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INVITED REVIEW

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P2X purinoceptors and sensory transmission

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Abstract The involvement of P2X purinoreceptors (P2X receptors) in somatosensory transmission is herein reviewed with a focus on those receptors that are expressed on sensory neurons to elucidate their roles in the initiation of sensory excitation from primary afferent neurons, in modulating synaptic transmission at the first sensory synapses formed between primary afferent central terminals and dorsal horn neurons, in directly mediating sensory synaptic transmission to the spinal cord dorsal horn, and in modulating synaptic transmission among spinal cord dorsal horn neurons. Research on P2X receptors has indicated that these receptors play a significant role in both physiological and pathological pain states. As a result, P2X receptors may serve as therapeutic targets for the treatment of pathological pain conditions associated with nerve injury, tissue inflammation, cancer, and other diseases.

Keywords ATP · P2X · Purinoceptor · Pain · Sensory transmission

Introduction

P2 purinoreceptors (P2 receptors) are membrane receptors that can be activated upon the binding of adenosine triphosphate (ATP) at extracellular receptor binding sites [10, 59, 65]. Long before the identification of P2 receptors, the sensory functions of extracellular ATP were proposed by Holton and Holton [33] based on their observation that primary afferent fibers could release ATP. It was, thus, proposed that ATP might function as an extracellular chemical messenger to transmit sensory information [33]. The algogenic actions of ATP (or ATP-induced painful sensations) were later observed in human blister base preparation [3]. Since then, more evidence has been accumulated to show the sensory actions of ATP in human as well as animal behavioral tests. For example, ATP produced weal and flare responses in a dose-dependent manner [21] when it was injected intradermally into the backs of human volunteers. ATP produced a modest burning pain sensation when it was delivered to the forearm skin of human volunteers, and the painful sensation began within 20 s and, thereafter, was maintained for several minutes [31]. ATP also caused an increase in blood flow when it was injected into human skin, which, thus, represented one aspect of the inflammatory responses [31]. ATP-induced painful sensation was dependent on the activation of nociceptive afferent fibers that were sensitive to capsaicin. Moreover, ATP-induced painful sensation was largely potentiated when ATP was injected into the skin after heat radiation [31]. Similar to the above-mentioned studies on human subjects, nociceptive behaviors were observed in animals after the subplantar injections of ATP or α,β-methylene ATP [2, 30, 69, 73]. These observations strongly suggested that ATP might, thus, be a strong inflammatory mediator involved in nociceptive signaling at peripheral sites.

The idea that ATP is a sensory mediator gained increasing support after the direct observations of ATP-induced depolarizing currents in both dorsal root ganglion (DRG) neurons and spinal cord dorsal horn neurons [38, 45]. The cloning of multiple subunits of P2X receptors during the last decade has been an important landmark in the exploration of the sensory functions of ATP and P2X receptors at molecular levels [42, 59–61]. The P2X-mediated actions of ATP on the sensory neurons were supported at a cellular level by the identification of P2X receptors expressed in both the primary afferent neurons

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and spinal cord dorsal horn neurons [18]. As suggested by an elegant study using a co-culture preparation of skin cells and nociceptive afferent neurons, endogenously released ATP after tissue damage may directly initiate the nociceptive impulses through the activation of P2X receptors expressed on primary afferent peripheral terminals [20]. The initiation of sensory impulses at peripheral nerve endings may only represent one part of the sensory functions of ATP and P2X receptors. P2X receptors have been found to be expressed at the central terminals of primary afferent fibers and their activation modulates sensory synaptic transmission into the spinal cord dorsal horn. In addition to their expression on primary afferent fibers, P2X receptors have been identified on spinal cord dorsal horn neurons at their pre- and postsynaptic sites. As a result, endogenous ATP or exogenous P2X receptor agonists and antagonists can exert their effects at all these sites along the sensory pathways (Fig. 1). Research on animal models has provided a great body of evidence indicating that ATP and P2X receptors are involved in a variety of pain conditions. In addition to P2X receptors, recent evidence for the presence of P2Y receptors on both primary afferent fibers and spinal cord dorsal horn neurons has led to a new expansion in research on the functions of purinoreceptors in sensory transmission and pain. The potential roles of P2Y receptors in nociception have been reviewed recently [11, 24] and, therefore, will not be reviewed in this article.

