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Painful Purinergic Receptors

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Abbreviations: ATP, adenosine triphosphate; ADO: adenosine; α,β-meATP: α,β-methyleneATP; CFA: complete Freund's adjuvant; IL-1: interleukin-1; DRG: dorsal root ganglion; BDNF: brain-derived neurotrophic factor; TRPV1: transient receptor potential-1.

Abstract

Multiple P2 receptor-mediated mechanisms exist by which ATP can alter nociceptive sensitivity following tissue injury. Evidence from a variety of experimental strategies including genetic disruption studies and the development of selective antagonists has indicated that the activation of P2X receptor subytpes, including $P2X_3$, $P2X_{2/3}$, $P2X_4$ and $P2X_7$, and P2Y (e.g. $P2Y_2$) receptors can modulate pain. For example, administration of a selective $P2X_3$ antagonist, A-317491, has been shown to effectively block both hyperalgesia and allodynia in different animal models of pathological pain. Intrathecally delivered antisense oligonucleotides targeting $P2X_4$ receptors decrease tactile allodynia following nerve injury. Selective antagonists for the $P2X_7$ receptor also reduce sensitization in animal models of inflammatory and neuropathic pain providing evidence that purinergic glial-neural interactions are important modulators of noxious sensory neurotransmission. Additionally, activation of $P2Y_2$ receptors leads to sensitization of polymodal TRPV1 receptors. Thus, ATP acting at multiple purinergic receptors, either directly on neurons (e.g. $P2X_3$, $P2X_{2/3}$ and P2Y receptors) or indirectly through neural-gial cell interactions ($P2X_4$ and $P2X_7$ receptors), alters nociceptive sensitivity. The development of selective antagonists for some of these P2 receptors has greatly aided investigations into the nociceptive role of ATP. This perspective highlights some of the recent advances to identify selective P2 receptor ligands, which has enhanced the investigation of ATP-related modulation of pain sensitivity.

Introduction

Pain is a multidimensional sensory process that, acutely, is physiologically adaptive in response to dangerous (e.g. sharp, hot, or chemical stimuli) stimuli in the environment. Persistent pain can range from increased sensitivity to mildly painful stimuli (hyperalgesia) or to otherwise innocuous stimuli (allodynia) (Honore and Jarvis, 2006a). It is well appreciated that distinct sensory mechanisms contribute to physiological pain, to pain arising from tissue damage (inflammatory or nociceptive pain) and to pain arising from injury to the nervous system (neuropathic pain). Nociceptive pain is caused by the ongoing activation of A-δ and C-nociceptors in response to a noxious stimulus. It can be further classified into visceral pain, superficial somatic pain, and deep somatic pain (Perl, 2007; Honore and Jarvis, 2006). Tissue injury results in the release of pronociceptive mediators that sensitize peripheral nerve terminals that can ultimately lead to increased excitability of spinal cord dorsal horn neurons. As such, injury-induced sensitization of peripheral nerves facilitates a sensitization of the central nervous system. A multitude of receptors, transmitters, second messenger systems, transcription factors, and other signaling molecules are now appreciated to be involved in pain pathways (Perl, 2007; Honore and Jarvis, 2006).

The ability of ATP (Figure 1), to modulate neural function has been well documented (Burnstock and Williams, 2000; Burnstock, 2007). Mechanistic understanding of the role for ATP in processing painful sensory information was initially indicated by early demonstrations that ATP was released from sensory nerves (Holton and Holton, 1954; Holton, 1959) and by subsequent data showing that ATP produces fast excitatory action potentials in dorsal root ganglionic (DRG) neurons (Jahr and Jessel, 1983). While the inhibitory effects of adenosine (ADO) and direct acting ADO receptor agonists on nociceptive neurotransmission and nocifensive behavior have been generally accepted (Sawynok, 2006; McGaraughty and Jarvis, 2006), the specific

mechanisms by which ATP serves to modulate neuronal function remained ambiguous until the discovery of distinct adenosine-sensitive P1 and ATP-sensitive P2 receptor classes, which allowed for initial investigations of the pharmacology of the individual receptor subtypes (Burnstock, 2007). It is now known that ATP receptor super-families comprise both G-protein coupled receptors (P1 and P2Y receptors) and ligand gated ion channels (P2X receptors) (North, 2002; Burnstock, 2007).

