Therefore, it is assumed that G protein–coupled P2X2 rather than ionotropic P2X receptors are involved.

The mixed P2X2/P2Y receptor antagonist suramin, which, however, at higher concentrations blocks NMDA receptors as well (von Kügelgen and Wetter 2000), at 30 μM did not alter the ATP-induced (300 μM) potentiation of NMDA (30 μM) currents (Fig. 1C; but see Wirkner and others 2002). In contrast, the mixed P2X2/P2Y receptor antagonist PPADS (30 μM) abolished the effect of ATP (Fig. 1Ab,C). Both suramin and PPADS were superfused at least 15 min before T1 and throughout the experiment. The responses to NMDA at T2 were depressed in the presence of both suramin and PPADS, when compared with recordings in the absence of these antagonists, allowing no definite conclusion about the operation of an endogenous ATPergic tone probably facilitating NMDA receptor function; however, the action of NMDA was reproducible both at T2 and T5 (Wirkner and others 2002). ATP (300 μM) was active in the overwhelming majority of layer V pyramidal cells of the rat PFC (46 out of a total of 52 cells). The fraction of pyramidal neurons responding to UDP or UTP applied by slow superfusion (100 μM each, 5 out of 10 and 14 out of 22 cells, respectively) appears to

Statistics

Means ± standard error of the mean are given throughout. Multiple comparisons with the control value were performed by the Kruskal-
on washout (Fig. 2Aa, B). UTP at 30 μM caused a slight depression of NMDA currents (Fig. 2B). We did not investigate the mechanism of this inhibitory action. In contrast, the facilitatory effect of UTP at a higher range of concentrations (100–300 μM) was unequivocally due to the stimulation of a P2Y receptor because PPADS (30 μM) was clearly antagonistic (Fig. 2Ab, B). A structural analogue of ATP, 2-MeSATP, at a high concentration of 300 μM inhibited rather than facilitated the NMDA current (Fig. 2B). This effect was mediated by adenosine A1 receptors, which may be activated by 2-methylthio adenosine, generated by enzymatic degradation of its mother compound 2-MeSATP. The ability of the highly A1-selective antagonist DPCPX (0.1) to abolish the effect of 2-MeSATP strongly supports this suggestion.

The missing effect of the nonselective P2X/P2Y receptor agonist 2-MeSATP (having some preference for P2Y1,12,15 receptors over the residual P2Y subtypes) in conjunction with

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**Figure 1.** Effects of ATP, UDP, and UTP on the NMDA-induced current in layer V pyramidal neurons of the rat PFC. Whole-cell patch-clamp recordings at a holding potential of –80 mV. NMDA (30 μM) or AMPA (3 μM) was applied 3 times (T1-T3) for 1.5 min every 10 min. All drugs were applied by changing the superfusion medium by means of 3-way taps. Current responses were reproducible at 1.5 min every 10 min. ATP (30 and 300 μM), concentration dependently, increased the NMDA-induced currents; this effect was completely reversible after washout (Fig. 2Aa, B). ATP at 30 μM caused a slight depression of NMDA currents (Fig. 2B). We did not investigate the mechanism of this inhibitory action. In contrast, the facilitatory effect of UTP at a higher range of concentrations (100–300 μM) was unequivocally due to the stimulation of a P2Y receptor because PPADS (30 μM) was clearly antagonistic (Fig. 2Ab, B). A structural analogue of ATP, 2-MeSATP, at a high concentration of 300 μM inhibited rather than facilitated the NMDA current (Fig. 2B). This effect was mediated by adenosine A1 receptors, which may be activated by 2-methylthio adenosine, generated by enzymatic degradation of its mother compound 2-MeSATP. The ability of the highly A1-selective antagonist DPCPX (0.1) to abolish the effect of 2-MeSATP strongly supports this suggestion.