P2X receptors on primary afferent neurons

The somatosensory system conveys and processes sensory signals including touch, heat, and pain from the body. In the somatosensory system, the first-order sensory neurons or primary afferent neurons are located on the dorsal side of the spinal column. These primary afferent neurons are also called dorsal root ganglion neurons (Fig. 1). Primary afferent neurons have two major processes, one extending to peripheral sites and the other to the spinal cord dorsal horn. Nerve endings at the peripheral sites touch the skin, muscles, joints, and other tissues, where sensory stimuli are detected by these nerve endings. The sensory stimuli can depolarize the peripheral nerve endings to initiate action potentials, which travel along the primary afferent fibers to the central terminals of primary afferent fibers, thereby evoking the release of neurotransmitters from the central terminals onto the dorsal horn neurons. At the periphery, it is conceivable that ATP can be released from damaged cells after tissue injury because all cells contain high millimolar concentrations of ATP [9]. ATP may also be released after tissue stretch and inflammation [4, 8]. It has been hypothesized that endogenously released ATP can directly initiate sensory impulses from peripheral nerve endings through activation of P2X receptors. However, ATP actions at the peripheral nerve endings have never been directly demonstrated because it is difficult to directly access peripheral nerve endings using electrophysiological methods or other functional approaches. Nevertheless, the sensory functions of many receptors can be studied on the somas of such primary afferent fibers. Similar to other sensory receptors such as the heat-sensing receptor TRPV1 [12, 71], studies on peripheral sensory functions of P2X receptors have so far been mainly performed on the somas of primary afferent neurons. This approach is based on the well-accepted assumption that if one type of receptors is expressed on a soma of a primary afferent neuron, then the peripheral nerve endings of the primary afferent fiber also express the same type of receptors. This assumption has recently been further extended to the central terminals of primary afferent fibers [28]. Although this assumption is likely to be true for most receptors, it should be noted that membrane receptors may not always be delivered to both ends of a primary afferent fiber. Primary afferent neurons can be functionally classified into two major categories, namely, nociceptive (pain sensing) and non-nociceptive neurons. A number of methods have been used to define nociceptive neurons in vitro, including capsaicin sensitivity, isolectin B4 (IB4) binding, and substance P immunoreactivity. Each of these methods only defines a subgroup of nociceptive neurons. For example, most noxious heat-sensitive primary afferent neurons are defined by capsaicin sensitivity, and these cells express TRPV1 receptors that can be activated by both noxious heat and capsaicin [12]. The mechanical nociceptive neurons are a group of primary afferent neurons that detect high-threshold noxious mechanical stimuli, but no cellular or molecular marker has yet been identified for this functional group of sensory neurons in mammals [81].

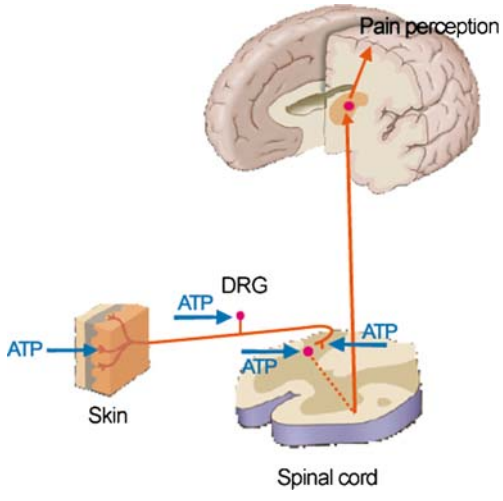

Fig. 1 ATP actions mediated by P2X receptors along the sensory transmission pathways to the spinal cord dorsal horn. This schematic diagram illustrates the potential sites where endogenous ATP exerts its actions through the activation of P2X receptors. These sites include the primary afferent nerve endings at peripheral sites, primary afferent ganglions, primary afferent central terminals within the spinal cord dorsal horn, and postsynaptic and presynaptic sites on dorsal horn neurons

P2X receptor expression has been studied on functional groups of primary afferent neurons [14, 47, 63, 64, 79], which, thus, provides primary information for the potential sensory functions of these receptors. Seven P2X subunits

($P2X_1$–$P2X_7$) have been identified and cloned so far [59], and these subunits can form at least 11 functional P2X subtypes in heterologous expression systems [60]. The biophysical and pharmacological properties of these recombinant P2X receptors have previously been well studied and extensively reviewed [42, 61]. Based on immunostaining and in situ hybridization studies, it appears that, except for $P2X_7$, all other P2X receptor subunits are expressed on primary afferent neurons in the dorsal root ganglia and trigeminal ganglia [18, 82]. Among these subunits, the $P2X_3$ subunit expression patterns were the most extensively studied. Molecular cloning and characterization of $P2X_3$ receptors were first successfully done in DRG neurons [14]. $P2X_3$ receptor subunit transcripts and proteins were identified to be predominantly present in a subset of DRG neurons that were small in size (<30 μm) and IB4-positive neurons. A small number of medium-sized DRG neurons were later found to express $P2X_3$ subunits, but the expression level appeared to be lower than those in small-sized IB4-positive neurons [7]. Most $P2X_3$-expressing DRG neurons also expressed the nociceptive heat-sensing receptor TRPV1 [77], which supports the observation that heat radiation can potentiate ATP-induced pain [31]. $P2X_2$ receptors were initially cloned from PC12 cells [6], but in situ hybridization has revealed that mRNAs for $P2X_2$ subunits are expressed on some medium-sized DRG neurons [77]. A small portion of $P2X_2$-expressing neurons were found to express mRNAs for $P2X_3$ subunits as well [43, 77]. Neither $P2X_2$ nor $P2X_3$ mRNAs were detectable in large-sized DRG neurons. No DRG neurons expressing other P2X receptor subunits have yet been well characterized.

