Review

Regulation of neuronal ion channels via P2Y receptors

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Abstract

Within the last 15 years, at least 8 different G protein-coupled P2Y receptors have been characterized. These mediate slow metabotropic effects of nucleotides in neurons as well as non-neural cells, as opposed to the fast ionotropic effects which are mediated by P2X receptors. One class of effector systems regulated by various G protein-coupled receptors are voltage-gated and ligand-gated ion channels. This review summarizes the current knowledge about the modulation of such neuronal ion channels via P2Y receptors. The regulated proteins include voltage-gated Ca^{2+} and K^+ channels, as well as *N*-methyl-D-aspartate, vanilloid, and P2X receptors, and the regulating entities include most of the known P2Y receptor subtypes. The functional consequences of the modulation of ion channels by nucleotides acting at pre- or postsynaptic P2Y receptors are changes in the strength of synaptic transmission. Accordingly, ATP and related nucleotides may act not only as fast transmitters (via P2X receptors) in the nervous system, but also as neuromodulators (via P2Y receptors). Hence, nucleotides are as universal transmitters as, for instance, acetylcholine, glutamate, or γ-aminobutyric acid.

Introduction

Many functional characteristics of neurons are similar or even identical to those of other cells, but there is one fundamental difference: The primary functions of neuronal cells are to receive, modify, and transmit messages. This flow of information occurs within one neuron as well as between neurons. Whether the neuronal communication is intra- or intercellular, it is always dependent on electrical activity which is provided by ligand- as well as voltage-gated ion channels. With respect to electrical properties, different neurons are by far not equal but rather characterized by prototypical synaptic responses that are governed by the expression of a certain set of ion channels. Even within one nerve cell, the electrical properties are not fixed, but may change with time depending on the presence of neurotransmitters, hormones, or growth factors. The basis for changes in the electrical responsiveness of a neuron are alterations in the opening and closure of ion channels. Such changes are in many cases triggered by neurotransmitters that act via heptahelical transmembrane receptors typically linked to heterotrimeric G proteins [1].

Adenine and uridine nucleotides are present in all types of cells and are released in response to various stimuli [2]. Once in the extracellular space, they activate one or more of at least 15 different membrane proteins which are categorized as P2 receptors. Alternatively, they are converted to other nucleotides or degraded down to nucleosides by different ectoenzymes [3]. The receptors for nucleosides are categorized as P1 receptors. Receptors for nucleotides comprise ionotropic P2X and metabotropic P2Y receptors. P2X receptors are ATP-gated cation channels composed of presumably three subunits out of a repertoire of at least seven different proteins which all have two presumed transmembrane domains with large extra-cellular loops and intracellular N- and C-termini [4, 5]. P2Y receptors, in contrast, are characterized by seven putative transmembrane domains typical of G protein-coupled receptors. Currently, at least eight different subtypes have been identified ($P2Y_{1, 2, 4, 6, 11, 12, 13, 14}$; [4, 6–8]. Thus, with respect to the modulation of ion channels, receptors for nucleotides may represent the regulated entity in the case of P2X receptors, and they may also be the regulating entity in the case of P2Y receptors. This review focuses on the interactions between P2Y receptors and both, ligand- as well as voltage-gated, ion channels and therefore deals with P2X receptors in only a very few instances.

Pharmacological and functional characteristics of P2Y receptors

A considerable number of heptahelical receptors that can be activated by extracellular nucleotides have been

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cloned from various species. According to the structural classification of P2 receptors they were categorized as P2Y receptors and numbered in chronologic order which led to the description of $P2Y_1$ through $P2Y_{15}$ receptors [9]. However, some of the receptors provided with P2Y receptor numbers were subsequently identified as species homologues of other P2Y receptors or as members of other families of G protein-coupled receptors [6]. The presumed $P2Y_{15}$ receptor is also activated by α-ketoglutarate [10] and may thus not be a true member of the P2Y family. Accordingly, only eight different mammalian subtypes are currently viewed as members of the P2Y family, namely $P2Y_{1, 2, 4, 6, 11, 12, 13, 14}$ [4, 6–8].