P2X receptors function as nonselective cation channels (permeable to Ca^{2+}, Na^{+} and K^{+}) and are expressed on a variety of excitable cells including neurons, glia, and smooth muscle cells (North, 2002; Khakh and North, 2006). Stimulation of P2X receptors can also lead to down-stream activation of voltage-operated calcium channels, and Ca^{2+}-stimulated tyrosine kinases that in turn activate MAP kinases (ERK1 and ERK2) to modulate transcriptional processing (Khakh and North, 2006). Extracellular ATP availability arises from a variety of mechanisms including mechanical stimulation, vesicular release with other neurotransmitters (e.g. acetylcholine, norepinephrine, glutamate, GABA, and neuropeptide Y) or by cellular damage (e.g. hypoxia) (Burnstock and Williams, 2000; North, 2002; Burnstock, 2007). Once released, the extracellular actions of ATP are limited by its rapid degradation by membrane bound and soluble nucleotidases (Burnstock, 2007). The metabolic degradation of ATP leads to increased extracellular levels of ADP, AMP and ADO, all of which have specific receptor-mediated activities. In the context of nociceptive neurotransmission, activation of P1 receptors by ADO decreases nociception, inflammation, and cellular excitability (McGaraughty and Jarvis, 2006) whereas P2X receptor activation by ATP stimulates cellular excitability, augments the release of excitatory amino acids, initiates nociceptive responses, and can lead to apoptosis (Burnstock and Williams, 2002; Burnstock, 2007). Activation of P2Y receptors also facilitates excitatory neurotransmission by modulating glial-neuron synaptic activity, sensitizing polymodal integrators such as the TRPV1 receptor

(Moriyama et al., 2003) and propagating calcium-dependent neuronal activity (Burnstock, 2007).

Historically, a significant limitation in the interpretation of P2 receptor biology has been due to the fact that few, if any, ligands showed meaningful pharmacological selectivity of individual P2 receptor subtypes. Essentially, all of the known P2X receptor agonists have pharmacological activity at multiple P2 receptor subtypes (Jacobson et al., 2002). Further, traditionally used antagonists such as suramin and PPADS (Figure 2) are generally weak blockers of P2 receptors and have a multitude of other pharmacological actions (Jacobson et al., 2002; Burnstock, 2007). As discussed below, the discovery of receptor-selective antagonists has helped provide greater clarity as to the specific functional roles of these receptors.

Recent investigations of how ATP modulates the processing of noxious sensory input indicates that there are multiple P2 receptor-based mechanisms by which ATP can facilitate nociceptive sensitivity following tissue injury. Evidence from a variety of experimental strategies including genetic disruption and the development of receptor selective antagonists has indicated that the activation of $P2X_3$ and $P2X_{2/3}$ receptors lead to direct neuronal activation. In contrast, activation of some other P2 receptors including $P2X_4$ and $P2X_7$ receptors, and some P2Y (e.g. $P2Y_2$) receptors can modulate pain neurotransmission via indirect mechanisms involving glial-neuronal interactions and/or modulation of other nociceptive specific receptors (e.g. TRPV1 receptors). This perspective highlights the recent developments in the identification of P2 receptor-selective antagonists and the role of individual P2 receptors in the sensory processing of noxious stimuli.

$P2X_3$ & $P2X_{2/3}$ Receptors

Intradermally administered ATP elicits pain in humans under normal conditions and enhances inflammatory-mediated pain (Bleehen and Keele, 1977, Hamilton et al.,

2000) by exciting both mechano-responsive and mechano-insensitive C-fibers (Hilliges et al., 2002). Experimentally, the nociceptive effects of intradermally administered P2X receptor agonists (e.g. ATP, α,β-methyleneATP (α,β-meATP), and BzATP; Figure 1) are short lasting (1-10 min) and similar in magnitude to that produced in the acute phase of the standard formalin test, a neurogenic inflammatory pain model in rodents (Jarvis et al., 2001). As has been observed in humans, both the potency and effectiveness of locally administered P2X receptor agonists to elicit nociceptive responses are increased in situations of peripheral inflammation-induced neuronal sensitization (Hamilton et al., 2000; Sawynok, 2007). Stimulation of spinal P2X receptors may also contribute to nociception as indicated by the ability of intrathecally (i.t.) administered P2X receptor agonists to increase sensitivity to acute and persistent noxious stimuli in rodents (Nakagawa et al., 2007).

