

Review

P2Y receptors and pain transmission

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Received 18 August 2004; accepted in revised form 23 September 2004

Key words: analgesia, dorsal root ganglion, electrophysiology, immunohistochemistry, P2Y receptor

Abstract

It is widely accepted that the most important ATP receptors involved in pain transmission belong to the P2X₃ and P2X_{2/3} subtypes, selectively expressed in small diameter dorsal root ganglion (DRG) neurons. However, several types of the metabotropic ATP (P2Y) receptors have also been found in primary afferent neurons; P2Y₁ and P2Y₂ receptors are typically expressed in small, nociceptive cells. Here we review the results available on the involvement of P2Y receptors in the modulation of pain transmission.

Abbreviations: 2-MeSADP – 2-methylthio ADP; 2-MeSAMP – 2-methylthio AMP; 2-MeSATP – 2-methylthio ATP; α,β -meATP – α,β -methylene ATP; A3P5P – adenosine-3'-phosphate-5'-phosphate; ADP – adenosine 5'-diphosphate; ADP- β -S – adenosine 5'-O-(2-thiodiphosphate); AMP – adenosine 5'-monophosphate; AR-C69931MX – N⁶-(2-methylthioethyl)-2-(3,3,3-trifluoropropylthio)- β,γ -dichloromethylene ATP; ATP – adenosine 5'-triphosphate; BDNF – brain-derived neurotrophic factor; [Ca²⁺]_i – intracellular Ca²⁺ concentration; CGRP – calcitonin gene-related peptide; CREB – cAMP-responsive element binding protein; DAMGO – D-Ala²,N-Me-Phe⁴,Gly-ol⁵-enkephalin; DPDPE – D-penicillamine(2,5)-enkephalin; DRG – dorsal root ganglion; EPSP – excitatory postsynaptic potential; GDP- β -S – guanosine 5'-O-(2-thiobisphosphate); GF109203x – PKC inhibitor; HEK – human embryonic kidney-derived; IB₄ – isolectin B₄; IP₃ – inositol 1,4,5-triphosphate; IR – immunoreactivity, immunoreactive; KCNQ2 – potassium voltage-gated channel, KQT-like subfamily, member 2; mRNA – messenger ribonucleic acid; MRS-2179 – N⁶-methyl-2'-deoxyadenosine-3',5'-bisphosphate; MRS-2279 – (1R,2S,4S,5S)-1-[(phosphato)methyl]-4-(2-chloro-6-aminopurin-9-yl) bicyclo [3.1.0]-hexane-2-phosphate; NG – nodose ganglion; P2X – ionotropic ATP receptor; P2Y – metabotropic ATP receptor; pCREB – phosphorylated CREB; PKC – protein kinase C; PLC – phospholipase C; PPADS – pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid; RB2 – reactive blue 2; RT-PCR – reverse transcription polymerase chain reaction; SGC – satellite glial cells; SP – substance P; TG – trigeminal ganglion; TRPV1 – vanilloid receptor 1; U-50488H – *trans*-(\pm -3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]-cyclohexyl)-benzeneacetamide; U73122 – PLC inhibitor; UDP – uridine 5'-diphosphate; UTP – uridine 5'-triphosphate; VACC – voltage-activated calcium channel

Introduction

It was observed in the late sixties that the endogenous P2 receptor agonist adenosine 5'-triphosphate (ATP) can evoke pain sensation [1, 2]. The most important ATP-sensitive receptors involved in pain transmission appear to be the ligand-gated P2X₃ and P2X_{2/3} subtypes, selectively expressed by a subpopulation of small diameter dorsal root ganglion (DRG) cells binding isolectin B₄ (IB₄) and possessing also capsaicin-sensitive vanilloid receptors (TRPV1) [3, 4]. Numerous studies have shown that P2X receptors play a crucial role in facilitating pain transmission at peripheral and spinal sites; a large number of reviews has recently been published on these receptors and

nociception [5–10]. However, possible roles for metabotropic P2Y receptors in nociceptive signaling have received limited attention. Here we review the results available on P2Y receptors relating to the sensory pathway and on the available evidence that P2Y receptors are also involved in the modulation of pain transmission.

P2Y receptors

The following G protein-coupled P2Y receptors have been characterized to date in humans: P2Y₁, P2Y₁₁, P2Y₁₂, and P2Y₁₃ (sensitive to the adenine nucleotides ADP and ATP); P2Y₄ and P2Y₆ (sensitive to the pyrimidine nucleotides UDP and UTP); P2Y₂ (sensitive to both ATP and UTP); P2Y₁₄ (sensitive to uridine sugars such as UDP-glucose and UDP-galactose) [11–16].