These P2Y receptors are activated not only by ATP, but also by other naturally occurring nucleotides or nucleotide sugars, such as ADP, UTP, UDP, and UDP glucose [4, 6–8]. At $P2Y_1$ receptors of various species, the rank order of agonist potency is 2-MeSADP ≥ 2-MeSATP > ADP ≥ ATP with uridine nucleotides being inactive. At $P2Y_2$ receptors, ATP and UTP are equipotent agonists, and ADP, UDP, or 2-methylthio derivatives have weak or no activity. $P2Y_4$ receptors are activated by UTP, and the rat and mouse receptors are also activated by ATP, whereas the human receptors are antagonized by ATP [11]. At $P2Y_6$ receptors, UDP is the most potent agonist and ADP, ATP or UTP are, if at all, only weak agonists. Human $P2Y_{11}$ receptors show the following rank order of agonist potency: ATP ≥ 2-MeSATP > ADP, and the observed agonistic activity of UTP depends on the signalling cascade that is activated by the receptor [12]. The nucleotide selectivity of canine $P2Y_{11}$ receptors is 2-MeSATP > ADP > ATP [13]. At $P2Y_{12}$ receptors, 2-MesADP is much more potent as an agonist than ADP, and the efficacy of ATP is species-dependent with high intrinsic activity at rat but not at human receptors [14, 15]. $P2Y_{13}$ receptors are activated by 2-MesADP, ADP, and ATP, but the rank order of agonist potency is different for human, murine, and rat receptors [16–18]. The $P2Y_{14}$ receptor is activated by various UDP sugars, but not by adenine or uridine nucleotides [8, 19].

According to the agonist profiles summarized above, P2Y receptors can be characterized as receptors for purines ($P2Y_{1, 11, 12, 13}$), for pyrimidines ($P2Y_{6, 14}$), or for both types of nucleotides ($P2Y_{2, 4}$). In parallel, these receptors can be classified as receptors preferring nucleoside tri- ($P2Y_{2, 4, 11}$) or diphosphates ($P2Y_{1, 6, 12, 13, 14}$). This latter categorization appears particularly important from a functional point of view, as extracellular nucleotides are released from a large variety of cells and rapidly converted by ectoenzymes [3]. The interconversion between different extracellular nucleotides is also a factor that needs to be taken into consideration, when P2Y receptors are characterized by the application of various nucleotides. For instance, the triphosphate sensitivity of $P2Y_2$ and $_4$ receptors can only be demonstrated unequivocally, if the conversion of nucleoside diphosphates to triphosphates is prevented [20]. Given these experimental difficulties with endogenous and exogenous agonistic nucleotides, the characterization of P2Y receptors should preferably rely on the use of specific antagonists. A considerable number

of compounds have been found to block P2Y receptors, but unfortunately the majority of antagonists is not sufficiently selective. For instance, the most widely used P2 receptor antagonists suramin and reactive blue 2 block not only a large number of P2X and P2Y receptors, but also unrelated receptors, such as NMDA receptors [21] and anion channels [22]. Nevertheless, for some P2Y receptor subtypes selective antagonists have been identified or developed. For instance, adenosine-2′-phosphate-5′-phosphate (A2P5P) and adenosine-3′-phosphate-5′-phosphate (A3P5P) were found to be partial agonists with low intrinsic activity at $P2Y_1$ receptors [23]. Derivatives thereof, such as N^6-methyldeoxyadenosine 3′,5′-biphosphate (MRS2179) or 2-chloro-N^6-methyldeoxyadenosine 3′,5′-biphosphate (MRS 2216) are selective and competitive antagonist at $P2Y_1$ receptors with nanomolar affinity [24]. The $P2Y_{12}$ receptor has been identified as the target of metabolites of the well-known antithrombotic drugs ticlopidine and clopidogrel [25]. ATP derivatives, such as AR-C69931MX, are also antagonists with high affinity at this receptor subtype and were developed for clinical use in patients with acute coronary syndromes [26]. Unfortunately, this latter agent is not absolutely selective for $P2Y_{12}$, but also blocks $P2Y_{13}$ receptors. However, at $P2Y_{12}$ this agent is a competitive, and at $P2Y_{13}$ a non-competitive, antagonist [17]. Recently, insurmountable antagonists with nanomolar affinities for $P2Y_6$ receptors, but no activity at $P2Y_{1,2,4}$ and $P2Y_{11}$ receptors, such as 1,4-di-[(3-isothiocyanato phenyl)-thioureido]butane (MRS 2578), have been developed [27].