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**Figure 2.** Effects of UTP and 2-MeSATP on the NMDA-induced inward current in layer V pyramidal neurons of the rat PFC. Whole-cell patch-clamp recordings at a holding potential of –80 mV. NMDA (100 μM) was applied 7 times (T1–T7) for 2 s every 60 s by means of a fast-pressurized superfusion system. Current responses were reproducible under these conditions. UTP (30, 100, and 300 μM) and 2-MeSATP (300 μM) were applied from 30 s before T7 until the end of T7. PPADS (30 μM) and DPCPX (0.1 μM) were superfused at least 15 min before T1 and throughout. (A) Potentiation by ATP of the current response to NMDA (a) and inhibition of ATP by PPADS (b). Representative tracings. The dotted line indicates the zero current level. (B) Effects of ATP on the AMPA- and NMDA-induced currents at T7, normalized with respect to the responses measured at T7. Concentration-dependent effect of ATP. The potentiation by ATP persisted in the nominal absence of extracellular Ca²⁺ or in the presence of TTX. (C) Effects of ATP alone and in the presence of suramin or PPADS as well as effects of UTP and UTP on the NMDA-induced currents at T7. Means ± standard error of the mean. The number of ATP-, UDP-, or UTP-responsive cells out of the total number of cells tested is indicated in each column. In the case of a complete blockade of the facilitatory effect of ATP, the number of all cells tested is indicated. *P < 0.05, statistically significant difference from controls at T7 (0%). #P < 0.05, statistically significant difference from the effect of ATP (300 μM) alone.

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be smaller than the ATP-sensitive fraction (Fig. 1C). Hence, the present experiments both confirmed and extended our previous results, thereby setting the stage for further investigations.

Because the complete exchange of the drug-free superfusion medium in the brain slice chamber by a drug-containing one is a slow process requiring a considerable time, desensitization of NMDA receptors during the 1.5-min contact period is unavoidable. To decrease the extent of desensitization, we ejected a higher concentration of NMDA (100 μM) onto the pyramidal cells investigated by means of a fast, local superfusion system for short periods of 2 s and every 1 min. The application of UTP (100, 300 μM), concentration dependently, increased the NMDA-induced currents; this effect was completely reversible after washout (Fig. 2Aa, B). ATP at 30 μM caused a slight depression of NMDA currents (Fig. 2B). We did not investigate the mechanism of this inhibitory action. In contrast, the facilitatory effect of UTP at a higher range of concentrations (100–300 μM) was unequivocally due to the stimulation of a P2Y receptor because PPADS (30 μM) was clearly antagonistic (Fig. 2Ab, B). A structural analogue of ATP, 2-MeSATP, at a high concentration of 300 μM inhibited rather than facilitated the NMDA current (Fig. 2B). This effect was mediated by adenosine A1 receptors, which may be activated by 2-methylthio adenosine, generated by enzymatic degradation of its mother compound 2-MeSATP. The ability of the highly A1-selective antagonist DPCPX (0.1) to abolish the effect of 2-MeSATP strongly supports this suggestion.

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**Figure 2.** Effects of UTP and 2-MeSATP on the NMDA-induced inward current in layer V pyramidal neurons of the rat PFC. Whole-cell patch-clamp recordings at a holding potential of –80 mV. NMDA (100 μM) was applied 7 times (T1–T7) for 2 s every 60 s by means of a fast-pressurized superfusion system. Current responses were reproducible under these conditions. UTP (30, 100, and 300 μM) and 2-MeSATP (300 μM) were applied from 30 s before T7 until the end of T7. PPADS (30 μM) and DPCPX (0.1 μM) were superfused at least 15 min before T1 and throughout. (A) Potentiation by ATP of the current response to NMDA (a) and inhibition of ATP by PPADS (b). Representative tracings. The dotted line indicates the zero current level. (B) Concentration–response relationship for UTP. Effects of UTP alone and in the presence of PPADS at T7, normalized with respect to the responses measured at T7. Effects of 2-MeSATP alone and in the presence of PPADS. Means ± standard error of the mean. The number of UTP-responsive and 2-MeSATP-responsive cells out of the total number of cells tested is indicated in each column. In the case of a complete blockade of the facilitatory effect of ATP, the number of all cells tested is indicated. *P < 0.05, statistically significant difference from controls at T7 (0%). #P < 0.05, statistically significant difference from the effect of UTP or 2-MeSATP (300 μM) each alone.
the comparable activity of equinuclor UTP and ATP concentrations indicate that ATP/UTP-sensitive P2Y2 or P2Y4 receptors may be involved. The blockade of the current responses to ATP and UTP by the P2Y4-preferential antagonist PPADS (acting also at P2Y4 receptors), but not by the P2Y1,-2-preferential antagonist suramin (von Kügelgen and Wetter 2000), brought out the participation of the P2Y4 subtype.