P2Y receptors, in common with other G protein-coupled receptors, have seven transmembrane domains, an extra-

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cellular N terminus and an intracellular C terminus. The P2Y₁, P2Y₂, P2Y₄ and P2Y₆ receptors are positively coupled to phospholipase C via G_{q,11} proteins, while the P2Y₁₂₋₁₄ receptors are negatively coupled to adenylyl cyclase via G_i proteins. P2Y₁₁ receptors are dually coupled to G_{q,11} and G_s proteins, activating both phospholipase C and adenylyl cyclase [11–16].

The ligand selectivity of different P2Y receptor subtypes has extensively been discussed elsewhere [17–19]. ADP, adenosine 5'-O-(2-thiodiphosphate) (ADP-β-S) and 2-methylthio ADP (2-MeSADP) are agonists at P2Y₁, P2Y₁₂ and P2Y₁₃ receptors. Among them, ADP and ADP-β-S act also at P2X_{1,2,3,4} receptors. UTP is selective for P2Y₂ and P2Y₄ receptors, whereas UDP preferentially activates the P2Y₆ receptor; UDP-glucose acts at P2Y₁₄ receptors. P2Y₁₁ receptors are activated by ATP and 2-MeSATP. Adenosine-3'-phosphate-5'-phosphate (A3P5P), N⁶-methyl-2'-deoxyadenosine-3',5'-bisphosphate (MRS-2179) and (1R,2S,4S,5S)-1-[(phosphato)methyl]-4-(2-chloro-6-aminopurin-9-yl) bicyclo [3.1.0]-hexane-2-phosphate (MRS-2279) are selective antagonists for the P2Y₁ receptor. Pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) also shows some preference for P2Y₁ receptors over the residual P2Y receptor-types. N⁶-(2-Methylthioethyl)-2-(3,3,3-trifluoropropylthio)-β,γ-dichloromethylene ATP (AR-C6931MX) is a selective antagonist at P2Y₁₂ and P2Y₁₃ receptors, whereas 2-MeSAMP selectively blocks the P2Y₁₂ receptor-type.

Expression of P2Y receptors in neurons of sensory ganglia

In contrast to P2X₃ receptors, which are mostly expressed in small neurons of the DRG and other sensory ganglia, P2Y receptors seem to be equally expressed in small, large and medium-sized sensory neurons. Reverse transcription (RT)-PCR demonstrated the presence of P2Y₁ receptor mRNA in rat DRG, nodose ganglion (NG) and trigeminal ganglion (TG) [20–22]. *In situ* hybridization studies showed the presence of P2Y₁ receptor mRNA in about 20% of both rat and mouse DRG neurons [23, 24], with the most intense staining in large, RT-97-immunoreactive (IR) neurons, and weaker staining in small, peripherin-IR neurons [25]. Immunostaining studies showed that P2Y₁ receptor antibodies stained over 80% of the DRG, NG and TG neurons [22]. In this study, the small-diameter neurons were stained much more intensely than the medium- and large-diameter neurons. The staining was evenly distributed throughout the cytoplasm of these cells and immunoreactive cells were randomly distributed throughout the ganglia. P2Y₁ receptor-IR has also been demonstrated in cell bodies of the rat NG [26]. Ligation of the vagus nerve led to accumulation of staining adjacent to the ligature and this accumulation was found central to the proximal ligature and peripheral to the distal ligature, suggesting anterograde and retrograde transport, respectively. These data imply that P2Y₁ receptors are transported to and expressed functionally on terminals of sensory nerves.