Functional P2X receptors have been studied using the patch-clamp recording technique with tests of P2X receptor agonists and antagonists [7, 48, 63, 64, 76, 77]. A comparison of the biophysical and pharmacological profiles of P2X-mediated membrane currents in primary afferent neurons with those recombinant P2X receptors has been used as an important means to evaluate functional P2X receptor subtypes on primary afferent neurons [64, 76]. When recorded from acutely dissociated DRG neurons, ATP and other P2X receptor agonists such as α,β-methylene ATP can evoke membrane currents from many small-sized and some medium-sized primary afferent neurons. The evoked currents show three distinct phenotypes, namely, a fast current, slow current, and mixed current with both fast and slow components [7, 48, 63, 64, 76, 77]. The fast current is manifested by a rapid desensitization in the range of milliseconds in the presence of agonists. In contrast, the slow current displays a weak or little desensitization in the range of seconds in the presence of agonists. Fast currents were usually seen in small-sized DRG neurons that were capsaicin sensitive and IB4 positive, and slow and mixed currents were found mainly in medium-sized DRG neurons that were not capsaicin insensitive and IB4 negative. Comparing the pharmacological and biophysical profiles of recombinant P2X receptors with P2X agonist-evoked currents in DRG neurons, it has, thus, been concluded that most fast currents were mediated by homomeric $P2X_3$ receptors. This is consistent with the expression pattern of $P2X_3$ subunits in DRGs [5, 77, 79]. This conclusion was further supported by studies on the primary sensory neurons from $P2X_3$-knockout mice [17, 70] in which no rapidly desensitizing ATP-induced currents were observed. However, the lack of fast P2X currents in $P2X_3$-knockout mice was inconsistent with the expression of $P2X_1$ receptors and the presence of $P2X_1$-like fast currents recorded in some DRG neurons in rats [17, 64, 70]. Slow currents evoked by α,β-methylene ATP were thought to be mainly mediated by heteromeric $P2X_{2/3}$, and mixed currents evoked by α,β-methylene ATP were believed to be due to the co-expression of homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors in the same cells. Using the retrograde labeling technique, Cook et al. [19] pre-identified nociceptive afferent neurons that innervate rat tooth pulps and showed that these nociceptive neurons responded to ATP through the activation of the $P2X_3$ subunit-containing receptors. In contrast to these nociceptive afferent fibers, the same study showed that stretch-sensitive afferent neurons innervating muscles responded to ATP through the activation of P2X receptors other than $P2X_3$-containing receptors. These P2X receptors were found to be insensitive to α,β-methylene ATP, but the exact subtype of these P2X receptors remains to be elucidated. $P2X_3$-containing P2X receptors are highly sensitive to α,β-methylene ATP. $P2X_3$-containing receptor-mediated initiation of nociceptive responses have been implicated in behavioral tests in which injection of α,β-methylene ATP into rat plantar surface produces mechanical allodynia along with nocifensive behaviors and thermal hyperalgesia [73]. It is interesting that the α,β-methylene ATP-induced thermal hyperalgesia was absent but the α,β-methylene ATP-induced mechanical allodynia remained in rats after neonatal capsaicin treatment to remove capsaicin-sensitive afferent fibers. Furthermore, the proportion of DRG neurons that responded to α,β-methylene ATP with slow current remained constant in capsaicin-treated rats, whereas the population that responded to α,β-methylene ATP with fast currents dramatically decreased. These findings suggested that the activation of homomeric $P2X_3$ receptors on capsaicin-sensitive afferent nerve endings could, thus, give rise to thermal pain sensations while the activation of heteromeric $P2X_{2/3}$ receptors on capsaicin-insensitive afferent fibers could yield mechanical pain sensations.

While $P2X_3$-containing receptors appeared to contribute to the ATP-evoked currents in many DRG neurons, other P2X receptor subtypes have also been indicated to play sensory roles. In a study testing the effects of TNP–ATP, a potent antagonist selective to $P2X_1$, $P2X_3$, and $P2X_{2/3}$ receptors, on α,β-methylene ATP-induced currents in acutely dissociated DRG neurons, it was found that the non-desensitizing currents evoked by 100 μM α,β-methylene ATP were not sensitive to the blockade by TNP–ATP in a subpopulation of DRG neurons, but the currents could be blocked by PPADS [76]. These results indicated that the α,β-methylene ATP-induced currents were not mediated by $P2X_3$-containing receptors, and the involvement of

$P2X_{4/6}$ or $P2X_{1/5}$ receptors were suggested based on both pharmacological and electrophysiological profiles [42]. The physiological and pathological sensory functions of these P2X receptors remain to be studied.

Presynaptic P2X receptor-mediated modulation of glutamatergic synaptic transmission at the first sensory synapses