Astrocytic Localization of P2Y Receptors Mediating the Facilitation by ATP of NMDA Current

Experiments with ATP in the presence of a Ca2+-free medium or TTX prompted the hypothesis that this nucleotide may activate P2Y receptors situated at astrocytes and in consequence initiate the release of a glial transmitter or signaling molecule. Whereas the exocytotic release of, for example, glutamate from neurons is absolutely dependent on extracellular Ca2+, the astrocytic release is mostly regulated by the intracellular free Ca2+ concentration and is thereby relatively independent of extracellular Ca2+ (Jeremic and others 2001). Other types of Ca2+-independent release mechanisms discussed for astrocytes are those via connexin hemichannels (Ye and others 2003), glutamate transporters operating in the reverse mode (Hansen and Nedergaard 1988), and P2X-receptor channels (Nedergaard and others 2002; Duan and others 2003; Illes and Ribeiro 2004).

Preincubation of PFC slices with BAPTA-AM (50 μM), which may rapidly buffer intracellular Ca2+ ([Ca2+]i) of astrocytes possibly by preferential intracellular loading (for ω-nitrophenyl-ETGA-AM, see Liu and others 2004), or CPA (10 μM), known to deplete [Ca2+]i of both neurons and astrocytes, abolished the ATP-induced (300 μM) potentiation of NMDA (30 μM) currents (Fig. 3C). Bafilomycin blocks a vesicle-associated H+-ATPase and in consequence the exocytotic, vesicular release of glutamate both from neurons and from astrocytes (Araque and others 2000). Preincubation with bafilomycin (1 μM) for 1 h did not modify the ATP effect (Fig. 3Aa); however, a 1-h preincubation and subsequent superfusion of PFC slices for at least 30 min before T1 was sufficient to exclude the facilitatory action of ATP (Fig. 3Ab). Apparently, the local pressurized application of bafilomycin markedly increased the effect of this compound, which even in cell culture preparations needs a considerable time to develop (Zhou and others 2000). Tetanus toxin (1 μg/ml, 2 h preincubation and subsequent superfusion for 30 min) known to specifically cleave the neuronal/astrocytic type of soluble N-ethylmaleimide-sensitive attachment receptor (SNARE) protein synaptobrevin and thereby to inhibit the exocytotic release of glutamate (Kreft and others 2001) also excluded the ATP-induced potentiation (Fig. 3C). Moreover, the effect of 7-nitroindazole (100 μM) added to the external medium, but not to the pipette solution, was inhibitory on the facilitation by ATP (Fig. 3Bc). 7-Nitroindazole, an NO synthase inhibitor, was reported to prevent the NO-induced release of vesicular glutamate from astrocytes (Bal-Price and others 2002). Hence, application of 7-nitroindazole via the superfusion medium, which reaches both astrocytes and the pyramidal neuron under investigation, abolished the potentiation of NMDA currents by ATP, whereas application via the patch pipette solution into the pyramidal neuron itself was ineffective. In addition, extracellular 7-nitroindazole (100 μM) alone did not alter the amplitude of NMDA currents at T2 (control, −135.0 ± 17.7 pA, n = 8; 7-nitroindazole, −155.6 ± 41.2 pA, n = 7; P > 0.05). Therefore, NMDA receptor channels did not appear to be under the regulatory influence of a continuous NO production. In conclusion, all these results perfectly agree with the P2Y receptor-mediated release of a signaling molecule from astrocytes; this molecule could, then, stimulate its own receptors at pyramidal neurons and activate a second messenger pathway, leading to the inhibition of NMDA receptor channels.

Finally, we used a pharmacological tool, fluorocitric acid (100 μM), to examine whether the ATP effect could be abated when the glial Krebs cycle (Clarke 1991) and possibly in consequence the ATP-dependent storage of a gliotransmitter were blocked (Gordon and others 2005). Brain slices were
incubated for 2 h with fluorocitric acid before they were set up in the recording chamber. Whereas the effect of ATP (300 μM) on the NMDA (30 μM) currents was abolished (−11.2 ± 3.4%, n = 7), ACPD (100 μM), which directly stimulates mGluRs, continued to act at all neurons investigated (68.2 ± 23.5, n = 7).