The co-expression of P2Y₁ mRNA with TRPV1 mRNA, thought to be characteristic for nociceptive DRG neurons, was investigated by *in situ* hybridization. Astonishingly only 2% of TRPV1 mRNA-expressing cells possessed also P2Y₁ mRNA and *vice versa* [24]. However, in another study, where the receptor proteins were identified by immunocytochemistry in combination with confocal laser scanning microscopy, co-staining for P2Y₁, TRPV1 and P2X₃ receptors was observed in 80% of small-diameter (20–35 μM) neurons in the rat DRG [27]. These data were confirmed by the finding that P2Y₁ receptors are often co-localized on the same cells with P2X₃ receptors [22]. A high number of P2X₃-IR cells were also found to be P2Y₁ receptor-IR in DRG, NG and TG; in general, more neurons appeared to express P2Y₁ receptors than P2X₃ receptors [22]. Double immunofluorescence histochemistry showed that 30% of P2Y₁ receptor-IR cells showed also NF200-IR in DRG, NG and TG. NF200 is an anti-neurofilament antibody that stains the large-diameter neurons with myelinated axons [22]. The P2Y₁ receptor was also co-localized with the neuropeptide calcitonin gene-related peptide (CGRP) in more than 60% of DRG, NG and TG cells. The P2Y₁ receptors were further highly co-expressed in IB₄ containing neurons; 80%, 60% and 20% of the IB₄ cells were also immunoreactive for the P2Y₁ receptor, in DRG, NG and TG cells, respectively [22].

RT-PCR revealed the presence of P2Y₂ mRNA in rat DRG, NG and TG [21, 22]. *In situ* hybridization showed that 80% of adult rat DRG neurons expressed P2Y₂ mRNA, but only half of these neurons responded to UTP by phosphorylating the cAMP-responsive element binding protein (CREB) [28]. P2Y₂ mRNA was expressed in neurons of all sizes, but was seen less frequently in large cells: 90% of small cells (<30 μm diameter) were positive, as were 40% of larger cells (>40 μm diameter). A similar distribution of P2Y₂ expression was observed in neurons of the rat TG [28]. However, another *in situ* hybridization study showed that only 20% of rat DRG neurons expressed P2Y₂ receptors and that a significant population of DRG neurons co-expressed P2Y₂ and TRPV1 mRNA [24].

RT-PCR confirmed the presence of P2Y₄ mRNA in rat DRG, NG and TG [21, 22]. Immunohistochemistry revealed that the number of neurons staining for the P2Y₄ receptor antibody was lower than that staining for the P2Y₁ receptor antibody, and the medium- and large-diameter neurons were stained much more frequently than the small-diameter neurons [22]. The co-localization of P2Y₄ and P2X₃ receptors was less frequent than that of P2Y₁ and P2Y₄ receptors. Double immunofluorescence histochemistry showed that many P2Y₄ receptor-IR cells showed also NF200-immunoreactivity in DRG, NG and TG. CGRP-IR cells co-expressed P2Y₄ receptors much less frequently than P2Y₁ receptors. Unlike P2Y₁ receptor-IR cells, P2Y₄-IR neurons lacked IB₄ in these sensory ganglia [22]. Considering these findings, it is likely that P2Y₄ receptors are preferentially expressed in large, IB₄- and CGRP-negative DRG neurons. These neurons are known to be implicated in the transmission of tactile allodynia but not acute pain or thermal hyperalgesia [29] suggesting that P2Y₄ receptors may play a role in this process.