$P2Y_{1, 2, 6, 13,}$ and $P2Y_{14}$ are widely expressed in a variety of tissues including the nervous system, whereas $P2Y_4$ and $_{11}$ show a restricted expression pattern that excludes neuronal tissues [4, 9, 19, 28]. $P2Y_1$ mRNA and protein is found at high levels in many regions of the central nervous system including the cerebral and cerebellar cortices, hippocampus, caudate nucleus, putamen, globus pallidus and midbrain [29–31]. In *in situ* hybridizations of brain sections, the expression pattern of $P2Y_{12}$ was consistent with a predominantly glial localization [25, 32]. $P2Y_{13}$ mRNA was also detected in different brain regions, such as the thalamus, caudate nucleus, substantia nigra, hippocampus and cerebellum [28].

In heterologous expression systems, all P2Y receptor subtypes were found to mediate increases in inositol phosphates (IPs) or in intracellular Ca^{2+}, thus indicating that they are coupled to phospholipase C (PLC) [4, 9, 28, 32]. The P2Y receptor-mediated increases in IPs did not involve pertussis toxin-sensitive G proteins in the cases of $P2Y_{1, 6}$ and $_{11}$, but were to various degrees reduced by that bacterial toxin in the cases of $P2Y_2$ and $P2Y_4$ [4]. With $P2Y_{12, 13}$ and $P2Y_{14}$ receptors, nucleotide-dependent increases in IPs were only detected when the receptors were co-expressed together with either $G\alpha_{16}$ or chimaeric G protein α subunits, and the actions mediated by these receptors were pertussis toxin-sensitive and thus mediated by inhibitory G proteins [19, 28]. $P2Y_{11}$ mediates increases in cyclic AMP in addition to the rises in IPs [9, 33], and the coupling of this receptor to different effector systems

displays different agonist sensitivities [12]. P2Y$_{12}$ and P2Y$_{13}$ mediate a pertussis toxin-sensitive inhibition of adenylyl cyclase [25, 28], and in pertussis toxin-treated cells, P2Y$_{13}$ mediates a stimulation of adenylyl cyclase [28].

Regulation of neuronal ion channels via G protein-coupled receptors

Ion channels and G protein-coupled receptors of neurons are encoded by several hundred genes, and the list is still growing. Accordingly, it is impossible to summarize all the interactions between them, and this chapter highlights only a few examples which are important for the understanding of mechanisms that may link P2Y receptors to various ion channels. The ion channels that will be described below include ligand-gated ion channels as well as voltage-gated Na$^+$, Ca^{2+} and K$^+$ channels.

Currently, at least nine different types of pore forming subunits of voltage-gated Na$^+$ channels are known and they contain several sites that may become phosphorylated by protein kinases A and C. On a functional level, phosphorylation by both kinases leads to reduced Na$^+$ currents [34]. In accordance with this, D$_1$-like receptors were found to reduce Na$^+$ currents in hippocampal neurons via protein kinase A [35], and this effect was enhanced at hyperpolarized membrane potentials and by co-activation of protein kinase C [36]. Moreover, activation of muscarinic receptors reduced Na$^+$ currents via protein kinase C in hippocampal [37] and neocortical neurons [38]. A similar effect was observed in neurons of the prefrontal cortex when 5-HT$_2$ receptors were activated [39]. In sensory neurons, however, hyperalgesic agents increase currents through tetrodotoxin-resistant Na$^+$ channels [40], and this effect was again mediated by protein kinase A [41]. Thus, several types of G protein-coupled receptors were found to modulate Na$^+$ channels and increases as well as decreases in currents were observed depending on the types of neurons that were investigated.