Based on the previous experiments, we hypothesized that ATP could promote the vesicular release of glutamate from astrocytes, which in turn may activate mGluRs at layer V pyramidal neurons of the PFC. There are at least 8 mGluRs (mGluR1–8); they can be divided into groups I, II, and III on the basis of sequence homology, signal transduction mechanisms, and pharmacological properties (Pin and Duvoisin 1995). The group I mGluRs mGluR1 and mGluR5 are coupled to Gq proteins, mediating increases in inositol phosphates and the subsequent release of Ca2+ from intracellular stores. In fact, the group I/II mGluR agonist ACPD (50 and 100 μM) superfused 5 min before Tt concentration dependently increased the effect of NMDA at Tt (Fig. 4B,C). The selective group I mGluR agonist DHPG also potentiated the NMDA currents (Fig. 4C). Further, the selective group I mGluR antagonist AIDA (300 μM) applied 15 min before Tt and throughout abolished the ATP-induced (300 μM) facilitation of the NMDA currents (Fig. 4A,C). In addition, AIDA (300 μM) alone did not alter the amplitude of NMDA currents at Tt (control, −91.3 ± 12.2 pA, n = 7; AIDA, −129.3 ± 30.5 pA, n = 7; P > 0.05). Hence, NMDA receptor channels do not appear to be under a tonic influence of group I mGluRs activated by endogenously released glutamate.

Whereas both ATP and ACPD acted in all neurons investigated, the subtype-selective antagonists CPCCoEt (100 μM; mGluR1) and MPEP (30 μM; mGluR5) abolished the response to ATP (300 μM) only in 4 out of 10 cells and 6 out of 10 cells, respectively. The remaining effect of ATP in the presence of CPCCoEt and MPEP was 42.7 ± 12.4% (n = 4) and 36.1 ± 7.7% (n = 6). However, a combination of CPCCoEt (100 μM) and MPEP (30 μM) abolished the ATP-induced (300 μM) potentiation in all cells investigated (1.1 ± 6.8%, n = 6), indicating that although probably both subtypes of mGluRs facilitate NMDA currents (Benquet and others 2002), some layer V pyramidal cells are endowed with mGluR1, whereas others possess mGluR5.

**Second Messenger Pathway of the P2Y, Metabotropic Glutamate, and NMDA Receptor Interaction**

Then, we aimed at identifying the second messenger pathway involved in the effect of ATP/ACPD application. In accordance with the transduction mechanisms of group I mGluRs, ATP via the release of glutamate and the subsequent activation of mGluRs as well as via the direct stimulation of mGluRs by ACPD appeared to cause phosphorylation of NMDA receptor channels by calcium/calmodulin kinase II and in consequence increase the conductance of these channels (Koles and others 2001). In fact, the blockade of G protein-dependent reactions by intrapipette GDP-β-S (300 μM) and the inhibition of phospholipase C by extracellular U-73122 (10 μM), but not by its inactive structural analogue U-73343 (10 μM), abolished the facilitatory effect of ATP (300 μM) on the NMDA (30 μM) currents (Fig. 5A). The desensitization of IP3 receptors by their continuous activation (intracellular IP3, 50 μM) or the blockade of IP3 receptors by xestospongin C (10 μM), both applied via the pipette solution, behaved in all respects identical to GDP-β-S and U-73122. Further, a more efficient buffering of [Ca2+]i by BAPTA (5.5 mM) or the blockade of calcium/calmodulin kinase II (CAMKII) by the intrapipette application of a CAMKII inhibitory peptide (50 μM) or KN-93 (3 μM) also abolished the ATP-induced potentiation of NMDA currents. Finally, we excluded an alternative signaling pathway by using genistein and PP1, known to block Src-type tyrosine kinases (Fig. 5B). However, neither drug altered the potentiation of NMDA currents by ATP.

In conclusion, by choosing key inhibitors of the Gq/phospholipase C/IP3/Ca2+/calmodulin kinase II pathway (GDP-β-S, 300 μM; BAPTA-AM, 50 μM; CAMKII inhibitory peptide, 50 μM), we proved that ACPD and ATP utilize identical transduction mechanisms (Fig. 5C).

**Exclusion of the Participation of P2Y2 Receptors in the Facilitation of NMDA Currents in P2Y2−/− Mice**

Rodent P2Y2 and P2Y1 receptors exhibit comparable sensitivity with ATP and UTP (von Kügelgen and Wetter 2000). Although our previous pharmacological approach based on the determination of the agonist (ATP and UTP) and antagonist (suramin
and PPADS) profiles suggested the involvement of P2Y<sub>4</sub> rather than P2Y<sub>2</sub> receptors; we definitely excluded the participation of the latter receptor type by utilizing P2Y<sub>2R</sub> mice. In the wild-type controls, both ATP (300 μM) and UTP (100 μM) potentiated the NMDA (30 μM) current in a PPADS-antagonizable (30 μM) manner (Fig. 6 A–C). Whereas layer V pyramidal neurons of the rat PFC were almost without exception sensitive to ATP, only about the half of their mouse counterparts responded to P2Y<sub>2</sub> or ATP in layer V pyramidal neurons of mice. P2Y<sub>2R</sub> mice are presently unknown. In conclusion, P2Y<sub>2</sub> receptors definitely do not mediate the effect of ATP or UTP in layer V pyramidal neurons of mice.

