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P2X Receptors and Nociception

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***Abstract*——The potential importance for nociception of P2X receptors, the ionotropic receptors activated by ATP, is underscored by the variety of pain states in which this endogenous ligand can be released. Several important findings have been made recently indicating that P2X receptors can be involved in pain mechanisms both centrally and in the periphery. The roles of ATP at these two sites and the P2X receptor subtypes involved appear to be different. In the periphery, ATP can be released as a result of tissue injury, visceral distension, or sympathetic activation and can excite nociceptive primary afferents by acting at homomeric $P2X_3$ or heteromeric $P2X_{2/3}$ receptors. Centrally, ATP released from central afferent terminals or second order neurons can modulate neurotransmitter release or postsynaptically activate neurons involved in central nociceptive transmission, with $P2X_2$, $P2X_4$, $P2X_6$, and some other receptors being potentially involved. Evidence from in vivo studies suggests that peripheral ATPergic mechanisms are most important under conditions of acute tissue injury and inflammation whereas the relevance of central mechanisms appears to be more limited. Furthermore, the release of ATP and P2X receptor-mediated afferent activation appear to have been implicated in visceral and neuropathic pain; the importance of the ATPergic component in these states needs to be investigated further. Thus, peripheral P2X receptors, and homomeric $P2X_3$ and/or heteromeric $P2X_{2/3}$ receptors in particular, constitute attractive targets for analgesic drugs. The development of selective antagonists of these receptors, suitable for a systemic in vivo use although apparently difficult, may prove a useful strategy to generate analgesics with a novel mechanism of action.**

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I. Introduction

P2X receptors are cation-permeable ion channels gated by ATP and some related nucleotides (Abbracchio and Burnstock, 1994; Fredholm et al., 1994; Khakh et al., 2001). During the last several years, a great body of evidence has been accumulated indicating that these receptors can be involved in pain mechanisms and thereby constitute possible targets for analgesic drugs. The potential importance of this target is emphasized by the ubiquitous presence of the endogenous ligand, ATP, in living cells, and the variety of states in which this ligand can be released activating its receptors. Unfortunately, development of selective antagonists suitable for in vivo use has been a challenge, and currently available compounds have limited selectivity, potency, or in vivo stability. Nevertheless, extensive work has been done to elucidate the role of P2X receptors in different pain states and to evaluate the potential of P2X receptor antagonists for the treatment of pain. Efforts using pharmacological tools have been recently substantiated by investigations of genetically modified animals with targeted deletion of P2X receptors in pain models. The aim of this review is, therefore, to analyze and to superimpose these various lines of evidence for the role of P2X receptors in different pain states and thus describe the profile of potential analgesics targeting these receptors.

Seminal work of Burnstock (1996) has attracted researchers' interest in the role of ATP and its receptors in peripheral tissues. There, large quantities of ATP may leave the intracellular space as a result of tissue trauma, tumor, inflammation, migraine, or visceral distension. The resulting P2X receptor activation is likely to contribute to the intense pain sensation occurring under these conditions. Furthermore, ATP is an important cotransmitter in peripheral and central noradrenergic neurons (von Kügelgen and Starke, 1985; Vizi and Burnstock, 1988; Poelchen et al., 2001). Its release from sympathetic postganglionic neurons could contribute to the condition of sympathetically maintained pain sometimes developing as a consequence of nerve injury. Interest in peripheral ATP mechanisms has been greatly intensified by findings that a subtype of ionotropic ATP receptors, the $P2X_3$ receptor, is expressed with considerable selectivity by a subset of nociceptive sensory neurons in dorsal root ganglia (Chen et al., 1995; Lewis et al., 1995). In addition to such a universal role of ATP as a 'pain molecule' in the periphery, central release of ATP from terminals of primary afferent fibers has been demonstrated. There, in addition to the modulatory role of presynaptic P2X receptors controlling neurotransmitter release from primary afferents, postsynaptic P2X receptors on second order neurons, might be involved in pain transmission.

Several short reviews have recently appeared summarizing the status of understanding of the role of P2X receptors in pain (McCleskey and Gold, 1999; Bland-Ward and Humphrey, 2000; Ding et al., 2000; Hamilton and McMahon, 2000; Burnstock, 2001; Salter and Sollevi, 2001). However, given the great interest in this analgesic target, the rate at which new reports appear, and some inevitable discrepancies and controversies of the data, particularly from in vivo studies, there is a continuing need for a detailed analysis of the accumulated information. In this review, after a summary on the localization and pharmacology of pain-relevant P2X receptors, we shall consider the evidence from mechanistic studies regarding the relative roles of peripheral and central P2X receptors. Further on, in vivo evidence for the involvement of ATP and P2X receptors in acute, inflammatory, neuropathic, and visceral pain will be analyzed separately for each of these states. Finally, implications for the development of novel analgesics will be discussed.

II. P2X Receptor Subtypes, Localization, and Pharmacology

Extracellular ATP has been shown to act at two P2 receptors belonging either to the P2X (ligand-activated cationic channel) or P2Y (G protein-coupled receptor) types (Abbracchio and Burnstock, 1994; Ralevic and Burnstock, 1998). The classification of P2X receptors as well as their localization and pharmacological properties have been extensively discussed previously (Nörenberg and Illes, 2000; North and Surprenant, 2000; Khakh et al., 2001). Only a brief account will be given here on these issues, with the focus on aspects pertinent to nociception.

A. Subtypes of Ionotropic Receptors for ATP

Of the seven subtypes of cloned mammalian P2X receptors ($P2X_1$–$P2X_7$), the respective mRNAs or subunit proteins all have been found in the central and peripheral nervous system (Collo et al., 1996; Le et al., 1998b; Loesch and Burnstock, 1998; Kanjhan et al., 1999); measurements of changes in the intracellular free Ca^{2+} concentration as well as recordings of membrane potential alterations or the underlying ionic currents evoked by P2X receptor activation confirmed the functional significance of these subunits (Edwards et al., 1992; Harms et al., 1992; Illes and Nörenberg 1993; Illes et al., 1996; Nörenberg and Illes, 2000). Studies on the localization of P2X receptors have confirmed the presence of at least six subtypes ($P2X_1$–$P2X_6$) in nervous structures involved in nociceptive transmission (see *Section II.B.*). Although $P2X_7$ receptor mRNA has been demonstrated in retinal ganglion cells and cochlear spiral ganglia of rats (Brändle et al., 1998, 1999), there is no functional evidence hitherto that these receptors could be involved in sensory transmission or nociception (Nörenberg and Illes, 2000). $P2X_7$ receptors are situated at immune cells such as macrophages, lymphocytes, and microglia where they mediate the release of proinflammatory cytokines

or the stimulation of transcription factors and are also regarded as being important for apoptosis (Burnstock, 2000; Illes et al., 2000; Di Virgilio et al., 2001). In this review, therefore, we shall focus on the first six members of the ionotropic ATP receptor family and their role in nociception.

$P2X_1$ through $P2X_5$ subunits form functional receptors when expressed in heterologous systems, whereas only small currents are seen with recombinant homomeric $P2X_5$ receptors, and the functionality of homomeric $P2X_6$ receptors still remains to be demonstrated (Collo et al., 1996; Le et al., 1998a; King et al., 2000). In addition, most subunits have been shown to form functional recombinant heteromeric receptors, e.g., $P2X_{1/5}$, $P2X_{2/3}$, $P2X_{2/6}$, and $P2X_{4/6}$ (Radford et al., 1997; Le et al., 1998a; Torres et al., 1998, 1999). The only subunit that was unable to form hetero-oligomeric assemblies was $P2X_7$ (Torres et al., 1999). It has recently been suggested that trimeric complexes of identical subunits seem to constitute an essential structural element of the P2X receptors channel ($P2X_1$ and $P2X_3$, Nicke et al., 1998; $P2X_2$, Stoop et al., 1999). However, the stoichiometry of the native receptor is at present unresolved. The comparative properties of recombinant and native P2X receptors are summarized in *Section II.C*.