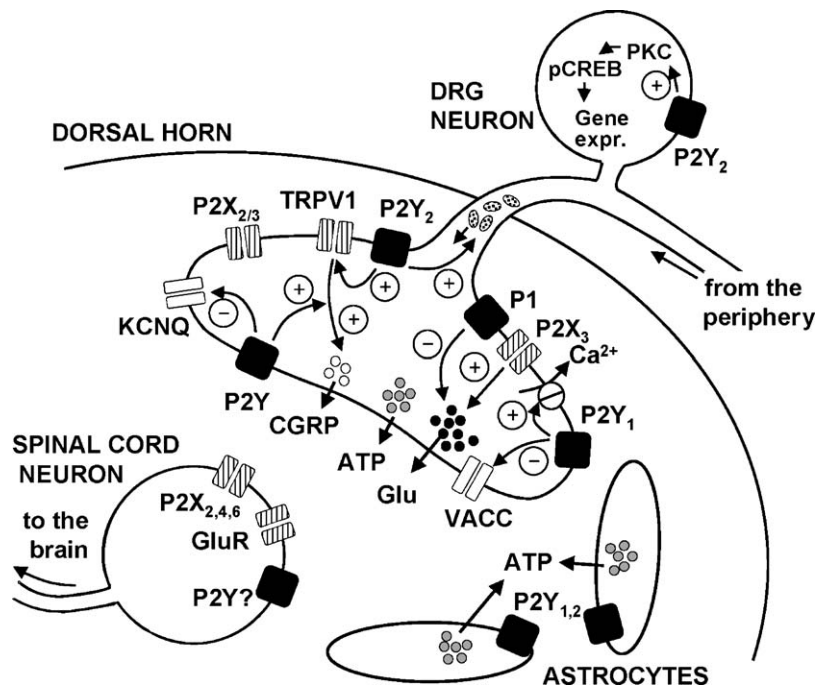


Figure 1. The role of P2Y receptors in pain pathways. The P2Y receptors are present on DRG cells, spinal cord astrocytes and probably on dorsal horn neurons. In DRG neurons, the P2Y₁ receptor stimulates the plasma membrane Ca²⁺ pump which removes Ca²⁺ from the cell. The P2Y₁ receptor further inhibits VACCs, which play a role in the vesicular release of glutamate from DRG neurons. The P2Y₂ receptor potentiates capsaicin-induced membrane currents through the vanilloid receptor 1 (TRPV1), increases the axonal transport of membrane-bound organelles and activates the phosphorylation of CREB in DRG cells. P2Y receptors of unidentified subtypes inhibit the M-type potassium currents through KCNQ channels in DRG cells and potentiate the capsaicin-induced release of CGRP from these cells. Of course, P2X₃ and P2X_{2/3} receptors are also present at the terminals of DRG neurons. P2X₃ receptors have been shown to positively modulate the release of glutamate, while P1 receptors of the A1-subtype depress glutamate release. Dorsal horn astrocytes are known to release ATP which is in turn able to activate neighboring cells via P2Y₁ and P2Y₂ receptors. Finally, spinal cord neurons are endowed with P2X_{2,4,6} and ionotropic glutamate receptors. The presence of P2Y receptors appears to be likely but has not been confirmed yet. ATP: adenosine-5'-triphosphate; CGRP: calcitonin gene-related peptide; CREB: cAMP-responsive element binding protein; DRG: dorsal root ganglion; Glu: glutamate; GluR: glutamate receptor; KCNQ: potassium voltage-gated channel; pCREB: phosphorylated CREB; PKC: protein kinase C; VACC: voltage-activated calcium channel.

RT-PCR demonstrated the presence of P2Y₆ mRNA in rat DRG, NG and TG, but the receptor was not confined to sensory neurons [21, 22]. ATP-induced Ca²⁺-release from inositol 1,4,5-triphosphate (IP₃)-sensitive Ca²⁺ stores in large (30–45 μM) but not in small (20–25 μM) mouse DRG neurons [30]. The effect was thapsigargin-sensitive; however, the involvement of P2Y receptor subtypes other than P2Y₆ was not investigated. In another functional study, UTP caused an increase of intracellular calcium concentration ([Ca²⁺]_i) in equal numbers of small (<30 μM), medium (30–40 μM) and large cells (>40 μM), suggesting the existence of P2Y₂ and/or P2Y₄ receptors at these neurons [21]. ADP-β-S evoked Ca²⁺ release from intracellular stores of small (20–35 μM) and IB₄-IR (nociceptive) DRG cells from the rat and this effect was antagonized by the P2Y₁ receptor antagonist MRS 2179 [31]. ATP, ADP and UTP were shown to inhibit voltage-activated calcium channels (VACCs) in cultured small-diameter (20–35 μM) DRG neurons from the rat, indicating the functional presence of P2Y₁, P2Y₂ and/or P2Y₄ receptors on these cells [27].

P2Y receptors in neurons of the dorsal spinal cord

There are only few and relatively controversial data available on the presence of P2Y receptors at dorsal horn neurons. It has been concluded that ATP increases [Ca²⁺]_i

in 30% of these neurons via P2Y receptor activation, since the selective P2X_{1,3} receptor agonist α,β-methylene ATP (α,β-meATP) had no effect [32]. However, ATP also acts via ionotropic P2X receptors of the P2X_{2,4,6} types which have been shown to be expressed on the somata of dorsal horn neurons [7]. The involvement of these receptors in ATP-induced [Ca²⁺]_i transients was not excluded by selective pharmacological ligands or omission of Ca²⁺ from the extracellular solution. In another study, the P2Y-selective 2-MeSADP caused no change in [Ca²⁺]_i in spinal cord cultured neurons [33]. The non-selective P2 agonists ATP and 2-MeSATP increased [Ca²⁺]_i in 60% of neurons [34]. The response was not abolished in Ca²⁺-free extracellular solution, and PPADS blocked the effect of 2-MeSATP. The possible influence of glutamate, released from neighboring cells, was ruled out using diverse glutamate receptor antagonists. In acutely isolated neurons which lost their dendrites, there was no P2Y response [34]. However, further experiments are needed to reliably evaluate the expression of P2Y receptors on spinal cord dorsal horn neurons (Figure 1).