**Interaction of P2Y and NMDA Receptors in Acutely Dissociated Pyramidal Neurons of the PFC**

In a following series of experiments, we investigated the interaction between P2Y and NMDA receptors in acutely dissociated pyramidal neurons of the rat PFC (Fig. 7). We have chosen individual neurons for recording, which did not form contacts with neighboring cells, that is astrocytes. Of course, it is unknown from which layer the pyramidal cells tested originate; however, some of them are expected to be layer V neurons. It is noteworthy that the process of enzymatic treatment and mechanical dissociation used by us decreases the number of ATP-responsive cells out of the total number of cells tested indicated in each column. In the case of a complete blockade of the facilitatory effect of ATP or UTP, the number of all cells tested is indicated.

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**Figure 5.** Effects of ATP and ACPD on the NMDA-induced current in layer V pyramidal neurons of the rat PFC. Whole-cell patch-clamp recordings at a holding potential of −80 mV. The experimental protocol for the application of NMDA (30 μM), ATP (300 μM), and ACPD (100 μM) as well as the calculation of standard error was similar to that described in the legend to Figure 1 for ATP. U-73122 (10 μM), U-73343 (10 μM), gerstein (30 μM), and BAPTA-AM (50 μM) were superfused at least 15 min before T<sub>1</sub> and throughout. GDP-β-S (300 μM), IP<sub>3</sub> (50 μM), xestospongin C (10 μM), BAPTA (5.5 mM), CAMKII inhibitor peptide (50 μM), KN-93 (3 μM), and PP1 (10 μM) were added to the pipette solution. (A) Inhibition of the potentiation by ATP of the NMDA current by GDP-β-S, U-73122 (but not U-73343), IP<sub>3</sub>, xestospongin C, and BAPTA. (B) Inhibition of the potentiation by ATP of the NMDA current by CAMKII inhibitor peptide and KN-93 but not gerstein and PP1. (C) Inhibition of the potentiation by ACPD of the NMDA current by GDP-β-S, BAPTA-AM, and CAMKII inhibitor peptide. Means ± standard error of the mean. The number of ATP- or ACPD-responsive cells out of the total number of cells tested is indicated in each column. In case of a complete blockade of the facilitatory effect of ATP or ACPD, the number of all cells tested is indicated. *P < 0.05, statistically significant difference from controls at T<sub>1</sub> (0%). #P < 0.05, statistically significant difference from the effect of ATP and ACPD alone, respectively.

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**Figure 6.** Effect of ATP on the NMDA-induced current in layer V pyramidal neurons of the mouse PFC. P2Y<sub>2R</sub> mice and the respective wild-type animals were used. Whole-cell patch-clamp recordings at a holding potential of −80 mV. The experimental protocol for the application of NMDA (30 μM), ATP (300 μM), and UTP (100 μM) as well as the calculation of data was similar to that described in the legend to Fig. 1. PPADS (30 μM) and MRS 2179 (10 μM) were superfused at least 15 min before T<sub>1</sub> and throughout. (A) Potentiation by ATP of the current response to NMDA (a) and interaction of ATP with PPADS (b) in wild-type mice. Representative tracings. (B) Effects of ATP and UTP on NMDA currents and their interaction with PPADS in wild-type mice. (C) Effects of ATP and UTP on NMDA currents and their interaction with PPADS or MRS 2179 in P2Y<sub>2R</sub> mice. Means ± standard error of the mean. The number of ATP- and UTP-responsive cells out of the total number of cells tested is indicated in each column. In case of a complete blockade of the facilitatory effect of ATP or UTP, the number of all cells tested is indicated. *P < 0.05, statistically significant difference from controls at T<sub>1</sub> (0%). #P < 0.05, statistically significant difference from the effect of ATP and UTP alone, respectively.
but does not abolish the sensitivity to NMDA of hippocampal CA1 pyramidal neurons of rats (Weber and others 2001). In the absence of ATP, the application of NMDA (1 mM) for 1 s every 1 min 6 times in total (T1–T6) initiated small current responses, which exhibited within the relatively short period of 6 min a run-down phenomenon (Fig. 7A, B). When ATP (30 and 300 µM) was applied immediately after the end of T2 until the end of T6, a pronounced and concentration-dependent depression, rather than potentiation of the NMDA current responses, was observed. However, ATP, both at 30 and 300 µM acted in most but not all neurons; a few cells were resistant to the effect of ATP alone.