P2X receptor antagonists (e.g. suramin and PPADS, Figure 2) have been demonstrated to reduce nociceptive sensitivity in a wide variety of animal models including tail-flick, chemically-induced persistent and inflammatory pain, and neuropathic pain (Inoue, 2006; Sawynok, 2007). However, their poor selectivity and weak potency has led to conflicting reports of both pronociceptive and antinociceptive effects following P2X receptor blockade (Jarvis, 2003). Interestingly, P2X agonist-induced receptor desensitization may also lead to reduced pain sensitivity following the initial pronociceptive effect (Inoue, 2006). TNP-ATP (Figure 2) is the most potent $P2X_3$ receptor antagonist (Jacobson et al., 2003). TNP-ATP has low nanomolar affinity for blocking $P2X_3$ receptors, but also has high affinity for $P2X_1$ receptors and is rapidly degraded *in situ* (North, 2002). Thus, *in vivo* studies of TNP-ATP as a pharmacological tool have been limited to direct intrathecal administration (Jarvis, 2003) or direct administration into a site of peripheral tissue damage (Jarvis et al., 2001; Honore et al., 2002).

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Several novel non-nucleotide small molecule $P2X_3$ antagonists have been reported. A-317491 (Figure 3) has nanomolar affinity for blocking both $P2X_3$ and $P2X_{2/3}$ receptors and is a competitive antagonist (Jarvis et al., 2002). RO-3 (Figure 3) is another recently identified antagonist that potently blocks $P2X_3$ receptors (pIC_{50} = 7.0) and exhibits at least 100-fold less activity across a wide range of kinases, receptors, and ion channels (Gever et al., 2006). Unlike TNP-ATP, A-317491 is not susceptible to metabolic degradation and shows high systemic bioavailability following subcutaneous administration, but lacks oral bioavailability. RO-3 has lower protein binding (48%) compared to A-317491 (99%) and good CNS penetration (Gever et al., 2006). Systemic administration of A-317491 effectively reduces nociception in inflammatory and neuropathic pain models (Jarvis et al., 2002). A-317491 also effectively blocked persistent pain in the formalin and acetic-acid induced abdominal constriction tests, but was generally inactive in models of acute noxious (thermal, mechanical, and chemical) stimulation. The less active R-enantiomer of A-317491, A-317334, was inactive in animal pain models (Jarvis et al., 2002). RO-3 has also been reported to reduce nociceptive sensitivity in animal pain models (Gever et al., 2006).

Systemic administration of the $P2X_3$ /$P2X_{2/3}$ antagonist, A-317491, utilizes both spinal and peripheral $P2X_3$ /$P2X_{2/3}$ receptors to affect different forms of pathological nociception. Direct peripheral and spinal application of A-317491 attenuates hyperalgesic responses in complete Freund's adjuvant (CFA)-inflamed animals and reduces formalin-induced nocifensive behaviors (McGaraughty et al., 2003) The spinal delivery of A-317491 was more efficacious than intraplantar administration in both of these models. Antagonism of spinal $P2X_3$ /$P2X_{2/3}$ receptors also leads to a broader spectrum of antinociception since intrathecal, but not intraplantar, injection of A-317491 effectively attenuates tactile allodynia caused by peripheral nerve injury (McGaraughty et al., 2003).

$P2X_4$ Receptors

$P2X_4$ receptors are widely expressed in a variety of cell types including both neurons and microglia in the central nervous system (Garcia-Guzman et al., 1997). Homomeric $P2X_4$ subunits constitute slow desensitizing calcium-permeable cationic channels that can be activated by ATP but are less sensitive to αβ-meATP and BzATP. $P2X_4$ mediated currents are relatively insensitive to blockade by suramin or PPADS (IC_{50} > 500 μM), which inhibit other P2X receptor-related currents in the low μM range (Khakh and North, 2006). Unlike other P2X receptors, $P2X_4$ currents evoked by ATP can be potentiated by ivermectin (Khakh and North, 2006). Co-expression of $P2X_4$ with $P2X_1$ ($P2X_{1/4}$) or $P2X_6$ ($P2X_{4/6}$) leads to the expression of cationic currents that are pharmacologically distinguishable from homomeric $P2X_4$-mediated currents (North, 2002). However, it remains to be elucidated whether heteromeric $P2X_{1/4}$ and $P2X_{4/6}$ receptors form functional channels in native tissues. In a very recent report (Guo et al., 2007), a functional heteromeric combination of $P2X_4$ and $P2X_7$ ($P2X_{4/7}$) receptors has been described in mouse macrophages.