Voltage-activated Ca^{2+} channels are classified by the genes encoding the pore forming α$_1$ subunits, and one can discern between three subfamilies named Ca$_V$1 to Ca$_V$3. Members of all three families contribute to the voltage-gated Ca^{2+} currents found in neurons. Channels formed by Ca$_V$1 proteins mediate L-type currents, those containing, Ca$_V$2.1 to Ca$_V$2.3 mediate P/Q-, N- and R-type currents, and those harbouring Ca$_V$3 subunits mediate T-type Ca^{2+} currents [42]. While channels providing N- and P/Q-type currents are involved in excitation secretion coupling in most nerve terminals, the other channels are rather found at somatodendritic regions of neurons [42]. Modulation via G protein-coupled receptors has been described for an exhaustive number of neurotransmitters and for all types of voltage-gated Ca^{2+} currents [43–46]. The modulation of L-type currents is either facilitatory or inhibitory and is in most cases mediated by diffusible second messengers and protein kinases [45]. In contrast, the modulation of N and P/Q-type channels is almost exclusively inhibitory and is frequently independent of diffusible second messengers

and protein kinases [43, 44], although there are some exceptions to this rule [47, 48]. The pathway that excludes diffusible second messengers is membrane-delimited and leads to a voltage-dependent inhibition of currents as revealed by the fact that large depolarisations attenuate the inhibition. This inhibition is in most, but not all, cases abolished by pertussis toxin [43], and is based on a direct interaction between G protein βγ subunits and Ca^{2+} channel proteins [44, 46]. Pathways involving the synthesis of diffusible second messengers most commonly lead to a voltage-independent reduction of Ca^{2+} currents and have been reported to involve α subunits of the G$_{q/11}$ protein family [49, 50].

The superfamily of voltage-dependent K$^+$ channels comprises many more members than those of Na$^+$ or Ca^{2+} channels, and the K$^+$ channels are, in addition, very heterogeneous [51]. Although quite a number of these various K$^+$ channels were reported to be modulated by neurotransmitters, the most intensively studied examples of K$^+$ channel regulation via G protein-coupled receptors are inward rectifier (Kir) channels and KCNQ channels which are now classified as K$_V$7 family [51]. In a variety of neurons, several neurotransmitters were found to cause hyperpolarizations by activating inwardly rectifying K$^+$ currents via receptors coupled to pertussis toxin-sensitive G proteins. These effects were found to involve proteins of the Kir3 family and G protein βγ subunits [52]. However, the regulation of G protein-coupled inwardly rectifying K$^+$ (GIRK) channels depends not only on βγ subunits, but also on other proteins and second messengers. G Protein α subunits may act as donors for βγ, on one hand, and directly block GIRK channels [53], on the other hand. Furthermore, the kinetics of the gating of GIRKs is determined by all three parameters, receptor type, G protein α, and G protein βγ subunits [54]. In addition, regulators of G protein signalling also determine the kinetics of GIRK activation [55]. Moreover, GIRK channels are activated by phosphatidylinositol 4,5-bisphosphate (PIP$_2$) [56], and the levels of PIP$_2$ are also regulated via G protein-coupled receptors and PLC [57]. As a consequence, activation of receptors linked to G$_q$ proteins may also contribute to the regulation of GIRK channels [58]. Finally, activation of Gs coupled receptors was reported to enhance currents through GIRK channels [59].

A number of neurotransmitters were found to depolarize neurons by reducing M-type K$^+$ currents (I_M) which are believed to be mediated by KCNQ channels [60]. These ion channels are opened in the subthreshold voltage range for action potentials and become completely activated when neurons are further depolarized. Hence, activated KCNQ channels keep neurons polarized, and closure of these ion channels causes depolarization and increased action potential discharge [61, 62]. The inhibition of I_M via M$_1$ muscarinic acetylcholine receptors involves α$_q$ subunits of heterotrimeric GTP binding proteins [63], and a reduction in PIP$_2$ through an activation of phospholipase C [64]. The inhibition of I_M via bradykinin B$_2$ receptors, in contrast, involves G proteins of the G$_q$ family, most likely G$_{11}$, and rather α then βγ subunits [50, 63, 65]. Via these

G proteins, phospholipase C-β4 is stimulated [66] to mediate the synthesis of IP3, which then causes liberation of Ca^{2+} from intracellular stores [67]. Cytosolic Ca^{2+} concentrations in the sub- to low micromolar range directly block K_M channels [68].