B. Distribution of P2X Receptors in Pain Relevant Neuronal Structures

1. Dorsal Root Ganglia and Trigeminal Ganglia. At least six ($P2X_1$–$P2X_6$) of the seven cloned mammalian P2X receptors are present in sensory ganglia, forming distinct distribution patterns in populations of sensory neurons (Nörenberg and Illes, 2000; Khakh et al., 2001). Neurons of dorsal root ganglia (DRG[2]), trigeminal, and nodose ganglia express $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, and $P2X_6$ mRNA, whereby the expression of the $P2X_3$ mRNA appears selective for a subpopulation of small-diameter cells (Chen et al., 1995; Lewis et al., 1995; Collo et al., 1996; Barden and Bennett, 2000). A similar distribution pattern is seen at the protein level (Vulchanova et al., 1996, 1997; Xiang et al., 1998). The predominant expression of the $P2X_3$ receptor compared with other P2X receptors in small-diameter neurons (Xiang et al., 1998) and the originally reported selectivity of this localization in the rat (Chen et al., 1995; Collo et al., 1996) have attracted interest in this receptor as a target for novel analgesics, although more recent data indicate that the distribution of this receptor both in rat (Xiang et al., 1998; Hansen et al., 1999; Zhong et al., 2000) and human tissues is less nociceptor-specific (Garcia-Guzman et al., 1997; Yiangou et al., 2000). In addition, the confinement of $P2X_3$ receptor immunoreactivity to brain structures involved in pain transmission (nucleus tractus solitarius, solitary tract, spinal trigeminal nucleus; Vulchanova et al., 1996, 1997) appears to be stringent in the adult rat bain in contrast to a more widespread distribution observed in the embryonic and neonatal rat brain (Kidd et al., 1998).

Immunocytochemistry studies on the localization of the $P2X_3$ receptor indicate its presence on a subpopulation of small-diameter nonpeptidergic neurons specifically binding the isolectin B_4; these neurons project to lamina II (inner) of the dorsal horn (Bradbury et al., 1998; Llewellyn-Smith and Burnstock, 1998; Vulchanova et al., 1998). Dorsal rhizotomy eliminates the $P2X_3$ receptor immunoreactivity in the spinal cord, confirming presynaptic localization on primary afferents (Bradbury et al., 1998; Vulchanova et al., 1998). In the lumbar DRG, $P2X_3$ receptor expressing sensory fibers innervate both skin and viscera, whereas muscle afferents have a very low expression level of these receptors (Bradbury et al., 1998). There is a relatively high level of colocalization of $P2X_3$ receptor immunoreactivity with the vanilloid receptor VR1 (Guo et al., 1999); because the latter confers sensitivity to noxious heat (Caterina et al., 1997; Tominaga et al., 1998; Caterina and Julius, 2001), the $P2X_3$ receptor might be expected to play a role in thermal nociception. Functional data on the existence and subtypes of P2X receptors on primary afferents are reviewed below (see *Section III.*).

2. Spinal Cord and Other Central Nervous System Areas. In the dorsal horn of the spinal cord, in addition to P2X receptors localized on primary afferent terminals (particularly $P2X_3$, $P2X_2$, and $P2X_1$, see above), both mRNA and the receptor protein for some P2X receptors have been found, indicating their presence on second order neurons. Here, as well as in other central nervous system regions, the $P2X_2$, $P2X_4$, and $P2X_6$ receptors appear to have the highest expression levels (Collo et al., 1996; Vulchanova et al., 1996; Le et al., 1998b) although $P2X_3$ receptors are also present (Vulchanova et al., 1997; Llewellyn-Smith and Burnstock, 1998). Functional evidence for the presence of P2X receptors and their role in synaptic transmission in the spinal cord is discussed below (see *Section IV.*).

C. Functional Properties of Different P2X Receptor Subtypes

A detailed account on the functional differences between individual P2X receptor subtypes in the desensitization rate and sensitivity to protons and other ions, as well as to agonists and antagonists, can be found in several recent reviews (Lambrecht, 2000; North and Surprenant, 2000; Khakh et al., 2001) (Table 1). These functional properties are frequently used to identify the subtype composition of native receptors in dissociated neuron or tissue preparations (e.g., see *Section III.*).

Inherent properties of recombinant and native P2X receptors are their different rates of desensitization in the continuous presence of ATP. $P2X_1$ (Valera et al.,

[2] Abbreviations: DRG, dorsal root ganglion; α,β-meATP, α,β-methylene-ATP; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid; TNP-ATP, 2′,3′-*O*-trinitrophenyl-ATP; IP_5I, diinosine pentaphosphate; EPSC, excitatory postsynaptic current.

TABLE 1
Comparative properties of some recombinant and native P2X receptors

Properties of Receptors	Recombinant Homomeric Receptors[a]						Recombinant Heteromeric Receptors[a]			Native Receptors[a]		
	$P2X_1$	$P2X_2$	$P2X_3$	$P2X_4$	$P2X_5$[b]	$P2X_6$	$P2X_{1/5}$	$P2X_{2/3}$	$P2X_{4/6}$	DRG	Nodose	SCG
Desensitization of responses to ATP[c]	Rapid	Slow	Rapid	Slow	Slow	Slow	Biphasic	Slow	Slow	Rapid, slow, biphasic	Slow	Slow
Modulation by H^{+}[d]	↓	↑	↓	↓			↓	↑ ↓		↑	↑	
Modulation by Ca^{2+}[d]	N.E.	↓	↑ ↓		↓		↓			↑ ↓		
Sensitivity to α,β-meATP[e]	+ (1 μM)	–	+ (1 μM)	–	–	–	+ (3 μM)	+ (1–3 μM)	+ (12 μM)	+ (1 μM)	+ (1 μM)	–
Sensitivity to PPADS[e]	+ (1 μM)	+ (1 μM)	+ (1 μM)	–	+ (3 μM)	–	+ (0.6 μM)	+ (3–300 μM)	+ (10 μM)	+ (10 μM)	+ (10 μM)	+
Sensitivity to suramin[e]	+ (1 μM)	+ (10 μM)	+ (3 μM)	–	+ (4 μM)	–	+ (1.6 μM)	+ (30–100 μM)	+ (10 μM)			
Sensitivity to TNP-ATP[e,f]	+ (6 nM)	± (1 μM)	+ (1 nM)	± (15 μM)			+ (0.4 μM)	+ (7 nM)		+	+ (RDC); – (NDC)	
Sensitivity to IP_5I[e]	+ (3 nM)	–	+ (3 μM)	– (↑)				–				

N.E., no effect.
[a] Data are from the following publications; Ralevic and Burnstock, 1998; Burgard et al., 1999; Lambrecht, 2000; Nörenberg and Illes, 2000; North and Surprenant, 2000; Khakh et al., 2001.
[b] Only very small ATP-evoked currents are observed with homomeric recombinant $P2X_5$ receptors.
[c] Desensitization is deemed slow if the time constant of the response decay is ≥10 s or if the amplitude of the remaining current at the end of agonist application (≥2 s) is >50%.
[d] ↑, potentiation; ↓, inhibition; ↑ ↓, biphasic dose-dependency of modulation.
[e] +, sensitive; –, insensitive; (↑), potentiation; values in parentheses are IC_{50}.
[f] RDC, rapidly desensitizing component; NDC, nondesensitizing component.

1994) and $P2X_3$ homomers (Lewis et al., 1995) in contrast to all other P2X receptors rapidly desensitize within tens or hundreds of milliseconds (Buell et al., 1996; Ralevic and Burnstock, 1998). There is disagreement on the mechanism of $P2X_3$ receptor desensitization, since on the one hand intracellular N-terminal (King et al., 1997a) or C-terminal domains (Koshimizu et al., 1999) were defined as possible sites of phosphorylation, and on the other hand the membrane-spanning hydrophobic segments were considered to be of primary significance (Werner et al., 1996). The $P2X_{2/3}$ heteromeric receptor exhibited a sustained response during a longer lasting exposure to ATP (Lewis et al., 1995).