Recent studies indicate that $P2X_4$ receptors may play a role in the development of neuropathic and inflammatory pain. $P2X_4$ mRNA expression has been observed in the dorsal root ganglion (DRG), spinal cord, and several regions of the brain (Kim et al., 2003). Following spinal nerve injury, $P2X_4$ receptor protein expression increased in spinal microglia but not in neurons or astrocytes (Inoue, 2006) whereas $P2X_4$ receptor expression remained unchanged in DRG neurons (Kim et al., 2003). $P2X_4$ gene knock-down studies have provided further insights into the role of $P2X_4$ receptors in neuropathic pain. Intrathecal administration of $P2X_4$ receptor antisense oligodeoxynucleotide decreased $P2X_4$ receptor expression and suppressed tactile allodynia caused by a peripheral nerve injury (Inoue, 2006). Conversely, intrathecal

infusion of ATP-stimulated microglia cells that express $P2X_4$ receptors produced allodynia in naïve rats (Inoue, 2006).

Following peripheral nerve injury, a trans-synaptic shift in anion gradient in spinal lamaina I neurons, due to the down-regulation of the potassium-chloride exporter, KCC2 (Coull et al., 2003), may transform normally inhibitory anionic synaptic currents to be excitatory, substantially driving up the net excitability of lamina I neurons. This enhanced excitability in spinal chord neurons may play an important role in developing nerve injury-induced pain. Although it is unclear whether $P2X_4$ receptor signaling is involved in down-regulation of KCC2, recent studies by Coull et al (2005) revealed that intrathecal injection of $P2X_4$-activated microglia increased intracellular Cl^- concentrations in lamina I neurons, mediated through brain-derived neurotrophic factor (BDNF) and TrkB receptor signaling pathways. This shift in the anion reversal potential in lamina I neurons induces neuronal hyperexcitability by means of reducing $GABA_A$-ergic and glycinergic inhibition (Coull et al., 2005).

The lack of selective $P2X_4$ antagonists has hindered the pharmacological validation of the role for $P2X_4$ receptors in pain. Recently, 5-(3-bromophenyl)-1,3-dihydro-2H-benzofuro[3,2-e]-1,4-diazepin-2-one (Figure 4) was shown to block $P2X_4$-mediated currents expressed in Chinese hamster ovary cells with an IC_{50} value of 0.5 μM (Fischer et al., 2004). It remains to be seen whether novel selective $P2X_4$ antagonists will elicit analgesic effects in neuropathic and inflammatory pain states.

$P2X_7$ Receptors

Homomeric $P2X_7$ receptors are activated by high concentrations of ATP (>100 μM) (North, 2002). Prolonged (> 60 sec) agonist activation leads to the formation of large cytolytic pores in the cell membrane (North, 2002; Burnstock, 2007; Di Virgilio, 2006). $P2X_7$ receptors are selectively expressed on cells of hematopoietic lineage including mast cells, lymphocytes, erythrocytes, and peripheral macrophages

(Surprenant et al., 1996; Burnstock, 2007). Within the CNS, $P2X_7$ receptors are localized on microglia and Schwann cells, as well as on astrocytes (North, 2002; Burnstock, 2007).

ATP acting at $P2X_7$ receptors serves as an efficient secondary stimulus for the maturation and release of IL-1β from pro-inflammatory cells (Ferrari et al., 2006; Mackenzie et al., 2001; Perregaux and Gabel, 1994). The activation of P2X receptors results in a rapid but reversible channel opening that is permeable to $Ca^{2+,}$ Na^+, and K^+ ions (North, 2002). $P2X_7$ receptor mediated increases in intracellular K^+ concentrations leads to the activation of caspase-1 and the rapid maturation and release of the pro-inflammatory cytokine, IL-1β (DiVirgilio, 2006; Perregaux and Gabel, 1994; Solle et al., 2001; Ferrari et al., 2006). $P2X_7$ (-/-) mice show a disruption in cytokine signaling cascades with perturbation of ATP-induced processing of pro-IL-1β in macrophages (Ferrari et al., 2006). $P2X_7$ (-/-) mice also show a decreased incidence and severity of arthritis as compared to wild-type control mice in a collagen monoclonal antibody-induced model of arthritis (Labasi et al., 2002). Collectively, these data have provided support for the hypothesis that $P2X_7$ receptor activation may function as a danger signal in the context of tissue trauma and inflammation (Ferrari et al., 2006).

The finding that disruption of $P2X_7$ receptors not only altered inflammatory pain, but also reduced pain associated with frank nerve injury (Chessell et al., 2005), is consistent with the mechanistic role of $P2X_7$ receptors in modulating IL-1β release and altered pain sensitivity (Wolf et al., 2004). Other genetic manipulations of the IL-1 system including targeted gene disruption of the IL-1 Type I receptor or the IL-1 accessory protein (IL-1acp), as well as transgenic over-expression of the IL-1 receptor antagonist (IL-1ra) (Wolf et al., 2004) or IL-1αβ double knockout (Honore et al., 2006a) have generated mice that show reduced nociceptive responses relative to wild-type animals.