Figure 7. Effect of ATP on the NMDA-induced current in dissociated pyramidal neurons of the rat PFC. Whole-cell patch-clamp recordings at a holding potential of −80 mV. NMDA (1 mM) was applied 6 times (T1–T6) for 2 s every 60 s by means of a fast-pressurized superfusion system. Under control conditions, current responses exhibited a slight run down. ATP (30 and 300 µM) was applied from immediately after T6 until the end of T6. PPADS (30 µM) was superfused at least 15 min before the start of the experiment and then throughout. The effect of ATP was expressed as a change in the NMDA current from T2 (100%) to T6. (A) Inhibition by a lower concentration of ATP (30 µM) of the current response to NMDA and interaction of ATP with PPADS. •, control; ○, ATP 30 µM; ▼, PPADS 30 µM + ATP 30 µM. Representative tracing of a control NMDA current (inset). (B) Inhibition by a higher concentration of ATP (300 µM) of the current response to NMDA and interaction of ATP with PPADS. •, control; ○, ATP 300 µM; ▼, PPADS 30 µM + ATP 300 µM. Means ± standard error of the mean. The number of ATP-responsive cells out of the total number of cells tested is indicated in each column. *P < 0.05, statistically significant difference from the respective controls. #P < 0.05, statistically significant difference from the effect of ATP alone.

(30 µM) abolished the inhibitory action of ATP (30 and 300 µM). Although we did not characterize the P2Y receptor type involved, we assume that the observed inhibition is due to the stimulation of P2Y1 receptors, which were reported to be situated at the layer V pyramidal neurons themselves and may inhibit NMDA receptor channels by immediate membrane-delimited interaction (Luthardt and others 2003). Still more importantly, the absence of a facilitatory ATP effect may be the consequence of the missing astrocytic-neuronal interaction after acute cell isolation.

Discussion

Regulation by P2Y Receptors of NMDA Receptor Channels of PFC Pyramidal Neurons

Functional investigations in the CNS indicate a role of P2X receptors in the mediation of fast synaptic transmission only in certain areas (Nörenberg and Illes 2000). In addition, ionotropic glutamate receptors had to be blocked to exclude a major glutamatergic component, before a minor ATPergic component of the EPSC could be identified. Although mRNA measurements and immunohistochemistry indicate a widespread distribution especially of the P2Y1 receptor message and its transcripts in the CNS (Moore and others 2000), there is only limited evidence for the functional significance of these receptors to directly modulate neuronal activity (Kawamura and others 2004; Rodrigues and others 2005). In contrast, many astrocytes possess P2Y receptors; they appear to mediate the propagation of Ca2+ waves in astrocytic networks as targets of the diffusible extracellular signaling molecule ATP (Fam and others 2000; Haydon 2001). Propagated Ca2+ waves may lead to the Ca2+-triggered release of various gliotransmitters such as ATP-, glutamate-, and GABA-modulating neuronal activity via astrocytic end-feets (Araque and others 2001; Fields and Stevens-Graham 2002; Newman 2003).

In brain slice preparations, we found both facilitatory (Wirkner and others 2002) and inhibitory interactions (Luthardt and others 2003) between P2Y and NMDA receptors in PFC pyramidal neurons. The inhibitory interaction appeared to be due to a membrane-delimited blockade of NMDA receptors by colocalized P2Y1 receptors; an analogous modulation of NMDA currents by mGlur1A has been reported previously (Yu and others 1997). However, both the mechanism of the facilitatory interaction and the subtype of P2Y receptors involved remained unresolved; it was the purpose of the present study to clarify these points. The finding that ATP upregulates NMDA receptor function in layer V but not in layer III pyramidal neurons of the PFC may be important for the processing of information carried by dopaminergic and glutamatergic projections terminating in layer V pyramidal neurons (Brozoski and others 1979; Groenewegen 1988).