P2Y receptors in glial cells

Sensory ganglia contain, in addition to neurons, glial cells that wrap around the neurons. These cells, also called

satellite glial cells (SGCs), are closely apposed to the neurons [35]. There is histological evidence that SGCs are affected in animal models for pain [36–39]. Specific P2Y receptor agonists (UTP, 2-MeSADP and ADP- β -S) but not the specific P2X_{1,3} receptor agonist α,β -meATP caused an increase in intracellular Ca²⁺ concentration in SGCs, indicating the functional presence of P2Y₁ and P2Y₂ or P2Y₄ receptors on these cells [40].

ATP, ADP, 2-MeSATP and 2-MeSADP all increased [Ca²⁺]_i in almost all astrocytes and in 20% of oligodendrocytes from cultured dorsal horn, whereas α,β -meATP had no effect [32, 33]. The action of the different P2Y₁ receptor agonists were independent of extracellular Ca²⁺ and sensitive to suramin, A3P5P and thapsigargin, indicating that these cells possess functional P2Y₁ receptors. In one of these studies, RT-PCR revealed the presence of P2Y₁ mRNA in the spinal cord [33]. Since 2-MeSADP caused no change in [Ca²⁺]_i of spinal cord neurons, it was concluded that the astrocytes are the source of P2Y₁ mRNA detected in the whole tissue. UTP was also shown to be able to increase [Ca²⁺]_i, although only in 30% of astrocytes from the dorsal horn [41]. The UTP-induced Ca²⁺ responses were thapsigargin-sensitive, pertussis toxin-insensitive, and both PPADS and suramin could antagonize them, demonstrating the functional expression of P2Y₂ receptors in dorsal horn astrocytes. Both the P2Y₁ and P2Y₂ receptors were found to be linked via G protein coupling to the release of [Ca²⁺]_i via the phospholipase C (PLC)/IP₃ pathway [42].

There are only sparse data available on the expression of P2Y receptors in spinal cord microglia. Application of the P2X/P2Y agonists ATP and 2-MeSATP on cultured microglia from rat spinal cord in the absence of extracellular Ca²⁺ induced an increase in [Ca²⁺]_i, whereas the P2X_{1,3,4}-selective β,γ -MeATP had no effect [43]. Therefore, ATP and 2-MeSATP may have increased [Ca²⁺]_i by activating a hitherto uncharacterized P2Y receptor-type.

P2Y receptors and pain

Excitation of DRG cells; possible noxious effects

In an early study on bullfrog DRG, ATP inhibited the M-type potassium current (Figure 1), leading to depolarization and initiation of action potentials [44]. Intracellularly applied guanosine 5'-O-(2-thiobisphosphate) (GDP- β -S), an inhibitor of G protein-mediated reactions, prevented the effect of ATP, suggesting the involvement of P2Y receptors. The M-current is a low-threshold, non-inactivating, voltage-sensitive K⁺ current, which is weak or undetectable at resting membrane potential and increases with membrane depolarization, inducing spike frequency adaptation and acting as a dynamic brake on neuronal excitability [45, 46]. M-currents emerge from homo- and heteromultimeric assemblies of KCNQ2-5 protein subunits [47]. Considering that agonists of M-currents have been shown to be antinociceptive [48], P2Y receptors inhibiting these currents may be able to stimulate the pain transmission.

In human embryonic kidney-derived HEK-293 cells, ATP, ADP, 2-MeSATP and UTP potentiated the capsaicin-evoked inward currents (Figure 1) [20]. PPADS, reactive blue 2 (RB2) and the protein kinase C (PKC) inhibitor calphostin C inhibited the stimulatory effect of ATP. Moreover, ATP treatment lowered the threshold temperature for TRPV1 activation from 42 to 35 °C, suggesting that TRPV1 activation could trigger the sensation of pain at normal body temperature in the presence of ATP. In further experiments on cultured rat DRG neurons, ATP and ADP caused the same potentiation of TRPV1 as on HEK-293 cells. This effect was independent of P2X receptors, since in cells with P2X receptor-mediated transient currents the magnitude of ATP-induced potentiation of capsaicin-evoked currents was indistinguishable from that seen in neurons lacking P2X responses. A following study from the same group revealed the importance of P2Y₂ receptors in nociception through TRPV1 [24]. It was reported that ATP-induced thermal hyperalgesia *in vivo* is abolished in mice lacking TRPV1, whereas it is preserved in P2Y₁ receptor-deficient mice. Moreover, patch-clamp analyses using mouse DRG neurons indicated the involvement of P2Y₂ rather than P2Y₁ receptors. The potentiation of capsaicin-evoked currents through ATP was still observed in P2Y₁-deficient mice, and UTP also potentiated TRPV1 in these cells. Suramin, which blocks P2Y₂ but not P2Y₄, abolished the effect of UTP.