Early pharmacological work by Dell'Antonio et al. (2002) showed that local administration of oxidized-ATP (Figure 2) reduced inflammation-induced mechanical hyperalgesia in rats, an effect that was attributed to pharmacological blockade of $P2X_7$ receptors. However, oxidized-ATP has weak affinity for $P2X_7$ receptors, slow kinetics, and many other pharmacological actions (Burnstock, 2007). More direct support for a role of $P2X_7$ receptors in pain modulation is provided by studies using selective antagonists (Honore et al., 2006b; Nelson et al., 2006; McGaraughty et al., 2007). Systemic administration of $P2X_7$ receptor-selective antagonists (e.g. A-438079, A-740003, Figure 5) produced dose-dependent antinociceptive effects in models of neuropathic (Honore et al., 2006b; McGaraughty et al., 2007; Nelson et al., 2006) and inflammatory pain (Honore et al., 2006b). Consistent with their *in vitro* potencies, A-740003 was more potent than A-438079 at reducing mechanical allodynia 2 weeks following spinal L5/L6 nerve ligation. These data illustrate the potential role of $P2X_7$ receptor modulation in reducing nociception in neuropathic pain models.

The robust antinociceptive effects of $P2X_7$ antagonists in inflammatory pain models does not appear to be secondary to an anti-inflammatory effect since A-740003 was more efficacious in reducing nociception than paw edema (Honore et al., 2006c). It should be noted, however, that the anti-inflammatory activity of $P2X_7$ antagonists may be more pronounced in arthritis models as compared to acute (carrageenan) and sub-acute (CFA) inflammatory models since the contribution of IL-1β to ongoing inflammatory processes is more prominent in chronic arthritis (Labasi et al., 2002).

Using in vivo electrophysiological recording techniques, the antinociceptive action of A-438079 was related to blocking mechanical and thermal inputs to several different classes of spinal neurons (McGaraughty et al., 2007). A-438079 reduced noxious and innocuous evoked activity of low threshold, nociceptive specific and wide dynamic range spinal neurons in neuropathic rats. Spontaneous activity of all classes of spinal neurons

was also significantly reduced by A-438079 in neuropathic but not sham rats. The effects of A-438079 on spontaneous and evoked firing were diminished or absent in sham-operated rats. Thus, the contribution of the $P2X_7$ receptor to spinal nociceptive processing is enhanced following a neuropathic injury and is likely to modulate a diverse spectrum of inputs affecting spinal neuronal excitability.

Studies with $P2X_7$ receptor-selective ligands provide direct evidence that acute *in vivo* blockade of $P2X_7$ receptors significantly reduced nociception in animal models of persistent neuropathic and inflammatory pain (Honore et al., 2006b; McGaraughty et al., 2007). Collectively, these data combined with growing evidence supporting the role of $P2X_7$ receptor modulation in pro-inflammatory IL-1 processing (Ferrari et al., 2006) indicates a specific role for $P2X_7$ receptors in neural-glial cells interactions associated with ongoing pain.

P2Y Receptors

In comparison to several of the P2X receptors, the contributions of the metabotropic P2Y receptors to normal and pathological pain have been less well examined. As with early P2X receptor research, this area of study is currently lacking selective ligands to interrogate the specific contributions of individual P2Y receptor subtypes in pain states. Eight mammalian P2Y receptors have been cloned ($P2Y_{1,2,4,6,11,12,13,14}$) that respond in varying degrees to the endogenous ligands ATP, ADP, UTP, and UDP (Figure 1) (Burnstock, 2007). Tissue localization for P2Y receptors is quite diverse. However, the expression of $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ mRNA in DRG neurons suggests that these receptors may be involved in peripheral somatosensory transmission (Burnstock, 2007; Moriyama et al., 2003). In addition to its primary afferent role, there is also some indication that spinal P2Y receptors may modulate pathological nociception (Okada et al., 2002). Of these four P2Y receptors, $P2Y_1$ and $P2Y_2$ have garnered the most scientific interest in nociception research.

Presently, there is limited evidence to suggest that $P2Y_4$ and $P2Y_6$ receptors have active roles in nociception. Indeed, it has been shown that $P2Y_6$ receptors do not influence the activity of primary afferent C-fibers (Stucky et al., 2004).