The presynaptic sites of primary afferent fibers are central terminals located in the spinal cord dorsal horns. The synapses formed between the primary afferent central terminals and dorsal horn neurons are the first sensory synapses in the somatosensory pathways. In all these synapses, glutamate is used as a fast excitatory neurotransmitter to convey sensory signals from the periphery to the spinal cord dorsal horn [85]. In addition, several neuropeptides such as substance P can also be released from the nociceptive afferent central terminals to the dorsal horn. The first sensory synapse is one of the most important places where sensory transmission from the periphery to the dorsal horn is regulated. A number of receptors are expressed at the center terminals of primary afferent fibers and these receptors play important roles in synaptic modulation of neurotransmitter release onto dorsal horn neurons. For example, the presynaptic inhibition at primary afferent central terminals have been known for many years, and it is mainly mediated by gamma-aminobutyric aci ($GABA_A$) receptors located at the central terminals of primary afferent fibers and activation of these presynaptically localized $GABA_A$ receptors causes inhibition of transmitter release from primary afferent central terminals [25, 52]. Inhibition of nociceptive transmission after the activation of opiate receptors on primary afferent central terminals is another example of presynaptic modulation of sensory transmission to the spinal cord dorsal horn [35, 39, 44]. In addition to such presynaptic inhibition, presynaptic facilitation at the central terminals of primary afferent fibers has also been observed. For example, *N*-methyl-D-aspartate receptors have been shown to be expressed at the central terminals of nociceptive afferent fibers and their activation facilitates substance P release [50, 53]. Using a model sensory synapse system made by co-cultures of DRG neurons and dorsal horn neurons, presynaptic localization of functional P2X receptors on DRG neuron processes were first identified by Gu and MacDermott [26]. The activation of these presynaptic P2X receptors was found to facilitate quantal release of glutamate due to direct Ca^{2+} entry into presynaptic terminals. This study further demonstrated that activation of P2X receptors at and/or near presynpatic sites directly elicited terminal action potentials and resulted in synchronized glutamate release from presynaptic terminals. Using spinal cord slices, a tissue preparation closely representing the in vivo conditions of synaptic transmission, presynaptic localization of P2X receptors at the central terminals of primary afferent fibers has been demonstrated in three different nociceptive processing regions in the spinal cord dorsal horn (see next section). Similar to those observed in the DRG and DH co-culture system, the activation of presynaptic P2X receptors facilitated quantal release of glutamate from primary afferent central terminals to dorsal horn neurons in the spinal cord slice preparations [46, 55]. Furthermore, the activation of presynaptic P2X receptors has been shown to strengthen sensory synaptic transmission after the stimulation of primary afferent fibers, and the synaptic strengthening was due to the presynaptic enhancement of synchronized glutamate release. The synaptic strengthening of sensory transmission mediated by presynaptic P2X receptors not only can be induced by exogenously applied P2X receptor agonists but also by endogenously released ATP after the repetitive stimulation of primary afferent fibers [55] (Fig. 2). In addition to the enhancement of presynaptic glutamate release, the activation of P2X receptors at or near the central terminals of primary afferent fibers in the spinal cord dorsal horn were also found to elicit substance P release, thus, resulting in the activation of neurokin receptor 1 (NK1R) and subsequent NK1R internalization on dorsal neurons [56]. The potentiation of sensory synaptic transmission after the activation of presynaptic P2X receptors may have important implications in pain conditions such as hyperalgesia, making central terminal P2X receptors potential therapeutic targets for the treatment of pathological pain conditions. Consistent with this idea, the intrathecal application of P2X

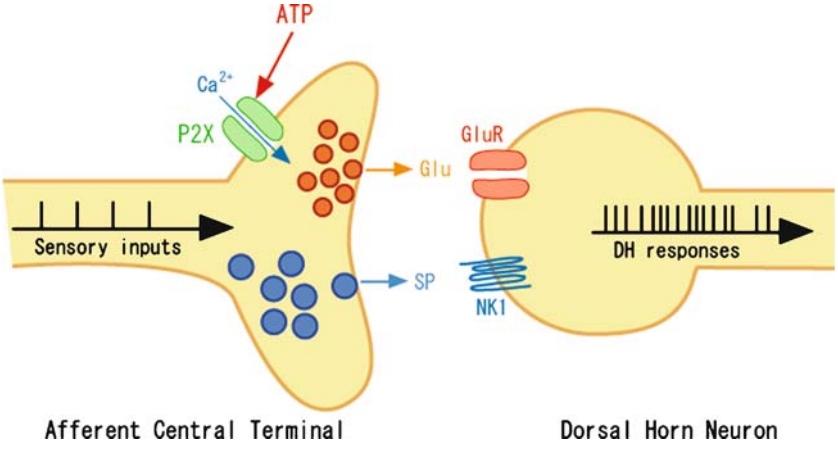

Fig. 2 Presynaptic P2X receptor-mediated modulation of sensory transmission at the first sensory synapses. This schematic diagram illustrates the sensory transmission between the nociceptive afferent central terminals and the dorsal horn neurons. The activation of P2X receptors on the central terminals of primary afferent fibers results in the release of glutamate and substance P in a Ca^{2+}-dependent manner, which in turn activates glutamate receptors and NK1 receptors on dorsal horn neurons

receptor antagonists [54, 72] or antisenses has been shown to alleviate allodynia in both inflammatory and neuropathic pain animal models [34].

P2X purinergic sensory inputs to different functional regions of the spinal cord dorsal horn

To better understand the nociceptive functions of P2X receptors on the primary afferent fibers under both physiological and pathological conditions, it is essential to identify the P2X-mediated nociceptive pathways and to identify where P2X-mediated nociceptive inputs are transmitted and processed in the spinal cord dorsal horn. The dorsal horn, comprising laminae I and II (superficial laminae), III and IV (intermediate part), and V and VI (deep laminae), is the primary central site for processing the somatic sensory inputs [67, 80]. Both the superficial and the deep laminae of the dorsal horn are responsible in the reception, processing, and transmission of nociceptive information [13, 22, 80]. In contrast, the intermediate part of the dorsal horn is mainly involved in processing non-nociceptive information [80].