The measurement of reversal potentials by the patch-clamp method indicated that recombinant homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors exhibit a considerably lower permeability for Ca^{2+} ions (Virginio et al., 1998a) than $P2X_1$ (Evans et al., 1996) or $P2X_4$ receptors (Buell et al., 1996). A direct determination of intracellular Ca^{2+} by fura-2 microfluorimetry also proved a lower peak response to $P2X_3$ than $P2X_1$ receptor activation in an expression system (gonadotropin-releasing hormone-secreting neurons; Koshimizu et al., 2000). Finally, extracellular Ca^{2+} has been reported to inhibit currents via $P2X_3$ and $P2X_{2/3}$ receptors with a lower affinity than via $P2X_2$ receptors (Virginio et al., 1998a). In contrast, Cook et al. (1998) described a marked potentiation of $P2X_3$ but not $P2X_{2/3}$ receptor currents in rat DRG neurons at higher external Ca^{2+} concentrations. It was suggested that Ca^{2+} binds to an extracellular site at the $P2X_3$ receptor causing a faster recovery from desensitization, which in turn leads to a larger availability of an agonist-sensitive receptor pool (Cook et al., 1998).

Zn^{2+} and Cd^{2+} failed to alter $P2X_3$ receptor currents although the ionic permeability of the $P2X_4$ receptor channels was slightly inhibited and that of the $P2X_2$ receptor channels was markedly potentiated (Nakazawa and Ohno, 1997). Both Cu^{2+} and Zn^{2+} potently enhanced the ATP-induced current in rat nodose ganglion neurons endowed with $P2X_{2/3}$ heteromers (Li et al., 1996b). Similarly, H^{+} enhanced the affinity of $P2X_2$ receptors for ATP (King et al., 1996, 1997b; Stoop et al., 1997), whereas the affinity of $P2X_1$, $P2X_3$, and $P2X_4$ receptors was decreased by acidification of the medium (Stoop et al., 1997; Wildman et al., 1999b). Most importantly, the $P2X_2$ subunit dominated the reaction of $P2X_{2/3}$ heteromeric receptors to changes of pH in that H^{+} caused facilitation (Stoop et al., 1997; Burgard et al., 1999). It is noteworthy that nodose ganglion neurons and DRG neurons possibly containing the $P2X_{2/3}$ heteromers show a similar effect to pH (Li et al., 1996a; Burgard et al., 1999). Since inflamed tissue has relatively low pH, sustained ATP responses mediated by $P2X_{2/3}$ heteromers (see *Section III.B.*) may manifest themselves preferentially in inflamed tissue (McCleskey and Gold, 1999). Substance P and bradykinin also potentiated currents via recombinant $P2X_3$ and $P2X_{2/3}$ channels in ac-

cordance with their known ability to modulate pain perception (Paukert et al., 2001). Eventually, cibacron blue, which has been reported to be an antagonist of ATP at recombinant $P2X_1$ and $P2X_2$ receptors (Surprenant, 1996), appeared to be an allosteric modulator of recombinant $P2X_3$ receptors (Alexander et al., 1999). Cibacron blue mediated a large increase in both the magnitude and the potency of the ATP-activated Ca^{2+} influx and transmembrane current. In contrast, ivermectin was a positive allosteric effector of the gating and kinetics of $P2X_4$ and probably $P2X_{4/6}$ but not of $P2X_2$, $P2X_3$, or $P2X_{2/3}$ channels (Khakh et al., 1999).

Ethanol has been shown to inhibit ATP-induced currents in DRG neurons of bullfrogs (Li et al., 1993). Further experiments suggested that the inhibitory action was due to an allosteric mechanism (Li et al., 1998a) and raised the possibility that it involves the extracellular domain of the ATP-gated ion channel (Weight et al., 1999). Since the receptors studied by Weight et al. (1999) did not show fast desensitization kinetics like $P2X_3$ receptors, they may belong to the $P2X_{2/3}$ type. Human recombinant $P2X_3$ receptors failed to react to ethanol but were inhibited by the active metabolite of the hypnotic drug chloral hydrate, trichloroethanol (Köles et al., 2000). The reported analgesic effect of chloral hydrate (Field et al., 1993) was suggested to be due to the modulation of pain transmission in DRG neurons (Köles et al., 2000). In contrast to $P2X_3$ receptors, recombinant $P2X_4$ receptors were sensitive to ethanol-induced inhibition (Xiong et al., 2000).

D. Available P2X Agonist and Antagonist Tools

The reader is referred to several recent reviews for more detailed information on the subtype selectivity profile of the available P2X receptor agonists and antagonists (e.g., Lambrecht, 2000; North and Surprenant, 2000; Khakh et al., 2001). The availability of pharmacological tools to study the role of P2X receptors in pain mechanisms is very limited. Most ligands that have been available so far have low affinity and/or selectivity. The agonist α,β-methylene-ATP (α,β-meATP) has been shown to act at homomeric $P2X_1$ (Valera et al., 1994, 1995) and $P2X_3$ receptors (Chen et al., 1995, Garcia-Guzman et al., 1997), as well as at hetero-oligomeric $P2X_{4/6}$ receptors (Le et al., 1998a) but not at the other P2X receptor subtypes (Ralevic and Burnstock, 1998). The finding that β,γ-methylene-L-ATP evoked fast inward currents in $P2X_1$ receptor-containing smooth muscle cells, but failed to do so in $P2X_{2/3}$ receptor-containing nodose ganglion neurons (see *Section III.B.*) supplies a further pharmacological tool for discrimination (Trezise et al., 1995). As far as antagonists are concerned, suramin (8-(3-benzamido-4-methylbenzamido)-naphthalene-1,3,5-trisulfonic acid) and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) are weak and nonselective for the various subtypes of P2X receptors and, in addition, have some other properties complicating the interpretation of the in vitro or in vivo data (e.g., inhibition of ectonucleotidases) (Ralevic and Burnstock, 1998). The heteromeric $P2X_{4/6}$ receptors have been shown to have a higher sensitivity to PPADS and suramin then their respective homomeric assemblies (Le et al., 1998a). Some antagonists with submicromolar affinity have recently been developed, e.g., 2′,3′-*O*-(2,4,6-trinitrophenyl)-ATP (TNP-ATP; Thomas et al., 1998; Virginio et al., 1998b), and diinosine pentaphosphate (Ip_5I; King et al., 1999; Wildman et al., 1999a; Dunn et al., 2000) that are potent and selective antagonists at $P2X_1$ and $P2X_3$ receptors; the former but not the latter compound also blocks the heteromeric $P2X_{2/3}$ receptor. Unfortunately, the use of TNP-ATP in whole tissue preparations or under in vivo conditions may be limited by its instability probably due to breakdown by ectonucleotidases (Lewis et al., 1998). The agonistic compounds ATP and α,β-meATP have been used in some in vivo experiments to inactivate P2X receptors by desensitization (see below).

III. Peripheral P2X Receptors and Nociception

A. Sources of Extracellular ATP in Peripheral Tissues

The importance of ATP as a pain-relevant molecule in peripheral tissues has been highlighted by Burnstock (1996), who hypothesized that ATP released from different cell types is implicated in the initiation of pain by acting on purinoceptors on sensory nerve terminals. According to this hypothesis, tissue trauma, tumor, inflammation, vascular or visceral distension, or sympathetic activation by nerve injury may all lead to accumulation of ATP in the extracellular space and activation of P2X receptors on sensory afferents (Bland-Ward and Humphrey, 2000; Burnstock, 2000, 2001; Ding et al., 2000; Hamilton and McMahon, 2000; Salter and Sollevi, 2001). It is noteworthy that ATP may be rapidly degraded by surface-located ectonucleotidases to adenosine (Zimmermann and Braun, 1999; Zimmermann, 2000), which acts at neuronal P1 receptors of the A_1- or A_2-type (Fredholm et al., 1994, 2000), thereby modulating pain transmission in the periphery as well as at central sites (Salter and Sollevi, 2001). The neuronal effects of adenosine are usually the opposite of the effects of ATP. For example, ATP is known to depolarize DRG neurons via $P2X_3$ and $P2X_{2/3}$ receptor activation (see *Section III.B.*), whereas adenosine inhibits high voltage-gated Ca^{2+} channels of DRG neurons by stimulating its own receptors of the A_1 type (Dolphin et al., 1986; Macdonald et al., 1986). In this review, data on the role of peripherally released ATP in different pain states will be discussed separately in sections dealing with the respective pain types (see *Section V.* and Table 2).