$P2Y_1$ receptor mRNA is upregulated in the lumbar DRG following peripheral axotomy indicating that $P2Y_1$ receptors may contribute to the heightened somatosensory sensitivity in this pathological state (Xiao et al., 2002). More specifically, the $P2Y_1$ receptor has been localized predominantly to small diameter neurons in the DRG and is co-expressed with $P2X_3$ and transient receptor potential–1 (TRPV1) receptors (Burnstock, 2007; Gerevich et al., 2004; 2005). Activation of $P2Y_1$ receptors on DRG neurons modulates currents generated through N-type ($Ca_v2.2$) calcium channels and $P2X_3$ receptors (Gerevich et al., 2004, 2005). Indeed, activation of N-type calcium channels in cultured DRG neurons was inhibited by ATP and even more potently by the $P2Y_{1,12,13}$ receptor agonist ADP (Gerevich et al., 2004). The effects of ATP were blocked by the selective $P2Y_1$ receptor antagonist, MRS 2179 (Figure 6), as well as by PPADS. Similarly, $P2Y_{1,12,13}$ receptor agonists inhibited currents evoked by activation of $P2X_3$ receptors in cultured DRG neurons from neonatal rats (Gerevich et al., 2005). Thus, $P2Y_1$ may serve as an "ATP counterbalance" following mutual activation of $P2Y_1$ and $P2X_3$ receptors. However, in human HEK293 cells transfected with $P2X_3$ receptors, inhibition of $P2X_3$ currents was reportedly mediated via a P2Y receptor other than $P2Y_1$ (Gerevich et al., 2007) leaving a putative $P2X_3$-$P2Y_1$ interaction in question. Nonetheless, the outcome of $P2Y_1$-related inhibition on N-type calcium channels or $P2X_3$ receptors is likely to result in a decreased release of nociceptive transmitters into the spinal cord (Burnstock, 2007).

Early work with TRPV1-transfected HEK293 cells suggested that the $P2Y_1$ receptor was responsible for the ATP modulation of TRPV1 responses to heat, capsaicin and protons (Tominaga et al., 2001). More recently, this hypothesis has been revised by

proposing that the $P2Y_2$ receptor is the key purinoceptor involved in the modulation of TRPV1 receptor sensitization (Moriyama et al., 2003). $P2Y_2$ receptors are expressed on small diameter capsaicin-sensitive DRG neurons (Moriyama et al., 2003; Stucky et al., 2004). Intraplantar injection of ATP reduced thermal thresholds in both wild type and $P2Y_1$ deficient mice but not in TRPV1 deficient mice (Moriyama et al., 2003). These results confirmed the link between ATP-induced thermal hyperalgesia and TRPV1 receptors and also demonstrated that $P2Y_1$ receptors are not necessary for this interaction. Moreover, the $P2Y_{2,4}$ receptor agonist, UTP, potentiated capsaicin-evoked currents in isolated mouse DRG neurons and induced thermal hyperalgesia after intraplantar injection (Moriyama et al., 2003). The effect on capsaicin currents was blocked by application of the antagonist suramin, which is somewhat more selective for $P2Y_2$ over $P2Y_4$ receptors. Lakshimi and Joshi (2005) also demonstrated that ATP, acting at $P2Y_2$ receptors, could activate TRPV1 receptors independent of other stimuli or endogenous ligands. Thus, $P2Y_2$ receptors appear to be the route through which ATP affects TRPV1 function.

The contributions of $P2Y_2$ receptors for pain transmission likely extend beyond interactions with TRPV1 receptors in primary afferent neurons. In the isolated skin-nerve preparation, 54 % of cutaneous C-fibers and 12 % of A-mechanoreceptors responded to UTP (about 70-80 % were capsaicin sensitive) (Stucky et al., 2004). However, an additional 22-26 % of large diameter Aβ fibers responded to UTP suggesting that $P2Y_2$ receptors also may be directly involved in the transmission of low threshold mechanical inputs to the spinal cord. It is also possible that activation of a recently described hetero-oligomeric $P2Y_2$/ADO A_1 receptor complex (Suzuki et al., 2006) may also negatively modulate the antinociceptive effects of ADO A_1 receptor agonists (McGaraughty and Jarvis, 2006).

Perspective

Acting via multiple P2 receptor subtypes across the neuroaxis, ATP is an important initiator and modulator of mammalian nociceptive sensitivity. Collectively, the recent discovery of receptor-selective ligands for several of the P2 receptors has provided significant new insights into the mechanisms by which ATP initiates and maintains heightened nociceptive sensitivity in mammals. Based on a series of local delivery (peripheral, spinal, and brain) studies using some of these compounds, as well as an analysis of receptor expression following peripheral nerve injury (McGaraughty and Jarvis, 2006), activation of homomeric $P2X_3$ receptors likely contributes to acute nociception and some aspects of acute inflammatory pain. In contrast, activation of heteromeric $P2X_{2/3}$ receptors appears to modulate longer-lasting nociceptive sensitivity associated with nerve injury or chronic inflammation (Jarvis, 2003; Nakagawa et al., 2007). Additionally, it is now clear that under conditions of persistent nociceptive input, activation of other P2 receptors (e.g. $P2X_4$ and $P2X_7$) receptors may also serve to maintain nociceptive sensitivity through complex neural-glial cell interactions or via sensitization (via $P2Y_2$) of other nociceptive receptors such as TRPV1 channels.