P2X purinergic sensory pathways to the dorsal horn have been studied on spinal cord sections by immunochemistry with P2X antibodies. In immunochemistry studies with $P2X_3$ antibodies, $P2X_3$-expressing primary afferent terminals [5, 29, 79] were found to be restricted to the inner part of lamina II (lamina IIi) in normal animals. The immunoreactivity of $P2X_3$ receptors was shown to be co-localized with IB4 staining and also with TRPV1 receptor immunoreactivity in the inner portion of lamina II [29, 79]. It should be noted that a significant portion of TRPV1 receptor immunoreactivity is located in lamina I where no immunoreactivity to $P2X_3$ receptors could be detected. When saporin-conjugated IB4 was injected into rat sciatic nerve to chemically ablate IB4-positive afferent neurons, the immunostaining for $P2X_3$ receptors almost completely disappeared in the spinal cord dorsal horn [58]. The immunoreactivity of $P2X_3$ receptors also substantially decreased after dorsal rhizotomy as well as after neonatal capsaicin treatment [5, 79]. The lamina distribution of afferent fibers that express other P2X subunits remains unclear, although there was a report that showed the immunoreactivity of $P2X_1$ and $P2X_2$ subunits in a superficial lamina as well [78]. Except for the $P2X_3$ subunit antibodies, it appears that antibodies for other P2X receptor subunits have limited usefulness for mapping P2X-expressing afferent central terminals in the spinal cord sections. In addition, a further limitation of immunochemistry is that it only reveals P2X subunits rather than functional P2X receptors [27].

The P2X purinergic sensory pathways to the dorsal horn have been studied using synaptic physiology with patch-clamp recording technique. This functional approach provides the opportunity to (a) assess functional P2X receptors that are expressed on primary afferent central terminals; (b) reveal the effects of P2X purinergic sensory inputs on dorsal horn neurons, i.e., the secondary order sensory neurons within the dorsal horn; and (c) identify neuronal circuits that are involved in processing P2X purinergic sensory inputs within the dorsal horn. To this end, we have studied the effects of P2X receptor activation on monosynaptic and polysynaptic transmission from primary afferent fibers to dorsal horn neurons located in lamina I, II, and V, three nociceptive processing regions. Patch-clamp experiments using spinal cord slice preparations showed that distinct subtypes of P2X receptors are located at afferent central terminals innervating lamina I, lamina II, and lamina V regions, where these P2X-expressing afferent central terminals synapse to dorsal horn neurons monosynaptically (Fig. 3). The activation of these central terminal P2X receptors enhanced glutamate release in all the three dorsal horn regions, but the release and pharmacological properties were found to be different among these regions [15, 55, 58]. In lamina II, P2X receptor-mediated enhancement of glutamate release was mainly transient [58]. In contrast, the P2X receptor-mediated enhancement of glutamate release was long lasting in the lamina I and lamina V regions [15, 55, 58]. Both transient and long-lasting types of enhancement of glutamate release could be pharmacologically induced by α,β-methylene ATP and blocked by low micromolar concentrations of suramin and PPADS, indicating the involvement of α,β-methylene ATP-sensitive P2X receptor subtypes. In lamina II, the transient modulation was abolished in the presence of nanomolar concentrations of TNP–ATP, suggesting that homomeric $P2X_3$ receptors were involved in presynaptic modulation of glutamate release from primary afferent central terminals to lamina II neurons. In lamina V region, the long-lasting modulation of glutamate release remained in the presence of high concentrations of TNP–ATP. Furthermore, the removal of $P2X_3$ receptor-expressing afferent terminals by saporin-conjugated IB4 or by the surgical removal of the superficial dorsal horn did not affect the presynaptic P2X receptor-mediated long-lasting modulation of glutamate release from primary afferent central terminals to the neurons located in lamina V region. These results indicated that, in contrast to the lamina II, P2X receptors on the afferent central terminals in the lamina V region are not $P2X_3$-containing P2X subtypes. This conclusion is consistent with anatomic studies that showed no detectable $P2X_3$ immunoreactivity in lamina V region. P2X receptors of afferent central terminals in lamina V appeared to be consistent with the $P2X_{4/6}$ or $P2X_{1/5}$receptors based on pharmacological properties of the recombinant P2X receptor subtypes [42]. In lamina I, the P2X receptor-mediated enhancement of glutamate release was also found to be long lasting, similar to those observed in lamina V. Although it remains to be elucidated, the P2X receptors expressed on the afferent central terminals innervating the lamina I region are also unlikely to be the $P2X_3$ subunit containing P2X receptors. The pharmacological profiles of these P2X receptors include (1) sensitive to the micromolar concentrations of α,β-methylene ATP, (2) non-desensitization to the prolonged application of ATP and α,β-

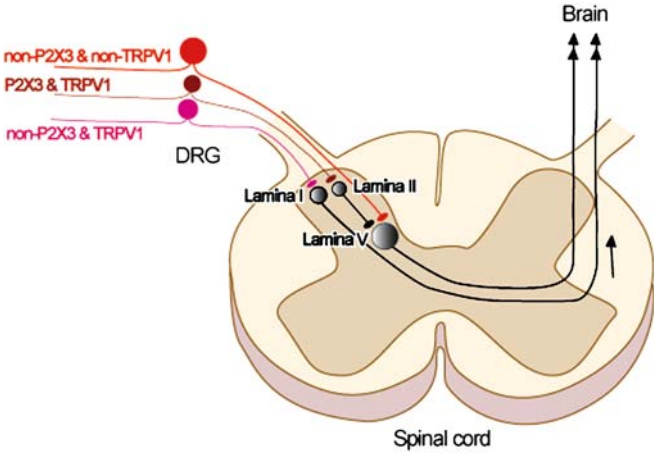

Fig. 3 Three distinct P2X purinergic sensory transmission pathways from the primary afferent fibers to the spinal cord dorsal horn. This schematic diagram illustrates three P2X-puringeric primary afferent pathways to lamina I, II, and V of the dorsal horn. The pathway to lamina I is derived from the primary afferent fibers that express P2X receptors lacking of $P2X_3$ subunits. These primary afferent fibers also express noxious heat receptor TRPV1 which are sensitive to capsaicin. The pathway to lamina II is derived from the primary afferent fibers expressing both $P2X_3$ and TRPV1 receptors. The pathway to lamina V is derived from the capsaicin-insensitive primary afferent fibers expressing $P2X_{1/5}$-like and/or $P2X_{4/6}$-like receptors

methylene ATP, and (3) sensitive to the block by suramin and PPADS [42].