The nicotinic acetylcholine receptor as a prototypical ligand-gated ion channel has been shown to be modulated via a variety of G protein-coupled receptors, and receptor activation was found to either enhance or reduce the function of that ion channel [69–73]. The inhibition of currents through nicotinic acetylcholine receptor was suggested to be mediated by an inhibition of proteinkinase A-dependent phosphorylation [73]. The enhancement of these currents involved pertussis toxin-sensitive G proteins, but not altered phosphorylation [72]. A direct interaction between G protein subunits and ligand-gated ion channels has been demonstrated for glycine receptors. Currents through these ion channels were enhanced by free G protein βγ subunits [74]. Like nicotinic acetylcholine receptors, glycine receptors were found to be inhibited by prostaglandin E2, and this effect involves proteinkinase A-mediated phosphorylation [75]. Another ligand-gated ion channel, the glutamatergic NMDA receptor, is also regulated by phosphorylation secondary to the activation of G protein-coupled receptors. In that case, the activation of muscarinic acetylcholine receptors enhanced NMDA receptor currents via protein kinase C and non-receptor tyrosine kinase [76]. However, D_2-like receptors reduce currents through NMDA receptors via transactivation of receptor tyrosine kinases [77]. In addition, D_1 receptors may also directly interact with NMDA receptors [78]. For the inhibitory transmitter GABA, a direct interaction of the ionotropic $GABA_A$ receptors and the metabotropic $GABA_B$ receptor has been reported to regulate receptor trafficking [79]. Hence, a multitude of mechanisms provide functional links between G protein-coupled receptors and various ligand-gated ion channels.

Regulation of Na$^+$ channels via P2Y receptors

ATP was found to enhance or inhibit tetrodotoxin-sensitive as well as -insensitive voltage-gated sodium channels in rat dorsal root ganglion neurons [80] and in a neuronal hippocampal cell line [81]. However, these effects did not appear to involve G proteins and were rather mediated by a direct interaction of ATP with the sodium channel. No additional evidence for a modulation of neuronal Na^+ channels via P2Y receptor could be found in the literature.

Regulation of Ca^{2+} channels via P2Y receptors

Native receptors

Since Dunlap and Fischbach [82, 83] first described transmitter-induced inhibition of voltage-gated calcium channels, a plethora of other neurotransmitters and hormones that exert their actions via G protein-coupled receptors have been shown to inhibit native Ca^{2+} currents (see above). First direct evidence for a nucleotide regulation of voltage-gated calcium channels was presented by Diverse-Pierluissi et al. [84], who showed that ATP and ADP, but not UTP, induce a pertussis toxin-sensitive inhibition of voltage-gated Ca^{2+} currents in bovine adrenal chromaffin cells. Subsequent studies by several other groups [85–87] corroborated these results, and further showed that this reduction of calcium current amplitudes was voltage-dependent, antagonized by reactive blue 2 and due to an inhibition of N- and P/Q-type calcium channels. Similar results were obtained in rat adrenal chromaffin cells, where ATP also inhibited voltage-gated Ca^{2+} currents in a PTX-sensitive manner and this effect was also antagonized by reactive blue 2 [88]. Another ATP-induced inhibition of Ca^{2+} currents, which was PTX-insensitive and voltage-dependent, was observed in frog sympathetic neurons [89]. In NG108-15 mouse neuroblastoma × rat glioma cells, not only adenine nucleotides, but also UTP and UDP, inhibited whole-cell Ca^{2+} currents [90], with the uridine nucleotides being even more potent agonists than the adenine nucleotides. In this case, not only an inhibition of N-type channels was responsible for the reduction of voltage-gated Ca^{2+} currents, but also L-type calcium channels were inhibited. Interestingly, only the N-type channel inhibition was PTX-sensitive, while currents through L-type channels were still modulated after PTX treatment. However, it remained unclear whether these two effects were mediated by two different receptor subtypes or by a single receptor that coupled to multiple G proteins.