Of particular note is the recent report of the existence of natively expressed heteromeric $P2X_{4/7}$ receptors (Guo et al., 2007; Dubyak, 2007). This finding provides a potential integrative mechanism of the complex nociceptive roles of both $P2X_4$ and $P2X_7$ receptors in chronic pain states. Both receptors share similar sequence homologies, chromosomal localizations, and cellular expression patterns (Dubyak, 2007). As noted above, they also contribute to similar aspects of ongoing inflammatory and neuropathic pain in experimental models. While the preliminary evidence indicates that the heteromeric $P2X_{4/7}$ receptor is sensitive to both $P2X_4$ and $P2X_7$ receptor antagonists, further research is needed to clearly differentiate the pharmacological properties of the heteromeric $P2X_{4/7}$ receptor from its homomeric partners.

To date, no selective P2X receptor antagonists have been evaluated clinically for the relief of pain. While reduced pain sensation was noted in a suramin phase 1 cancer clinical trial (Ho et al., 1992), the clinical utility of receptor-selective P2 antagonists for pain relief has not yet been established. The emerging data on P2 receptor selective antagonists provides intriguing promise that potentially useful drug candidates can be found that specifically target individual P2 receptor subtypes. While the P2 receptor-selective compounds identified to date have proven to be useful pharmacological tools in preclinical studies, further effort is needed to identify compounds with the drug-like required to interrogate the potential clinical utility of P2 receptor antagonists for pain.

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Footnotes

* All authors contributed equally to this perspective.

Figure Legends

Figure 1. Structures of prototypical P2 receptor agonists.

Figure 2. Structures of prototypical and nonselective P2 receptor antagonists.

Figure 3. Structures of $P2X_3$ receptor selective antagonists.

Figure 4. Structures of $P2X_4$ receptor selective antagonists.

Figure 5. Structures of $P2X_7$ receptor selective antagonists.

Figure 6. Structure of the $P2Y_1$ receptor selective antagonist, MRS 2159.