A synaptic physiological approach has also been applied to identify whether P2X receptor-expressing primary afferent fibers innervating lamina I, II, and V are capsaicin-sensitive afferent fibers. The test of capsaicin sensitivity is because almost all capsaicin-sensitive primary afferent fibers are nociceptive afferent fibers [51, 84]. In both lamina I and lamina II, the P2X-expressing afferent central terminals were found to be capsaicin-sensitive (Fig. 3) and, similar to the P2X agonists, capsaicin increased glutamate release from these afferent center terminals to neurons in lamina I and lamina II. On the other hand, P2X-expressing central terminals in lamina V were shown to be derived from the capsaicin-insensitive Aδ - primary afferents [15, 57]. Although the lamina V neurons were not shown to receive any direct afferent inputs from the $P2X_3$ receptor-expressing/capsaicin-sensitive fibers, these sensory inputs were found to be polysynaptically transmitted to the lamina V neurons. Furthermore, these polysynaptic inputs converged with the monosynaptic inputs from the ATP-sensitive/capsaicin-insensitive inputs onto lamina V neurons [57] (Fig. 3), which produced temporal summation in the individual lamina V neurons. As the simultaneous activation of the P2X and TRPV1 receptors may be common under many pathological conditions, it has been hypothesized that the excitatory synaptic convergence of P2X purinergic inputs and capsaicin-sensitive inputs may lead to the development of hyperactivity in the deep dorsal region, thereby inducing hyperalgesia. P2X-expressing/capsaicin-sensitive afferent fibers that innervate lamina I also play a very interesting role in the pain mechanism because the majority of lamina I neurons are nociceptive specific neurons that directly relay nociceptive information to the brain through the ascending pathways [37]. Two important issues that remain to be addressed are what subtypes of P2X receptors are expressed on these afferent fibers and whether these P2X receptors significantly play a role in physiological and pathological pain states [15].

P2X receptors on dorsal horn neurons and their functions in mediating and modulating synaptic transmission in the dorsal horn

The presence of functional P2X receptors on the dorsal horn neurons were first demonstrated in cultured spinal dorsal horn neurons by Jahr and Jessell [38]. In their study, it was found that ATP excited a subpopulation of these neurons due to ATP-induced depolarizing membrane currents. After the cloning of the seven P2X receptor subunits, studies showed that except $P2X_3$, mRNAs for all other P2X receptor subunits were distributed in the spinal cord dorsal horn, and mRNAs for $P2X_2$, $P2X_4$, and $P2X_6$ were found to be strongly expressed in superficial laminae [18]. Functional P2X receptors were examined in acutely dissociated spinal cord dorsal horn neurons [1]. The use of this type of cell preparation was done to avoid potential receptor phenotype changes under culture conditions. It was, thus, found that, similar to cultured dorsal horn neurons, ATP evoked inward currents in a small population of acutely dissociated cells from superficial laminae of the dorsal horn. The ATP-evoked currents showed slow desensitization and they could also be blocked by P2X receptor antagonists suramin and PPADS, thus, indicating the expression of functional P2X receptors on these dorsal horn neurons. P2X receptors on these cells appeared to be insensitive to α,β-methylene ATP [1]. These characteristics are consistent with a heterogeneous population of P2X receptors, the composition of which includes $P2X_2$, $P2X_4$, and $P2X_6$ receptor subtypes. Calcium imaging experiments showed that ATP evoked Ca^{2+} transients in those dorsal horn neurons in the presence of La^{3+}, a blocker of voltage-gated calcium channels [1]. This suggests that the P2X receptors on superficial dorsal horn neurons are highly permeable to Ca^{2+}. In spinal cord slice preparations, application of ATP induced fast inward currents which were associated with potentiation of excitatory synaptic currents in lamina II neurons. This effect of ATP was interpreted as postsynaptic P2X receptor-mediated sensory potentiation [49]. Postsynaptic localization of P2X receptors was first demonstrated in the medial habenula of rat brains, where P2X receptors were shown to mediate fast excitatory transmission [23]. In a small population (~5%) of dorsal horn neurons, fast excitatory postsynaptic synaptic currents (EPSCs) were also elicited in the superficial laminae by focal electrical stimulation in the presence of ionotropic glutamate receptor antagonists CNQX and APV, and the EPSCs were not inhibited by blockers of a number of other receptors for classical