Release of Astrocytic Glutamate by P2Y Receptor Activation

We suggest that the potentiation of NMDA currents is due to the exocytotic release of a transmitter of possibly astrocytic origin. For this purpose, inhibitors of the vesicular release mechanism (bafilomycin, tetanus toxin; Araque and others 2000; Kreft and others 2004), compounds buffering or depleting intracellular Ca2+ (BAPTA-AM, CPA; Liu and others 2004), the NO inhibitor 7-nitroindazole, known to depress NO-induced glutamate release from astrocytes (Bal-Price and others 2002), and fluoroacetate...
acid, a selective blocker of the astrocytic Krebs cycle (Clarke 1991; Gordon and others 2005), were utilized. Both the independence of the P2Y–NMDA receptor interaction on extracellular but not intracellular Ca\(^{2+}\) (Jeremic and others 2001) and the strong depression of this response by BAPTA-AM, which may be taken up preferentially into astrocytes (Liu and others 2004), suggest an astrocytic source of transmitter release. A further argument for this mode of action arises from the use of fluorocitric acid, an assumedly selective blocker of the ATP-dependent storage of gliotransmitters in astrocytes (Clarke 1991; Gordon and others 2005).

We believe that this transmitter is glutamate because the group I mGluR antagonist AIDA or a combination of selective mGluR1 (CPCCOEt) and mGluR5 (MPEP) antagonists abolished the facilitatory effect of ATP on the NMDA responses (compare with Benquet 2002). The pyramidal neurons may belong to 2 subpopulations, one of them endowed with mGluR1 and the other with mGluR5. Therefore, the blockade of one receptor subtype decreased only the number of the responsive neurons, whereas the blockade of both subtypes was needed to abolish the ATP effect. In addition, the mGluR agonists ACPD (group I) and DHPG (group I) both imitated the effect of ATP. A Ca\(^{2+}\)-, dependent vesicular release of glutamate from astrocytes in response to various neurotransmitter molecules including ATP was repeatedly demonstrated (Parpura and others 1994; Araque and others 2001; Jeremic and others 2003). Astrocytes possess a vesicular compartment that is competent for regulated exocytosis, after an increase in \([Ca^{2+}]_i\), astrocytic vesicles undergo rapid Ca\(^{2+}\)- and SNARE-dependent exocytotic fusion leading to glutamate release (Beczi and others 2004). All these data are in perfect agreement with our suggestion that ATP induces the exocytotic secretion of glutamate from astrocytes, leading to the activation of type I mGluRs at layer V pyramidal neurons.

**Transduction Pathway of mGluR-Mediated Potentiation of NMDA Receptor Channels**

A cross talk between type I mGluRs and NMDA receptors has repeatedly been demonstrated in various types of CNS neurons (hippocampal CA3 pyramidal cells, Benquet and others 2002; cultured cortical neurons, Heidinger and others 2002; spinal dorsal horn neurons, Guo and others 2004). Two alternative mechanisms were shown to produce NMDA current enhancement. These were 1) the phosphorylation of the NMDA receptor subunits \(\Delta \& B\) (NR2A/B) by protein kinases (protein kinase \(C\), tyrosine [Src] kinase) (Benquet and others 2002; Heidinger and others 2002; Guo and others 2004) increasing channel open probability or 2) the phosphorylation of a synaptosomal-associated protein, termed synaptosomal-associated protein 25 (SNAP-25), mediating increased recruitment of new channels to the plasma membrane via regulated exocytosis (Lan and others 2001). We found in partial agreement with these results that following ATP-induced glutamate release and activation of type I mGluRs, the \(G_\gamma/\phi\) phospholipase \(C/IP_\gamma/Ca^{2+}/CAMKII\) pathway may be instrumental in phosphorylating one of the subunits of the NMDA receptor channels or an associated protein and in consequence increasing the ionic conductance of these channels. Our suggestion was confirmed by sequentially inhibiting each step in the transduction cascade by pharmacological tools (GDP-\(\beta\)-S for \(G\) proteins; U-73122 for phospholipase \(C\); xestospongin \(C\) for \(IP_\gamma\) receptors; BAPTA for Ca\(^{2+}\) buffering; and CAMKII inhibitor peptide and KN-93 for CAMKII). In addition, the blockade of the key steps in the transduction cascade (\(G\) proteins, Ca\(^{2+}\) release, CAMKII activity) equally abolished the functional consequences of both ATP application and the direct stimulation of type I mGluRs by ACPD. The involvement of an alternative signaling pathway in the effect of ATP by the activation of Src-family tyrosine kinases via the release of intracellular Ca\(^{2+}\) (Guo and others 2004) was excluded by using the wide-range tyrosine kinase inhibitor genistein and the Src-type tyrosine kinase inhibitor PP1.