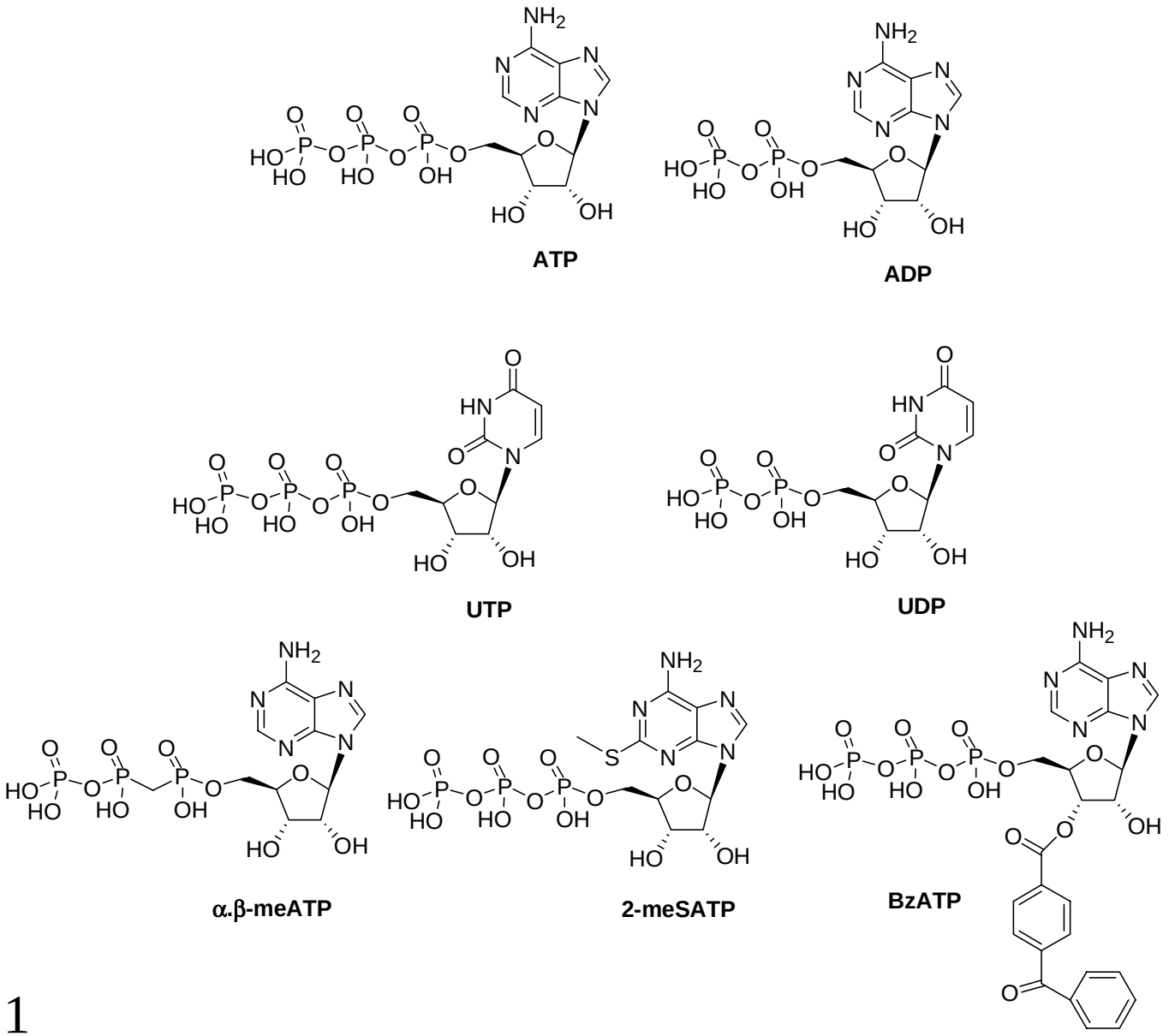
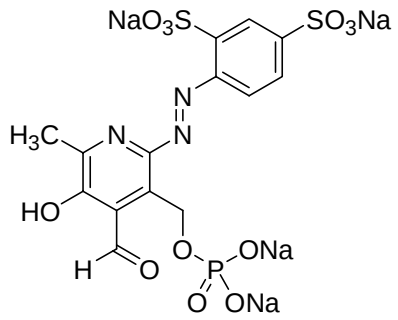
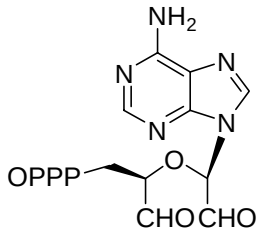


Figure 1



PPADS Tetrasodium salt



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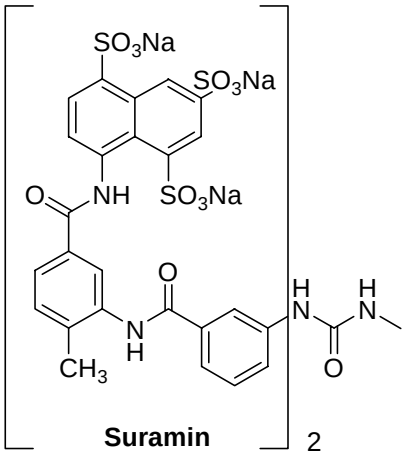

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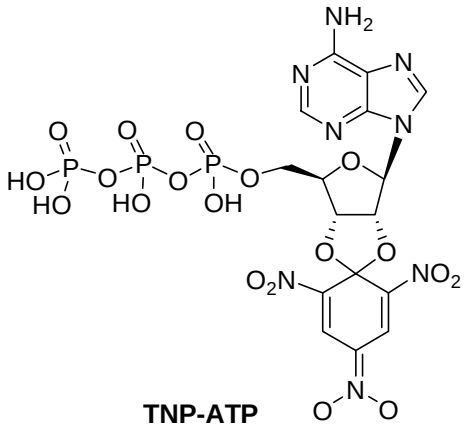

TNP-ATP

Figure 2

A-317491 **RO-3**

Figure 3

Figure 4

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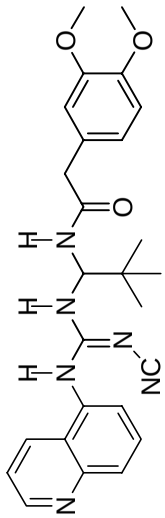

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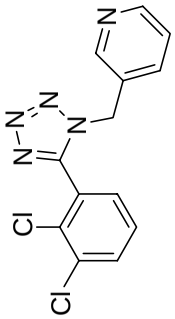

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Figure 5

Figure 6