transmitters such as 5-HT and acetylcholine [1]. The EPSCs, however, were inhibited by suramin and PPADS. This finding suggests that P2X receptors are expressed at postsynaptic sites of some superficial lamina neurons in the dorsal horn to mediate fast synaptic transmission. It remains unclear as to whether, at these synapses, the P2X receptors are co-localized with glutamate receptors at the postsynaptic sites and whether ATP and glutamate are co-released from these presynaptic terminals. P2X receptor-mediated fast EPSCs have been found to be much smaller than glutamatergic EPSCs, raising an issue whether P2X-mediated fast EPSCs are significant in directly transmitting sensory information. One hypothesis is that postsynaptic P2X receptors provide a route of Ca^{2+} entry to postsynaptic sites, which subsequently modulates the postsynaptic glutamate receptors to change synaptic plasticity at sensory synapses in the dorsal horn of the spinal cord. P2X receptors have also been reported to mediate fast synaptic transmission in many GABAergic synapses in cultured dorsal horn neurons [40], and it was, thus, suggested that ATP and GABA are co-transmitters in these inhibitory synapses. However, it is unclear whether ATP–GABA co-transmission occurs in vivo and, if so, what the synaptic functions are for both the excitatory and inhibitor transmission through the same synapses.

In addition to postsynaptic localization, P2X receptors have been indicated to be present at the presynaptic sites of inhibitory dorsal horn neurons and the activation of these presynaptic P2X receptors modulates the inhibitory synaptic transmission between the dorsal horn neurons. The functions of the presynaptic P2X receptors at inhibitory synapses have been demonstrated by an experiment using a synaptic bouton preparation that consists of acutely dissociated dorsal horn neurons with attached presynaptic boutons [68]. The recordings from the dissociated neurons in such cell preparations revealed a facilitation of glycine release from presynaptic boutons onto the dorsal horn neurons after the application of P2X receptor agonists ATP or 2MeSATP. The effects of ATP were not affected by *N*-ethylmaleimide, a G-protein inhibitor, but they were completely abolished by suramin and PPADS. Furthermore, the increases in the spontaneous glycine release by ATP required extracellular Ca^{2+}, but entry of Ca^{2+} through voltage-gated Ca^{2+} channels were not essential. These pharmacological properties, thus, suggested that $P2X_2$ receptors might be present at the presynaptic sites of glycinergic synapses and their activation, thus, resulted in a direct Ca^{2+}entry through presynaptic P2X receptors, which in turn increased the spontaneous releases of glycine. The presynaptic P2X-mediated modulation of GABA release was demonstrated using cultured neurons of the superficial dorsal horn [36]. In this study, ATP increased the frequency of the miniature inhibitory postsynaptic currents (mIPSCs) mediated by $GABA_A$ receptors in a subpopulation of the dorsal horn neurons. The P2Y receptors agonists UTP or ADP-β-S, on the other hand, did not have any effects on mIPSCs. The presynaptic effects of ATP were inhibited by the P2X receptor antagonists suramin, PPADS, and reactive blue. These results indicated that the activation of P2X receptors increased the presynaptic GABA release probability. The presynaptic facilitation was also reflected by ATP-induced potentiation of the amplitude of GABAergic IPSCs after electrical stimulation, and the potentiation was due to the enhancement of presynaptic GABA release probability rather than postsynaptic modulation. Increases in the inhibitory transmitter release probability after the activation of presynaptic P2X receptors may represent a novel inhibitory mechanism for sensory processing in the spinal cord dorsal horn. It remains to be elucidated whether this inhibitory mechanism plays a significant role in controlling nociceptive transmission.

P2X receptor functions in pathological pain conditions

Pathological pain conditions are often seen in such disorders as arthritics, cancer, neuropathy, and traumatic injury. Two common causes of pathological pain conditions are tissue inflammation and nerve injury. The pathological pain conditions are major clinical problems because they are poorly managed with current pain medications. Cyclooxygenase 2 inhibitors such as Vioxx, Celebrex, and Bextra have previously been widely prescribed for patients with arthritic pain, but their cardiac side effects have raised a major health concern for the use of such pain medications. There is currently no highly effective medication for neuropathic pain sensations. As a result, the development of new pain medicines that are highly effective for inflammatory and/or neuropathic pain with little or acceptable side effects has become an important goal in pain research, and P2X receptor antagonists are candidates of new pain medicines to bring new hope for patients suffering from pathological pain.

Animal studies have indicated that P2X receptors are involved in inflammatory pain conditions and are potentially therapeutic targets for the treatment of these pain conditions in clinic. For example, the co-administration of ATP or α,β-methylene ATP with formalin, carrageenan, or complete Freund's adjuvant (CFA) into the rat hindpaw strongly enhanced nociceptive behaviors in rodents [69]. Using in vitro skin-nerve preparation, it was found that both ATP and α,β-methylene ATP specifically activated nociceptors at the peripheral terminals of Aδ and C-primary afferent fibers, and these agonists also elevated the ongoing activities in C-mechanoheat nociceptors after carrageenan-induced tissue inflammation [32]. In contrast to P2X receptor agonists, intrathecal administration of P2X receptor antagonists PPADS or TNP–ATP suppressed both the formalin- and capsaicin-induced nociceptive behaviors in mice [72]. The intrathecal administration of $P2X_3$ receptor antisense oligonucleotide similarly significantly decreased the nociceptive behaviors observed after the injection of CFA, formalin, or α,β-methylene ATP into the rat hindpaws [34]. Furthermore, the ablation of the $P2X_3$ gene, which resulted in the loss of rapidly desensitizing ATP-activated currents in DRG neurons [17, 70], caused a reduction in the formalin-induced pain behavior [17]. These results suggested that the activation of $P2X_3$ receptors

contributed to the expression of chronic inflammatory states and that relief of chronic inflammatory pain may be achieved by the selective blockade of $P2X_3$ receptor activation. A highly selective antagonist for $P2X_3$ and $P2X_{2/3}$ receptors, A-317491, has recently been developed. Consistent with previous observations on other $P2X_3$ receptor antagonists, both the intraplantar and intrathecal injections of A-317491 produced a dose-related antinociceptive effect in the chronic hyperalgesia induced by CFA [54].