**Characterization of the P2Y Receptor Type Involved**

Further, we characterized the subtype of P2Y receptors involved by means of both pharmacological tools and knockout techniques and, in addition, searched for the astrocytic and/or neuronal localization of these receptors by immunohistochemical methods. The use of the nonselective agonist ATP and the \(P2Y_{2,4}\)-preferential agonist UTP, as well as investigations with antagonists selective (MRS 2179; \(P2Y_1\)) or preferential (suramin, \(P2Y_{1,2}\); PPADS, \(P2Y_{1,4}\)) for certain receptor subtypes, suggested the exclusive involvement of the \(P2Y_1\) receptor type. Both these experiments and experiments with \(P2Y_{1,2}\) mice unequivocally excluded the participation of \(P2Y_2\) receptors. Unfortunately, because of the unavailability of \(P2Y_{1,2}\) mice, the final proof for the involvement of this receptor type is hitherto missing.

Based on reverse transcriptase–polymerase chain reaction measurements, western blotting analyses, and single-cell Ca\(^{2+}\) imaging studies, it was suggested that, of P2Y receptors, cultured cortical astrocytes mostly express the \(P2Y_1\), \(P2Y_2\), and \(P2Y_4\) subtypes (Fumagalli and others 2003). Under in vivo conditions, cortical astrocytes of rats exhibited \(P2Y_{1,2,4}\) immunoreactivity, which was upregulated by mechanical injury (Franke and others 2004). Hence, all these data are compatible with the modulation of PFC pyramidal cell function by nearby astrocytes endowed with \(P2Y_1\) receptors.

**Absence of P2Y–NMDA Receptor Interaction in Acutely Dissociated PFC Pyramidal Neurons**

Individual, acutely dissociated PFC pyramidal neurons were chosen for patch-clamp recordings; these neurons did not establish contacts with neighboring astrocytes. Under these conditions, ATP failed to cause a rapidly desensitizing inward current in all neurons investigated and, in addition, depressed the NMDA current amplitudes in a PPADS-reversible fashion. It is interesting to note that previous results indicate that ATP or its enzymatically stable analogue ATP-\(\gamma\)-S induces, in pyramidal neurons of the hippocampus (CA1, Pankratov, Lalo, and Krishtal 2002; CA3, Mori and others 2001) or somatosensory cortex (layer II/III, Pankratov, Lalo, Krishtal, and Verkhratsky 2002), inward current. By contrast, ATP failed to cause current responses either in hippocampal CA1 pyramidal neurons (Khakh and others 2003) or layer V pyramidal neurons of the PFC (present study). It is possible that differences in the species investigated (rat and mice), the maturity of the neuronal system (acute slices and slice cultures), or the speed of the local superfusion may be the reasons for these discrepancies. Finally, a regional variation of P2X receptor endowment may exist, with an absence of this receptor type in layer V pyramidal neurons of the PFC. Hence, we conclude for the PFC that, in the absence of astrocyte–neuron interactions, the effect of ATP may be due to the activation of PPADS-sensitive neuronal \(P2Y_1\) receptors directly interacting with the NMDA receptor channels (Luthardt and others 2003).
In conclusion, ATP may exert 2 opposing effects on the NMDA receptor channel conductance, namely, inhibition via neuronal P2Y\textsubscript{1} and facilitation via astrogliotic P2Y\textsubscript{1} receptors. When in PFC brain slice preparations, layer V pyramidal neurons maintain their contacts with neighboring astrocytes, indirect effects of ATP through the astrogliotic release of glutamate predominating.

**Notes**

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**References**


Cepeda C, Radisavljevic Z, Peacock W, Levine MS, Buchwald NA. 1992. NMDA receptor channel conductance, namely, inhibition via neuronal P2Y\textsubscript{1} and facilitation via astrogliotic P2Y\textsubscript{1} receptors. When in PFC brain slice preparations, layer V pyramidal neurons maintain their contacts with neighboring astrocytes, indirect effects of ATP through the astrogliotic release of glutamate predominating.