TABLE 2
The role of peripheral P2X receptors in different painful conditions

Type of Pain	P2X Receptor Agonist, Allosteric Enhancer, or Antagonist (Route of Application)		Model, Species	Response (Modulation or Induction)	Blockade by Antagonist (Route of Application)	Reference
Acute pain	Agonists	ATP, α,β-meATP (i.a. or i.art.)	Electrophysiological recordings, anesthetized rat	Firing of articular afferents (↑)	PPADS (i.a.)	Dowd et al., 1998
		α,β-meATP, β,γ-meATP (i.pl.)	Electrophysiological recordings, anesthetized rat	Firing of spinal dorsal horn neurons (↑)		Stanfa et al., 2000
		ATP, α,β-meATP (i.pl.)	Behavioral observation, rat	Paw lifting and licking (↑)	Local anesthetic bupivacaine (i.pl.); desensitization by pretreatment with α,β-meATP or capsaicin (i.pl.)	Bland-Ward and Humphrey, 1997
		ATP, α,β-meATP (i.pl.)	Mechanical threshold testing, rat	Mechanical allodynia (↑)	PPADS (i.pl.)	Tsuda et al., 2000
		BzATP (i.pl.)	Behavioral observation, rat	Paw flinching (↑)	TNP-ATP (i.pl.)	Jarvis et al., 2001
		Cibacron blue (i.pl.)	Behavioral observation, rat	BzATP-induced paw flinching (↑)		Jarvis et al., 2001
		Cibacron blue (i.pl.)	Formalin test, rat	Early phase of nociceptive behavior (↑)		Jarvis et al., 2001
		ATP, α,β-meATP, 2-methylthio-ATP (i.pl.)	Behavioral observation and thermal threshold testing, rat	Paw lifting and thermal hyperalgesia (↑)		Hamilton et al., 1999
		ATP (iontophoresis onto skin)	Pain sensation rating, man	Modest burning pain		Hamilton et al., 2000
	Antagonists or desensitizing agonists	α,β-meATP pretreatment (i.pl.)	Formalin test, rat	Early phase of nociceptive behavior (N.E.)		Bland-Ward and Humphrey, 1997
		Suramin (i.pl.)	Formalin test, rat	Early phase of formalin test (N.E.)		Sawynok and Reid, 1997
		TNP-ATP (i.pl.)	Formalin test, rat	Early phase of nociceptive behavior (↓)		Jarvis et al., 2001
	Agonists	ATP, α,β-meATP (i.a.)	Electrophysiological recordings in anesthetized rat with CFA-induced arthritis	Firing of articular afferents (↑) similar to non-inflamed rats	PPADS (i.a.)	Dowd et al., 1998
		Cibacron blue (i.pl.)	Formalin test, rat	Late phase of nociceptive behavior (↑)		Jarvis et al., 2001
	Agonists	ATP, α,β-meATP (i.pl.)	Formalin test, rat	Late phase of nociceptive behavior (↑)	PPADS	Sawynok and Reid, 1997
		ATP, α,β-meATP, 2-meSATP (i.pl.)	Behavioral observation and thermal threshold testing, rat	(↑) Agonist-induced paw lifting and thermal hyperalgesia in rats with carrageenan- or UV-light-induced skin inflammation vs. non-inflamed side		Hamilton et al., 1999
		ATP (onto blister bases of skin)	Pain sensation rating, man	Painful sensation (↑)		Bleehen and Keele, 1977
Inflammatory pain	Agonists	ATP (iontophoresis onto skin)	Pain sensation rating, man	(↑) ATP-induced pain in subjects with UV-light-induced skin inflammation vs. non-inflamed skin		Hamilton et al., 2000
	Antagonists or desensitizing agonists	α,β-meATP pretreatment (i.pl.)	Formalin test, rat	Late phase of nociceptive behavior (N.E.)		Bland-Ward and Humphrey, 1997
		Suramin (i.pl.)	Formalin test, rat	Late phase of nociceptive behavior (N.E.)		Sawynok and Reid, 1997

TABLE 2
Continued

Type of Pain	P2X Receptor Agonist, Allosteric Enhancer, or Antagonist (Route of Application)		Model, Species	Response (Modulation or Induction)	Blockade by Antagonist (Route of Application)	Reference
		TNP-ATP (i.pl.)	Formalin test, rat	Late phase of nociceptive behavior (↓)		Jarvis et al., 2001
Neuropathic pain	Agonists	ATP (i.v.)	Electrophysiological recordings in anesthetized rats after CCI	Ectopic discharges in A fibers at the site of nerve injury (↑)	Reactive blue, but not aminophylline (i.v.)	Chen et al., 1999
	Antagonists	Suramin + phentolamine (i.p.)	Mechanical sensitivity testing in rats after SNL	Mechanical allodynia (↓)		Park et al., 2000
Visceral pain	Agonists	ATP α,β-meATP (i.a.)	Electrophysiological recordings in anesthetized rat	Mesenteric afferent nerve discharge (↑)	PPADS, suramin (i.v.)	Kirkup et al., 1999
		ATP α,β-meATP (i.a.)	Electrophysiological recordings in i.a. perfused rat tongue prep.	Capsaicin-sensitive tongue afferents (↑)	PPADS, suramin (i.a.)	Rong et al., 2000
	Antagonists or desensitizing agonists	α,β-meATP pretreatment (into bladder)	Electrophysiological recordings in isolated rat bladder-pelvic nerve prep.	Afferent nerve discharge in response to bladder distension (↓)		Namasivayam et al., 1999
		Suramin (into bladder)	Electrophysiological recordings in isolated rat bladder-pelvic nerve prep.	Afferent nerve discharge in response to bladder distension (↓)		Namasivayam et al., 1999

i.art., intra-articular; i.pl., intraplantar; prep., preparation; CFA, complete Freund's adjuvant; CCI, chronic constriction sciatic nerve injury; SNL, lumbar 5/6 spinal nerve ligation; ↑, potentiation or induction; ↓, inhibition; N.E., no effect.

B. Characteristics of P2X Receptors on Primary Afferents

The functionality of P2X receptors localized on peripheral nociceptive neurons is best demonstrated by electrophysiological studies on sensory ganglion cells. In such experiments, the kinetics and pharmacology of responses mediated by native receptors can be compared with those obtained in recombinant receptor systems, allowing the analysis of receptor subtypes involved. Numerous reports have documented that application of ATP or its analogs to the cell bodies of acutely dissociated or cultured sensory neurons results in depolarization or inward current, the effect being blocked by the P2X receptor antagonists suramin and PPADS (Jahr and Jessell, 1983; Krishtal et al., 1983, 1988a,b; Bean, 1990; Robertson et al., 1996; Rae et al., 1998; Ueno et al., 1999). The current is mediated by a cation channel with a relatively high calcium permeability (Krishtal et al., 1983; Bean et al., 1990; Virginio et al., 1998a), which leads to an increase in intracellular calcium concentration (Bouvier et al., 1991). These reports mention that a variable but generally high proportion of sensory neurons responds to ATP and its analogs. It must be noted, however, that the majority of such studies has been performed with acutely dissociated or cultured DRG cells. These results should be interpreted with some caution because one study has demonstrated that the relative number of cells responding to ATP and α,β-meATP in intact DRG preparations is substantially lower compared with acutely dissociated DRG neurons (Stebbing et al., 1998).

ATP-sensitive neurons of sensory ganglia are not homogeneous with respect to the presence of functional P2X receptor subtypes. Using retrogradely labeled tooth pulp nociceptors and muscle stretch receptors in culture, Cook et al. (1997) have characterized several types of sensory neurons on the basis of the kinetics of their responses and sensitivity to P2X receptor agonists and antagonists. In nociceptors, one group showed rapidly desensitizing and slowly recovering responses to ATP, which could be antagonized by suramin, and sensitivity to the $P2X_1/P2X_3$ receptor agonist α,β-meATP. The second group of nociceptors was also sensitive to both α,β-meATP and suramin, but the kinetics of their activation and desensitization was slow. Consistent with the predominant localization in small sensory neurons, the two populations of nociceptors were concluded to express functional homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors, respectively, whereas receptors other than $P2X_3$ (likely to be $P2X_5$) were presumed to mediate ATP responses of the homogeneous group of proprioceptive afferents.