The mechanisms by which P2X receptors are involved in inflammatory pain have been previously explored. P2X receptor upregulation has been suggested to be one mechanism contributing to inflammatory pain. For example, peripheral inflammation enhanced the expression of $P2X_2$ and $P2X_3$ receptors on DRG neurons, thus, resulting in large increases of ATP-activated currents [83]. This study also showed that the increase in ATP response could give rise to large depolarization to exceed the threshold of action potentials in inflamed DRG neurons. As a result, the upregulation of P2X receptors may contribute to sensory sensitization under inflammatory conditions. P2X receptor upregulation during inflammation appeared to be involved in nerve growth factors (NGF), which are known to have an elevated level during inflammation. The number of $P2X_3$-positive neurons in both cervical and lumbar DRGs was consistently found to significantly increase after the intrathecal administration of NGF or Glia cell line-derived neurotrophic factor (GDNF). In spinal cord sections where $P2X_3$ expression was restricted to primary afferent central terminals located in the inner II in control animals, NGF treatment induced expansion of $P2X_3$ expression with intense immunoreactivity on axons projecting to lamina I and to the ventro-medial afferent bundle beneath the central canal [66]. The abnormal expression of $P2X_3$ receptors on the primary afferent fibers innervating lamina I has raised an interesting question as to whether the $P2X_3$-mediated pathological pain conditions are associated with abnormal expression pattern after tissue inflammation. In addition to nerve growth factors, several inflammatory mediators have also been shown to directly modulate P2X receptors within a short time period. For example, the currents mediated by homomeric $P2X_3$ and heteromeric $P2X_{2/3}$ receptors were potentiated by substance P and bradykinin [62]. These effects were mimicked by phorbol ester and blocked by inhibitors of protein kinases. These results suggest that inflammatory mediators may directly sensitize nociceptors through phosphorylation of $P2X_3$ and $P2X_{2/3}$ receptors.

Similar to inflammatory pain, the roles of P2X receptors in neuropathic pain have been studied and the potential uses of P2X receptor antagonists in treating neuropathic pain have been explored in animal models. Several lines of evidence have indicated the association of $P2X_3$-containing receptors with neuropathic pain. For example, anatomical studies showed both an upregulation and a downregulation of $P2X_3$ receptors in DRG neurons after peripheral nerve injury, $P2X_3$ upregulation was found to be on spared intact DRG neurons while the downregulations were found on injured neurons after peripheral nerve injury [75]. In spinal nerve ligation in rats exhibiting neuropathic pain, there was a significant reduction in the number of small-sized DRG neurons that were responsive to α,β-methylene ATP [41]. On the other hand, $P2X_3$ immunoreactivity and $P2X_3$-like responses were detected in a subset of larger-sized DRG neurons after spinal nerve ligation. After spinal nerve ligation, some axotomized afferent neurons developed ongoing discharges (ectopic discharges) that originated in DRGs, and the ectopic discharges enhanced their activity after application of ATP or α,β-methylene ATP [86]. This ATP-induced enhancement of ectopic discharges was significantly blocked by P2X receptor antagonists. As a result, purinergic sensitivity developed in the DRG neurons after chronic axotomy of peripheral nerve, thus, suggesting that P2X receptors play a role in generating the nociceptive impulses after nerve injury. $P2X_3$ receptors have been used as therapeutic targets to treat neuropathic pain in experimental animals. The intrathecal administration of $P2X_3$ receptor antisense oligonucleotide reduced the mechanical allodynia induced by spinal nerve ligation [34]. Intrathecal delivery of A-317491 also attenuated the mechanical allodynia in both chronic constriction injury and spinal nerve ligation models of neuropathy [54].

Concluding remarks

P2X receptors on neuronal cells have displayed multiple sensory functions, from nociception to non-nociception, from acute pain to chronic pain, and from inflammatory pain to neuropathic pain. At the neuronal level, these physiological and pathological functions of P2X receptors rely on their roles in initiating sensory impulses at primary afferent peripheral nerve endings as well as in mediating and modulating sensory synaptic transmission in the spinal cord dorsal horn. $P2X_3$-containing receptors on neuronal cells have been clearly shown to play an important role in the pathological pain conditions and they are potential therapeutic targets for pain management. Other functional P2X receptor subtypes are also present on neuronal cells and they are involved in sensory signaling, transmission, and modulation. Future studies need to pinpoint the roles of these P2X receptors under physiological and pathological conditions. While P2X receptors on neuronal cells remain to be the main focus for exploring P2X receptor functions in pain, P2X receptors such as $P2X_7$ and $P2X_4$ subtypes located on non-neuronal cells have recently shed some new lights on the purinoreceptor pain mechanisms [16, 74]. As a result, the P2X receptor subtypes on both neuronal and non-neuronal cells may serve as targets for treating pain conditions associated with different disorders.

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