ATP, but not UTP, accelerates Ca²⁺ efflux from rat DRG cells by stimulating the plasma membrane Ca²⁺ pump (Figure 1) [49]. Ca²⁺ entering the cell during excitation must be removed from the cytoplasm to maintain normal Ca²⁺ homeostasis; the plasma membrane Ca²⁺ pump is a key component of the Ca²⁺ extrusion machinery in neurons [50]. The stimulatory effect of ATP was prevented by PPADS, the P2Y₁ antagonist A3P5P, the phospholipase C inhibitor U73122 and by inhibition of PKC through GF109203x. This acceleration of the plasma membrane Ca²⁺ pump via P2Y₁ receptors caused a decrease in amplitude and duration of the action potential after-hyperpolarization and thereby an increase in the excitability of DRG cells.

UTP evoked a slow-onset depolarization and sustained firing in dissociated DRG neurons from the rat that persisted for tens of seconds after removal of UTP from the medium [28]. In a subsequent study, UTP activated sustained action potential firing in 50% of C fibers using the skin nerve preparation from the adult mouse [51]. UDP had no effect, pharmacologically excluding the involvement of P2Y₆ receptors. The majority (70%–80%) of UTP-sensitive C- and AM fibers respond strongly to capsaicin, whereas UTP-insensitive fibers were largely capsaicin-insensitive. Most of the UTP-sensitive C- and AM fibers (80%–100%) also responded to the P2X agonist α,β -meATP, indicating that P2Y and P2X receptors are widely co-expressed on these fibers. These data also support the idea that P2Y₂ receptors on the terminals of capsaicin-sensitive and P2X₃-expressing cutaneous sensory neurons effectively stimulate nociceptive transmission [51].

Furthermore, UTP and ATP, but not the P2Y_{1,12,13} preferential ADP, increased phosphorylation of the transcrip-

tion factor CREB in 40% of the DRG neurons (Figure 1), in a PLC-dependent way [28]. Phosphorylated CREB (pCREB) is known to induce the expression of numerous neuropeptides (CGRP, substance P [SP] and brain-derived neurotrophic factor [BDNF]) that are upregulated in sensory neurons in response to injury or inflammation [52–54]. Ninety-five percent of the pCREB-IR neurons also displayed peripherin staining, indicating that DRG neurons with unmyelinated axons are involved. One-third of these cells also expressed CGRP and 40% IB₄. These data suggest that UTP evokes CREB phosphorylation in a subset of nociceptive DRG neurons. The UTP-induced phosphorylation of CREB was inhibited by suramin, but not by cibatron blue, an antagonist of P2Y₄ but not of P2Y₂ receptors, indicating an action via P2Y₂ receptors. It seems likely that P2Y₂ receptors, increasing the excitability of sensory neurons in the short term, may also activate a transcription factor that is likely to foster the expression of nociceptive neurotransmitters in the long term.

UTP has been shown to stimulate CGRP release from rat DRG neurons in the absence of extracellular Ca²⁺, and suramin abolished this stimulatory action [21]. These data suggest that P2Y₂ receptor activation and the subsequent increase of [Ca²⁺]_i may be accompanied by the release of the neuropeptide CGRP from the first order sensory neurons stimulating pain transmission. In contrast, in a more detailed study, neither ATP, UTP nor 2-MeSATP increased the basal release of SP or CGRP from cultured embryonic rat DRG neurons [55]. However, the concentration of UTP used in this study was lower than that used by Sanada et al. [21] (10 vs 100 μM) which can explain the inconsistency between the results. Similarly, ATP had little direct stimulatory effect on the release of CGRP from the nerve terminals in isolated rat dura mater but augmented proton-induced release of CGRP [56]. In embryonic rat DRG neurons, the ability of P2Y receptor agonists to modulate the excitability to capsaicin was also investigated [55]. ATP and UTP, but not 2-MeSATP or α,β-meATP, augmented the release of SP and CGRP evoked by capsaicin (Figure 1). The sensitizing effect of ATP was antagonized by suramin but not by PPADS and was inhibited by the PKC inhibitor bisindolylmaleimide. Together, these data suggest that ATP, acting at P2Y receptors in sensory neurons, can augment their sensitivity to other stimuli in a PKC-dependent manner.