A similar pattern has been observed in DRG. Consistent with the localization pattern of $P2X_3$ receptors (Burgard et al., 1999; Ueno et al., 1999), small-diameter, capsaicin-sensitive, isolectin B_4-positive neurons most frequently display rapidly desensitizing agonist-evoked currents and sensitivity to α,β-meATP and to the P2X receptor antagonists suramin, PPADS, and TNP-ATP (Burgard et al., 1999; Ueno et al., 1999; Li et al., 1999; Petruska et al., 2000a,b), the characteristics most closely matching those of recombinant homomeric $P2X_3$

receptors (Burgard et al., 1999; Liu et al., 2001; see also *Section II.C.*). Medium-sized capsaicin-insensitive neurons have response characteristics of $P2X_{2/3}$ heteromers and express both $P2X_2$ and $P2X_3$ receptor mRNA (Li et al., 1999; Ueno et al., 1999; Petruska et al., 2000a,b). Some authors have also observed mixed kinetics of responses (Burgard et al., 1999; Grubb and Evans, 1999; Ueno et al., 1999; Petruska et al., 2000a), which would be in agreement with the reported variety of functional profiles of cells with different relative levels of the $P2X_2$ and $P2X_3$ receptor expression in $P2X_{2/3}$ heteromultimers (Liu et al., 2001). Confirming the results of the pharmacological analysis, acutely isolated DRG neurons of $P2X_3$ receptor null mice did not show any rapidly desensitizing responses to ATP or α,β-meATP, indicating that these responses are mediated by the $P2X_3$ receptor (Cockayne et al., 2000, Souslova et al., 2000).

Vagal afferents appear to have a different profile of functional P2X receptors. Immunohistochemical studies have shown that peripheral terminals of these afferents, as well as neurons in nodose ganglia where their cell bodies are localized, express both $P2X_2$ and $P2X_3$ receptors (Vulchanova et al., 1997; Virginio et al., 1998b; Brouns et al., 2000). Electrophysiologically, however, the slow kinetics of response desensitization and relatively high sensitivity to inhibition by extracellular calcium indicate that homomeric $P2X_3$ receptors are absent in these cells (Khakh et al., 1995; Virginio et al., 1998a). Nodose ganglion neurons are either sensitive or insensitive to the $P2X_1$ and $P2X_3$ receptor antagonist TNP-ATP and most likely to express heteromeric $P2X_{2/3}$ or homomeric $P2X_2$ receptors, respectively (Thomas et al., 1998). Consistent with that, nodose ganglion neurons isolated from the $P2X_3$ receptor knock-out mouse did not respond to α,β-meATP, and the response to ATP was much reduced (Cockayne et al., 2000; Souslova et al., 2000).

Although the presence of functional P2X receptors on isolated nociceptors appears thus well established (see, however, Stebbing et al., 1998), one important question remains whether activation of these receptors can excite intact nociceptive afferents. To address this issue, direct recordings of primary afferent activity have been performed, yielding somewhat controversial results. In vivo, the P2X receptor agonist α,β-meATP was unable to excite corneal nociceptors (Dowd et al., 1997) or tooth pulp afferents in the cat (Matthews et al., 1997). In contrast, intra-arterial or intra-articular injections of either ATP or α,β-meATP into the knee joint in the anesthetized rat evoked a rapid and short-lasting excitation of C- and A_δ-fibers in afferent nerves innervating this joint (Dowd et al., 1998). These agonists were also able to evoke discharges in vitro in nociceptive afferents in the skin-nerve preparation (Hamilton and McMahon, 2000) and in capsaicin-sensitive fibers in a preparation of intra-arterially perfused rat tongue (Rong et al., 2000). Recordings from mesenteric nerves in the anesthetized rat have also shown that ATP and α,β-meATP can directly excite visceral afferents, confirming the presence of functional P2X receptors (Kirkup et al., 1999). The reasons for these discrepancies remain unclear; of those discussed, species differences and limited access of test compounds to relevant sites seem most plausible (Dowd et al., 1998).

Behavioral studies also support the existence of functional P2X receptors on peripheral nociceptors. Intraplantar applications of ATP, as well as of the $P2X_1$/$P2X_3$ agonist α,β-meATP, have been shown to evoke nocifensive behavior or to cause thermal or mechanical hypersensitivity in rats (see *Section V.A.* and Table 2). These behavioral changes could be abolished by a local anesthetic or by nociceptor desensitization using topical application of capsaicin (Bland-Ward and Humphrey, 1997), indicating their primary afferent origin. Neonatal treatment with capsaicin, which is known to selectively destroy fine heat-sensitive nociceptor afferents, selectively abolished α,β-meATP-induced thermal hyperalgesia without affecting the mechanical allodynia (Tsuda et al., 2000). This treatment also decreased the proportion of DRG neurons showing rapid desensitization of responses to α,β-meATP, whereas the percentage of cells with slowly desensitizing responses was not changed. Together with electrophysiological evidence discussed above, these data confirm that functional $P2X_{2/3}$ receptors are localized on medium-caliber, mechanosensitive afferents, whereas homomeric $P2X_3$ receptors are predominant on small-diameter heat-sensitive nociceptors, contributing to ATP-evoked mechanical allodynia and thermal hyperalgesia, respectively (Tsuda et al., 2000). Recent data from $P2X_3$ receptor null-mutant mice suggest that most of the nociceptive response to peripheral ATP is mediated by homo- or heteromeric $P2X_3$ receptors, although some contribution of other PPADS-sensitive P2X receptors was also observed (Cockayne et al., 2000; Souslova et al., 2000).

Some behavioral evidence that P2X receptor function in the periphery can be increased under conditions of inflammation also indirectly points at the functional presence of proton-sensitive P2X receptors, such as $P2X_{2/3}$ heteromeric assemblies (see *Section V.B.* and Table 2).

IV. Central P2X Receptors and Nociceptive Transmission

A. ATP As Nociceptive Neuromodulator or Neurotransmitter

In addition to sensing ATP in the periphery, P2X receptors can also be involved in the processing of nociceptive stimuli in the spinal cord. The role of ATP as nociceptive neurotransmitter has been suggested by reports showing its release from the terminals of primary afferents in the spinal cord. Early reports demonstrated the release of ATP from peripheral endings of primary

sensory neurons and hypothesized that the same may also occur centrally (Holton and Holton, 1954: Holton, 1959). This was later confirmed by findings that exposure to depolarising concentrations of potassium (White et al., 1985) or capsaicin (Sweeney et al., 1989) led to release ATP from dorsal spinal cord synaptosomes in a calcium-dependent manner. Prior dorsal rhizotomy greatly reduced, but did not fully abolish the release of ATP in this preparation, indicating that, although it was largely originating from central terminals of primary afferent neurons, a proportion of second order spinal neurons could also release ATP (Sawynok et al., 1993).

ATP released in the spinal cord upon peripheral noxious stimulation may act at presynaptic and/or postsynaptic P2X receptors. These different populations of spinal ATP receptors are functionally distinct and appear to have different subtype composition.

B. P2X Receptor Involvement in Spinal Nociceptive Transmission

Consistent with the predominant expression of $P2X_1$, $P2X_2$ and $P2X_3$ receptors on small DRG neurons (see *Section II.B.*), these receptors are localized on the central terminals of thin nociceptive fibers in the superficial dorsal horn (lamina II) of the spinal cord (Vulchanova et al., 1996, 1997, 1998). Their presynaptic localization suggests a role in controlling neurotransmitter release; indeed, there is some evidence that they may facilitate glutamate release onto second order neurons. Whole cell recordings from substantia gelatinosa neurons in spinal cord slices showed that bath applied ATP can evoke a fast inward current and potentiate both glutamate- and synaptically-induced currents (Li and Perl, 1995). In a DRG-dorsal horn coculture system, focal applications of ATP to DRG neurons were found to induce glutamate release onto dorsal horn neurons (Gu and MacDermott, 1997; Labrakakis et al., 2000). The time-courses of ATP-gated currents recorded at the cell bodies were mirrored by the time-courses of transmitter release from the DRG nerve terminals, indicating similar P2X receptor properties on the soma and their associated terminals (Labrakakis et al., 2000). Another study in spinal cord slices demonstrated that the P2X receptor antagonist PPADS can inhibit glutamatergic excitatory postsynaptic currents (EPSCs) in superficial dorsal horn neurons evoked by primary afferent stimulation (Li et al., 1998b). The alteration of responses to paired-pulse stimulation reported in this work suggested the involvement of a presynaptic PPADS-sensitive facilitatory mechanism. Consistent with these in vitro data, in the mouse spinal cord in vivo, the hyperalgesia evoked by intrathecally applied α,β-meATP could be antagonized by the exocytosis inhibitor botulinum neurotoxin B and NMDA receptor antagonists (Tsuda et al., 1999b).

All these findings are consistent with the role of synaptically released ATP as a positive modulator of glutamatergic nociceptive transmission in the spinal cord via presynaptic P2X receptors. However, the involvement of this mechanism in segmental nociceptive transmission appears to be variable and probably limited to some populations of lamina II neurons. Some authors saw little effect of PPADS on evoked or miniature glutamatergic EPSCs in dorsal horn lamina II neurons in spinal cord slices (Gu et al., 1998). In our hands, the selective $P2X_1/P2X_{2/3}/P2X_3$ receptor antagonist TNP-ATP (up to 10 μM) did not alter the population motoneuron EPSP evoked by high intensity dorsal root stimulation in the hemisected spinal cord preparation of the immature rat in vitro (Chizh, unpublished observations). Thus, the overall contribution of these receptors in acute segmental nociceptive transmission in the spinal cord appears to be limited.

In addition to presynaptic receptors, the existence of postsynaptic P2X receptors on second order spinal cord neurons has been shown (see *Section II.B.*). Neurons in the spinal trigeminal nucleus and dorsal horn of the spinal cord showed excitation in response to iontophoretic ATP administration in vivo (Salt and Hill, 1983; Fyffe and Perl, 1984; Salter and Henry, 1985). An inward current was observed in cultured (Jahr and Jessell, 1983; Hugel and Schlichter, 2000) or acutely dissociated (Bardoni et al., 1997; Rhee et al., 2000) dorsal horn neurons exposed to ATP. At the mRNA level, the most abundant P2X receptors in the spinal dorsal horn are of $P2X_2$, $P2X_4$ and $P2X_6$ subtypes (Collo et al., 1996), suggesting their role in fast purinergic transmission in the spinal cord. Consistent with that, acutely dissociated dorsal horn neurons showed nondesensitizing responses to ATP that were variably inhibited by the P2X receptor antagonists suramin and PPADS, and were largely insensitive to the $P2X_1/P2X_3$ receptor agonist α,β-meATP (Bardoni et al., 1997). These properties of the native receptors would be generally compatible with those of homomeric $P2X_2$ and heteromeric $P2X_{4/6}$ receptors, as well as homomeric assemblies of $P2X_4$ and $P2X_6$ receptor (see *Section II.C.*); the variability of the antagonist sensitivity suggests that the population of dorsal horn neurons was not homogeneous (the homomeric $P2X_2$ and heteromeric $P2X_{4/6}$ receptors are sensitive, and the homomeric $P2X_4$ and $P2X_6$ are largely insensitive to suramin and PPADS, see *Section II.C.*).

Although all these findings indicate a potential role of postsynaptic P2X receptors in spinal nociceptive transmission, studies directly addressing this issue have shown that only a small proportion of dorsal horn neurons have purinergic synaptic input. Bardoni et al. (1997) have found that only $<5\%$ of the tested lamina II neurons showed ATP-mediated EPSCs in response to dorsal root stimulation. Another group could not detect any residual current in superficial dorsal horn neurons in spinal cord slices after blocking the glutamatergic component of monosynaptic EPSCs evoked by afferent fiber stimulation (Li et al., 1998b).

Another potential source of synaptic ATP in the spinal cord is spinal interneurons. The presence of ATP-ergic neurons in the dorsal horn is suggested by the fact that prior dorsal rhizotomy did not completely abolish the release of ATP from dorsal spinal cord synaptosomes (Sawynok et al., 1993). Whole cell patch-clamp recordings from cultured neurons of superficial laminae of the dorsal horn have demonstrated that approximately half the cells utilize ATP as a fast excitatory transmitter acting at suramin- and PPADS-sensitive P2X receptors (Jo and Schlichter, 1999). All of these cells coreleased the inhibitory neurotransmitter GABA with ATP, and vice versa, the majority of neurons releasing GABA also released ATP, thus suggesting that GABAergic interneurons represent a major source of synaptic ATP in the spinal cord.

Both presynaptic action of ATP at P2X receptors enhancing glutamate release and its excitatory effects via postsynaptic P2X receptors on second order neurons would be generally consistent with its role in conveying nociceptive information in the spinal cord. However, ATP has also been shown to facilitate glycine- (Rhee et al., 2000) and GABAergic (Hugel and Schlichter, 2000) spontaneous inhibitory postsynaptic currents at dorsal horn neuron synapses; in both reports the effects were sensitive to the P2X receptor antagonists suramin and PPADS. Thus, P2X receptor-mediated activation of inhibitory interneurons by synaptically released ATP could inhibit nociceptive transmission. It is conceivable that a fine balance of excitatory and inhibitory components of ATPergic transmission may exist under normal conditions; it is also likely that this balance could shift under conditions of chronic pain, when major morphological changes in spinal cord organization, such as loss of inhibitory interneurons, take place. Thus, the role of P2X receptors in pain, and, respectively, the efficacy of the receptor antagonists may be expected to rise upon transition from acute to chronic states. The evidence for this comes largely from in vivo studies and will be discussed below (see also Table 3).

V. Role of ATP and P2X Receptors in Different Pain States

Generally, the progress in pharmacological characterization of the role of P2X receptors in pain in vivo has been greatly hampered by lack of selective, potent and stable tools that could be used systemically. For that reason, most of the studies in pain models have utilized topical (largely intraplantar and intrathecal) administration of agonists and antagonists. With this approach, high concentrations of compounds are used that cannot be directly compared with those active in vitro, which potentially confounds the results. This is at least one factor that may have contributed to the numerous discrepancies between the existing in vivo findings. On the other hand, topical peripheral and spinal administration of tool substances allows to unravel the roles in nociception of peripheral and spinal P2X receptors, respectively (see Tables 2 and 3). In some pain models this approach has proved successful in demonstrating the role of P2X receptors and endogenous ATP. The degree of this involvement, however, appears to vary depending on the painful condition investigated.

A. Acute Pain

Peripheral administration of P2X receptor agonists rapidly causes nocifensive behavior in experimental animals and pain sensation in humans. Thus, subcutaneous injection of the P2X receptor agonist α,β-meATP evoked hindpaw lifting and licking immediately after the administration (Bland-Ward and Humphrey, 1997). Benzoylbenzoyl-ATP, another P2X receptor agonist, has been shown to cause similar behavioral responses after injection into the rat paw; the effect was potentiated by a selective allosteric enhancer of $P2X_3$ and $P2X_{2/3}$ receptors, cibacron blue, and inhibited by the $P2X_1/P2X_3/P2X_{2/3}$ receptor antagonist TNP-ATP (Jarvis et al., 2001). In human volunteers, iontophoretic application of ATP onto skin evoked dose-related pain sensation (Hamilton et al., 2000). In addition to the ability to directly evoke pain or nociceptive behavior, both ATP and α,β-meATP have been demonstrated to cause a hypersensitivity to thermal or mechanical stimuli (Tsuda et al., 2000). Coadministration of ATP and α,β-meATP with formalin has also been shown to enhance the flinching response, especially in the second phase; the effect was antagonized by the P2X receptor antagonists suramin and PPADS (Sawynok and Reid, 1997).

Despite the apparent functionality of P2X receptors on nociceptors and the ability of the agonists to evoke nocifensive behavior or pain sensation, their role in physiological nociception seems to be limited. In studies using standard models of acute pain, such as tail-flick or hot-plate, little change in the latency of nociceptive responses was observed after intrathecal administration of the P2X receptor antagonist PPADS (Driessen et al., 1994; Li et al., 1998b). No inhibition of C-fiber-evoked responses of spinal dorsal horn neurons was observed in anesthetized rats after spinal administration of PPADS or suramin, the nonselective P2 receptor antagonist (Stanfa et al., 2000). These in vivo findings are in agreement with the limited involvement of P2X receptors in nociceptive synaptic transmission in the spinal cord in vitro (see *Section IV.*). Theoretically, the role of P2X receptors in acute pain should increase if extracellular levels of ATP are elevated, e.g., as a result of tissue damage. Lysates of human erythrocytes contain ATP and produce pain when applied to a human blister base (Bleehen et al., 1976). A direct activation of nociceptors via P2X receptors by endogenous ATP released by skin cell injury has recently been demonstrated in vitro (Cook and McCleskey, 2000). However, the importance of these mechanisms in vivo still needs to be demonstrated in experiments with selective antagonists.