ATP and UTP also increased axonal transport of membrane-bound organelles in anterograde and retrograde directions in cultured adult mouse DRG neurons (Figure 1); suramin completely blocked this effect of ATP [57]. ADP, α,β-meATP and 2-MeSATP were ineffective, suggesting the involvement of P2Y₂ receptors. Synaptic functions including neurotransmitter release and up-/downregulation of receptors require axonal transport [58, 59]. In primary sensory neurons from the rat DRG, axonal transport delivers receptors, ion channels, sensory neuropeptides, glutamate and aspartate in both central and peripheral directions [59]. It was suggested that blockade of axonal transport can inhibit the development of neuropathic pain by blocking the signaling of peripheral injury to the central

neurons [60]. In this case, P2Y₂ receptor activation through agonists would be pronociceptive through stimulation of the axonal transport.

There are several lines of evidence indicating that spinal cord glial cells can create and maintain exaggerated pain states [61–63]. These cells become activated in response to substances released by both the primary afferent terminals (ATP, SP, CGRP, and glutamate) and by spinal cord neurons (nitric oxide, prostaglandins). It is well known that the ATP-induced activation of glial cells is mediated by both P2X and P2Y receptors [64, 65]. Once activated, glial cells can release a variety of neurotransmitters and neuromodulators including ATP, which in turn can further stimulate neighboring glial cells and neurons, and is capable of altering pain transmission. This effect is due both to an enhanced release of SP and glutamate from the central terminals of DRG neurons [66, 67] and to an increased excitability of spinal cord neurons [63]. One important way by which astrocytes regulate their own activity is via Ca²⁺ signals. A rise in [Ca²⁺]_i limited to one part of an astrocyte may propagate within the rest of the cell, and Ca²⁺ responses can be transmitted from one astrocyte to others leading to Ca²⁺ waves that spread within astrocyte networks [68]. There are two main routes by which Ca²⁺ waves can propagate from one cell to another: Through gap junction channels [69] and through the extracellular space involving the release of pharmacologically active substances from one cell activating surface receptors on neighboring cells. A growing body of evidence indicates that a principal mechanism for the propagation of Ca²⁺ waves between astrocytes is by release of ATP, which acts as a diffusible extracellular messenger [70–72]. Release of ATP from astrocytes during Ca²⁺ wave propagation has been demonstrated [72]. In spinal cord astrocytes, Ca²⁺ wave propagation was found to be mediated by P2Y₁ and P2Y₂ receptors (Figure 1) [33, 73]. Both receptors were necessary and sufficient for the propagation of Ca²⁺ waves but characteristics of the propagation were different: Ca²⁺ waves propagating via P2Y₂ receptors travel faster and further than those propagating via P2Y₁ receptors. It was suggested that mediation of Ca²⁺ waves by ATP leading to activation of two subtypes of receptor, P2Y₁ and P2Y₂, may be a general principle for gliotransmission in the central nervous system [73].

Antinociceptive effects

P2Y receptor agonists were found to inhibit cytokine release from activated spinal cord microglia [43]. The rank order of inhibitory potency on cytokine release was: 2-MeSATP = ATP > ADP-β-S = ADP ≫ UTP > UDP. However, P2Y antagonists or further P2X and P2Y selective substances were not used in this study. It is known that activated microglia and astrocytes in the spinal cord can create and maintain chronic pain states [61, 74]. P2Y receptors inhibiting microglia overstimulation through the reduction of cytokine release from these cells could interrupt chronic pain development and continuation.

Repetitive stimulation of the dorsal root, with an intensity adequate to activate C-fibers but not A-fibers, induced slow depolarization of substantia gelatinosa neurons in the adult rat spinal cord transverse slices with dorsal root attached [75]. This effect was inhibited by the μ -opioid receptor agonist DAMGO, but not by DPDPE and U-50488H, δ - and κ -opioid agonists, respectively, indicating that the repetitive stimulation of C-fibers of the dorsal root is relevant to spinal nociception. ATP and UTP, but not α, β -meATP, were found to inhibit the slow depolarization of the substantia gelatinosa neurons in the dorsal horn [76]. The inhibitory effect of ATP was not reversed by suramin, the only antagonist used in this study.

In a subsequent study from the same laboratory, UTP and UDP were shown to be analgesic when applied intrathecally (i.t.) to rats [77]. In the paw pressure test using normal rats, i.t. administration of UTP and UDP elevated the mechanical nociceptive threshold, whereas ATP and α, β -meATP lowered it. Similarly, in the tail-flick test, UTP and UDP prolonged the thermal nociceptive latency. In the von Frey filament test on rats, while ATP induced a significant and long-lasting tactile allodynia, UTP and UDP were ineffective. In the neuropathic pain model, in which the sciatic nerves of rats were partially ligated, UTP and UDP were found to be antiallodynic. Furthermore, UTP and UDP caused no motor deficit in the inclined plane test, indicating that the elevation of mechanical nociceptive threshold and prolongation of the thermal nociceptive latency were likely due to antinociceptive effects, rather than deficits of motor function. Taken together, i.t. administration of UTP and UDP had antinociceptive effects on acute mechanical and thermal nociception in normal rats via P2Y₂, P2Y₄, and P2Y₆ receptors. The mechanism underlying these effects was not investigated.

In addition to the pyrimidine nucleotides acting on P2Y₂, P2Y₄, and P2Y₆ receptors, the ADP analog ADP- β -S has also been found to cause analgesia [27]. ADP- β -S, applied intrathecally to rats, prolonged the nociceptive threshold in the tail-flick test. Furthermore, in the same study, the possible mechanism of action was also investigated and ATP was found to inhibit the N-type VACCs and the subsequent increase in $[Ca^{2+}]_i$ in rat DRG neurons (Figure 1). No other Ca^{2+} channel types were altered. This effect of ATP was antagonized by the P2Y₁ selective antagonists MRS 2179 and PPADS and was abolished by intracellular application of GDP- β -S, a competitive G protein inhibitor, suggesting the participation of P2Y₁ receptors. Investigation of the secondary messenger pathway revealed that the inhibition was mediated through the $\beta\gamma$ subunit of the G protein, since a strongly depolarizing pre-pulse applied shortly prior to the test pulse and known to dissociate $G_{\beta\gamma}$ from the VACC prevented the inhibitory effect of ATP. Furthermore, ADP- β -S inhibited the dorsal root-evoked polysynaptic population EPSPs in the hemisectioned spinal cord from rats, indicating that the site of action of the P2Y₁ receptors is the central terminal of the DRG cells. It is known that N-type calcium channels play a major role in the release of neurotransmitters and ADP, which is generated by the enzymatic degradation of ATP, may

inhibit these channels through P2Y₁ receptors resulting in decreased sensory transmitter (glutamate) release at the central terminals and as a consequence in reduced spinal pain transmission.

In the same study, UTP was also tested and was found to inhibit the VACCs [27], suggesting that the analgesic effect of UTP [77] may be mediated by the same mechanism as that of ADP- β -S. It is interesting to note that *in vitro* UTP has also been found to excite DRG neurons (see above). Hence, these opposing effects of UTP may finally lead to an inhibition of pain transmission.

Conclusion

The demonstration of P2Y receptor gene expression in sensory cells and the presence of functional receptors on the cell surface suggests that these receptors can play a role in the nociceptive transmission. Co-localization of the different P2Y receptor-subtypes with markers for nociceptive neurons showed that the P2Y₁ and P2Y₂ receptors are typically expressed in small, nociceptive cells, whereas the P2Y₄ subtype seems to be present in large neurons involved in the transmission of tactile allodynia. Spinal cord astrocytes known to play a significant role in the modulation of pain transmission also express functional P2Y₁ and P2Y₂ receptors. The investigation of different P2Y receptor agonists acting selectively on the P2Y₁ or P2Y_{2/4} subtypes showed that these receptors can both stimulate and inhibit the excitability of sensory neurons without significant subtype specificity. On the other hand, *in vivo* studies revealed that i.t. administration of the P2Y_{2/4} agonist UTP and of the P2Y₁ agonist ADP- β -S inhibits pain transmission. It was suggested that P2Y receptors inhibit the release of glutamate from the central terminal of the DRG neurons via inhibition of the VACCs.

Acknowledgement

Financial support by the Deutsche Forschungsgemeinschaft, Bonn (DFG; IL 20/11-1) is gratefully acknowledged.

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