TABLE 3
The role of spinal P2X receptors in different pain states

Type of pain	P2X Receptor Agonist or Antagonist (i.t. Application)		Model, Species	Response (Modulation or Induction)	Blockade by Antagonist (i.t. Application)	Reference
Acute pain	Agonists	α,β-meATP, 2-methylthio ATP	Tail-flick model, rat	Withdrawal latency ($\downarrow$)	Suramin, Evans blue	Driessen et al., 1994
		α,β-meATP	Thermal nociceptive threshold testing, mouse	Withdrawal threshold ($\downarrow$) and thermal hyperalgesia	PPADS, TNP-ATP or desensitization by pretreatment with α,β-meATP	Tsuda et al., 1999b
		β,γ-meATP, but not α,β-meATP	Electrophysiological recordings in anesthetized rat	C-fiber-evoked responses of spinal dorsal horn neurons ($\uparrow$)		Stanfa et al., 2000
	Antagonists or desensitizing agonists	Suramin, Evans blue, reactive blue, trypan blue, but not PPADS	Tail-flick model, rat	Withdrawal latency ($\uparrow$)		Driessen et al., 1994
		Reactive red 2	Electrophysiological recordings in anesthetized rat	C-fiber-evoked activity of thalamic neurons ($\downarrow$)		Driessen et al., 1998
		PPADS	Tail-flick and hot-plate tests, rat	Withdrawal latency (N.E.)		Li et al., 1998
		PPADS, TNP-ATP or desensitization by pretreatment with α,β-meATP	Capsaicin and formalin tests, mouse	Capsaicin- and formalin-(early phase) induced nociception ($\downarrow$)		Tsuda et al., 1999a
		Suramin, PPADS	Electrophysiological recordings in anesthetized rat	C-fiber-evoked responses of spinal dorsal horn neurons (N.E.)		Stanfa et al., 2000
Inflammatory pain	Antagonists or desensitizing agonists	Desensitization by pretreatment with α,β-meATP	Formalin test, rat	Nociceptive behavior (N.E.)		Driessen et al., 1994
		Suramin	Formalin test, rat	Nociceptive behavior ($\downarrow$)		Driessen et al., 1994
		PPADS, but not TNP-ATP	Formalin test, mouse	Late phase of nociceptive behavior ($\downarrow$)		Tsuda et al., 1999a
		Suramin, PPADS	Electrophysiological recordings in anesthetized rats with carrageenan-induced paw inflammation	C-fiber-evoked responses of spinal dorsal horn neurons ($\downarrow$)		Stanfa et al., 2000
Neuropathic pain	Antagonists	PPADS	Thermal nociceptive threshold testing in rats with PCI	Thermal hyperalgesia in animals (N.E.)		Liu and Tracey, 2000
		Suramin, PPADS	Electrophysiological recordings in anesthetized rats with SNL	C-fiber-evoked responses of spinal dorsal horn neurons (N.E.)		Stanfa et al., 2000

i.t., intrathecal; PCI, partial constriction injury of the sciatic nerve; SNL, lumbar 5/6 spinal nerve ligation; $\uparrow$, potentiation or induction; $\downarrow$, inhibition; N.E., no effect.

Formalin-induced nociception can be considered as a model of chemically induced tissue injury. When the role of peripheral P2X receptors in this model was studied using intraplantar injections of α,β-meATP to produce their selective desensitization, no inhibition of nociception was observed, although this pre-emptive treatment was able to completely abolish the agonist-evoked nociception (Bland-Ward and Humphrey, 1997). Intrathecal administration of the P2X receptor-selective antagonists PPADS and TNP-ATP in mice caused a mild inhibition of the nociceptive behavior in the first "acute" phase of the formalin test (Tsuda et al., 1999a). Both the first and second phases of formalin-evoked nociceptive behavior in the rat were potentiated by peripheral administration of the selective allosteric $P2X_3$ receptor enhancer Cibacron blue and inhibited by the $P2X_3$ receptor antagonist TNP-ATP (Jarvis et al., 2001). Formalin-induced nociception, especially in the late phase, appears to involve tissue inflammation and nerve injury components (Tjølsen et al., 1992); the role of P2X receptors in inflammatory and neuropathic pain is discussed below (see *Sections V.B.* and *V.C.*).

Thus, both agonist and antagonist behavioral studies suggest that P2X receptors contribute to acute nociception, but only under conditions of tissue injury. The selectivity profile of the used ligands suggests a predominant role for homomeric $P2X_3$ and/or heteromeric $P2X_{2/3}$ receptors. Consistent with the pharmacological findings discussed above, analysis of $P2X_3$ receptor knock-out mice did not reveal any role of this receptor subtype in responses to noxious mechanical or thermal stimuli (Cockayne et al., 2000; Souslova et al., 2000). The formalin-induced pain behavior in these mutants was, however, significantly attenuated in both the early and late phases compared with wild-type mice, thus confirming the involvement $P2X_3$ receptors in pain induced by tissue injury. The role of P2X receptors in acute visceral pain is discussed below (see *Section V.D.*). $P2X_1$ receptor knock-out mice have also been described (Mulryan et al., 2000); however, no nociception-related phenotypic difference from wild-type animals was mentioned in the report.

B. Inflammatory Pain

Tissue inflammation has been demonstrated to potentiate nociception evoked by peripherally applied P2X receptor agonists. In an in vitro skin-nerve preparation, nociceptor responses to α,β-meATP were greatly enhanced in the presence of carrageenan-induced skin inflammation (Hamilton and McMahon, 2000). Nociceptive responses and hyperalgesia induced by intraplantar injection of ATP or α,β-meATP were substantially potentiated in rats with skin inflammation caused by carrageenan or ultraviolet irradiation (Hamilton et al., 1999). In human volunteers, the original observations on the algogenic action of ATP were done using blister base applications, i.e., under conditions of inflammation (Bleehen et al., 1976; Bleehen and Keele, 1977). In a more recent human study, pain caused by electrophoretic application of ATP to the skin was markedly enhanced by ultraviolet irradiation (Hamilton et al., 2000). On the contrary, Dowd et al. (1998) did not find any change in the C- and A_δ-afferent fiber response to intra-articular α,β-meATP injection into the knee joint after the induction of chronic arthritis in the anesthetized rat. Thus, peripheral P2X receptors may play different roles in inflammatory pain of different origin.

P2X receptor antagonists appear to be antinociceptive in several models of inflammatory pain in vivo. Several studies have reported inhibition of nociceptive behavior in the formalin test after intrathecal administration of suramin, PPADS, or TNP-ATP (see *Section V.A.*). The nonselective P2 antagonist suramin reduced C-fiber-evoked activity of dorsal horn neurons after spinal administration in carrageenan-inflamed, but not normal rats; the P2X receptor antagonist PPADS produced a similar, although not statistically significant, inhibition (Stanfa et al., 2000).

One potential mechanism of inflammatory hyperalgesia is accumulation of ATP in the periphery (Burnstock, 1996; Burnstock and Wood, 1996). Elevated levels of ATP in inflamed tissues have been observed in experimental animals and in arthritic patients (Gordon, 1986; Ryan et al., 1991; Park et al., 1996). Additionally, a functional up-regulation of P2X receptors in inflammation may occur. This could be due to the proton sensitivity of some P2X subtypes, e.g., of the $P2X_2$ and $P2X_{2/3}$ receptors (see *Section II.C.*), whereby tissue acidosis could substantially potentiate nociceptor activity triggered by these receptors. The importance of this mechanism can be expected to be higher in the periphery, where tissue inflammation has been shown to be able to shift the pH to values as low as 5.5 (Reeh and Steen, 1996). Other endogenous agents that can be released during inflammation can also potentiate nociceptor responses to ATP. Thus, substance P and bradykinin have been shown to substantially enhance responses of small DRG neurons to ATP (Hu and Li, 1996). Behaviorally, responses to intraplantar injection of ATP or α,β-meATP in rats were substantially potentiated after pretreatment with prostaglandin E_2 (Hamilton et al., 1999). Therefore, peripheral rather than central administration of P2X receptor antagonists could be expected to have a greater effect on inflammatory hypersensitivity; whether this is the case remains to be investigated.

A substantial attenuation of the nociceptive response to formalin has recently been reported in $P2X_3$ receptor null-mutant mice (Cockayne et al., 2000; Souslova et al., 2000). Surprisingly, carrageenan inflammation evoked greater rather than lower hyperalgesic responses in the $P2X_3$ receptor knock-out mice compared with their wild-type controls (Souslova et al., 2000). This raises the question of a possible up-regulation of other receptor or ion channel systems because of $P2X_3$ receptor genetic deletion. An up-regulation of, e.g., $P2X_2$ receptors in these

mice could manifest as an increased inflammatory hypersensitivity because of their proton sensitivity (see above).

C. Neuropathic Pain

There are several lines of evidence indicating that P2X receptors could contribute to neuropathic pain. Peripheral nerve injury has been shown to regulate the expression of peripheral $P2X_3$ receptors, although the direction of the change appears to vary depending on the kind of injury. Thus, a profound (>50%) glial-derived neurotrophic factor-dependent down-regulation of $P2X_3$ receptor expression in DRGs of the lumbar segments 4 and 5 was observed after sciatic nerve axotomy (Bradbury et al., 1998). Similarly, a significant decrease in numbers of $P2X_3$ receptor-like immunoreactive neurons was observed in human DRG after central axotomy in patients with brachial plexus injuries (Yiangou et al., 2000). In contrast, an increase of the number of $P2X_3$ receptor-immunoreactive DRG neurons was demonstrated after a chronic constriction injury of the sciatic nerve (Novakovic et al., 1999). P2X receptor immunoreactivity was also increased in the spinal dorsal horn ipsilateral to the nerve injury, consistent with up-regulation of these receptors on intraspinal terminals of primary afferents. Chronic constriction injury of the inferior alveolar nerve has also been demonstrated to cause a substantial increase of the number of $P2X_3$ receptor-immunoreactive neurons in trigeminal ganglia (Eriksson et al., 1998). Importantly, accumulation of $P2X_3$ receptor immunoreactivity was observed in nerve endings at the site of injury. This local up-regulation of P2X receptors may be responsible for the development of ectopic purinergic sensitivity at the sites of nerve injury. Indeed, intravenous injection of ATP has been reported to excite afferents in the nerve with chronic constriction injury without affecting nerve fibers on the contralateral side; the effect was antagonized by the P2 receptor antagonist reactive blue 2 (Chen et al., 1999).

The potential contribution of P2X receptors to neuropathic pain is frequently discussed in the context of sympathetically maintained pain (Burnstock and Wood, 1996; Burnstock, 2000). The involvement of the sympathetic nervous system in some patients with neuropathic pain has been documented (Roberts, 1986). Indeed, sympathectomy or sympathetic nerve block has been shown to alleviate pain in such patients (Richards, 1967; Bonica, 1990) and in some animals with spinal nerve injury (Kim and Chung, 1991; Kim et al., 1993; Kinnman and Levine, 1995). Adrenergic antagonists, however, showed more limited efficacy in reducing neuropathic pain in these models than sympathectomy (Kim et al., 1993, Lee et al., 1997), implying that the release of ATP from sympathetic postganglionic neurons sprouting into dorsal root ganglia may contribute to this process (Burnstock, 1990, 1996; Burnstock and Wood, 1996). One recent study analyzed the effects of sympathectomy and of block of adreno- and purinoreceptors in the spinal nerve ligation model of neuropathic pain in the rat (Park et al., 2000). Indeed, a clear division of neuropathic animals into "responders" and "nonresponders" to a combined systemic administration of the P2 antagonist suramin and the α-adrenoreceptor antagonist phentolamine was observed. In the responder subpopulation, consistent antiallodynic effects of the drug combination were observed after repeated treatments. Unfortunately, suramin is not a selective P2X receptor antagonist, and one cannot conclude from this study which P2X receptors are particularly involved in this sympathetically maintained allodynia. Other authors have failed to demonstrate any effect of suramin or a more selective P2X receptor antagonist PPADS in various models of neuropathic pain (Liu and Tracey, 2000; Stanfa et al., 2000). The known variability of the sympathetic component of neuropathic pain could be one explanation for these negative findings. Moreover, these other studies used intrathecal administration of antagonists; peripheral sites of ectopic purinergic sensitivity (see above) are unlikely to have been accessed by the antagonist given by this route. The reported lack of antiallodynic effect of intrathecally administered P2X receptor antagonists after peripheral nerve injury does imply that spinal P2X receptors play little role in the maintenance of this form of neuropathic pain. Nevertheless, given the limitations of the compounds used in these studies, this issue will need to be revisited when more potent, stable, and selective antagonists become available.

D. Visceral Pain

Burnstock (1996, 1999, 2001) has proposed that ATP plays an important role in visceral pain perception. According to his hypothesis, ATP can be released from epithelial cells upon distension of hollow visceral organs leading to activation of $P2X_3$ and/or $P2X_{2/3}$ receptors on visceral afferents (Burnstock, 1999). Thus, excessive distensions would release ATP in amounts sufficient to reach the receptors on extrinsic sensory nerves that would convey this information to the central nervous system, with colic pain as a consequence, whereas moderate distensions would only activate P2X receptors on intrinsic sensory fibers and contribute to peristalsis (Barthó et al., 1999; Burnstock, 2001). Some evidence has been accumulated supporting this hypothesis. Thus, ATP-mediated synaptic potentials have been observed in enteric neurons (Galligan and Bertrand, 1994). ATP release from urothelial cells has been documented upon changing the hydrostatic pressure in the bladder (Ferguson et al., 1997; Grygorczyk and Hanrahan, 1997). In an in vitro rat bladder-pelvic nerve preparation, increases in the nerve discharge have been recorded in response to bladder distensions; this activity was inhibited by the P2 receptor antagonist suramin, indicating a possible involvement of ATP (Namasivayam et al., 1999). In this preparation, desensitization of P2X receptors by infusion of the $P2X_1/P2X_3/P2X_{2/3}$ receptor ago-

nist α,β-meATP into the bladder also inhibited evoked afferent nerve discharges, confirming the involvement of functional P2X receptors on visceral afferents in this response. In an in vivo study in the anesthetized rat, ATP or α,β-meATP injections into the arteries supplying the gut evoked a biphasic increase of mesenteric afferent nerve activity (Kirkup et al., 1999). The second burst of activity was parallel to, and probably caused by, an increase in the intrajejunal pressure; both the early and the late phase of the afferent nerve response and the agonist-evoked rise in pressure were antagonized by systemic administration of the P2X receptor antagonists suramin or PPADS.

Of the P2X receptor subtypes, the homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors seem to be the likely candidates to mediate the action of ATP in visceral pain. Immunohistochemical studies have shown the presence of $P2X_3$ receptors on small-diameter DRG neurons with projections into the pelvic nerve (Bradbury et al., 1998) and on sensory neurons innervating the bladder (Elneil et al., 1999; Cockayne et al., 2000). Neurons in nodose ganglia, where the somata of vagal afferents are localized, express functional $P2X_2$ and $P2X_{2/3}$ receptors (see *Section III.B.*). Recently, an important role of $P2X_3$ receptors in bladder sensitivity to distension has been demonstrated in the $P2X_3$ receptor knock-out mice (Cockayne et al., 2000). The $P2X_3$ receptor null mice showed a significant increase in bladder capacity and decrease in micturition frequency, as well as greatly reduced distension-evoked bladder contractions. The involvement of the $P2X_3$ receptor in the afferent control of physiological bladder regulation warrants further studies under painful conditions, e.g., cystitis.

VI. Conclusions

Activation of certain P2X receptor types by ATP appears to be an important factor in several pain states. P2X receptors, in particular $P2X_3$ and $P2X_{2/3}$ receptors, represent attractive targets for the treatment of inflammatory, visceral, and possibly also neuropathic pain. Further progress in this area is hampered by the lack of potent and selective antagonists sufficiently resistant to enzymatic degradation under in vivo conditions.

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