More recently, several other examples for Ca^{2+} channel modulation via endogenous P2Y receptors were reported. Vartian and Boehm [91] found that adenosine nucleotides inhibited voltage-gated Ca^{2+} currents in PC12 cells via a voltage-dependent and PTX-sensitive mechanism, but at that time the pharmacological profile of the receptor involved did not correspond to any of the known P2Y receptors. However, in subsequent studies the receptor responsible for this inhibition of voltage-gated Ca^{2+} currents in PC12 cells could be identified as the $P2Y_{12}$ receptor [92, 93]. In hamster submandibular neurons, the $P2Y_2$ receptor was shown to mediate a voltage-dependent and PTX-sensitive inhibition of voltage-gated Ca^{2+} currents [94]. Moreover, $P2Y_1$ receptors in rat dorsal root ganglion neurons [95] and $P2Y_{12}$ receptors in rat sympathetic neurons [96] were both shown to mediate a voltage-dependent and pertussis toxin-sensitive inhibition of N-type calcium channels.

However, not only inhibitory effects of nucleotides on Ca^{2+} channels have been observed. ATP has also been shown to increase calcium currents in rat cardiac cells [97] and in hippocampal neurons [98]. Unfortunately, the signalling cascades involved in these effects have not been elucidated in detail.

Recombinant receptors

Based on their findings that UTP activates pertussis toxin-sensitive as well as -resistant pathways in NG108-15

neuronal hybrid cells [90, 99], Alexander Filippov, Eric Barnard, David Brown and collaborators extended their investigations on the coupling of P2Y receptors to neuronal ion channels by using rat superior cervical ganglion neurons as an expression system for molecularly defined receptor subtypes. All of the P2Y receptors that were investigated ($P2Y_{1, 2, 4, 6, 12}$) did couple to voltage-gated Ca^{2+} channels. As expected for a $G_{i/o}$ coupled receptor, the inhibition of voltage-gated Ca^{2+} currents by $P2Y_{12}$ was voltage-dependent and PTX-sensitive, and thus appeared to involve only the membrane-delimited pathway [14]. By contrast, the inhibition of voltage-gated Ca^{2+} currents mediated by $P2Y_{1, 2, 4}$ and $_6$ included two components: (1) the voltage-dependent and PTX-sensitive membrane-delimited pathway as well as a (2) PTX-resistant component. The $P2Y_2$ receptor-mediated inhibition included a voltage-dependent and PTX-sensitive component, on one hand, and a voltage-independent and PTX-resistant component, on the other hand [100]. By contrast, when $P2Y_6$ [101] or $P2Y_1$ [102, 103] were overexpressed, the pertussis toxin-resistant component appeared to be voltage-dependent, since it was facilitated by a large depolarizing prepulse. Interestingly, the authors found that the inhibition mediated by the $P2Y_6$ receptor is much more pronounced in perforated patch (73%) as compared to whole-cell (53%) recordings. Moreover, the inhibition observed in the perforated-patch configuration was hardly altered by PTX, while in whole-cell recordings it was reduced by 60% after treatment with this toxin. Similar results were obtained when $P2Y_4$ receptors were overexpressed [104]. In that case almost no inhibition ($\sim$10%) was observed in the disrupted whole-cell mode, while currents recorded in the perforated-patch mode were reduced by $\sim$45% in a PTX-sensitive and voltage-dependent manner. Taken together, these findings indicate that even the membrane-delimited, PTX-sensitive pathway may require a soluble co-factor, and that a modulation of voltage-gated Ca^{2+} currents should rather be investigated in the perforated-patch than the whole-cell mode of the patch clamp technique. Unfortunately, most of our current knowledge concerning the regulation of neuronal Ca^{2+} channels via P2Y and other G protein-coupled receptors stems from whole-cell experiments only.

Regulation of K$^+$ channels by P2Y receptors

Native receptors

Besides voltage-gated Ca^{2+} channels, a variety of K^+ channels have consistently been shown to be modulated by nucleotides. One of the first examples was the inhibition of I_M by UTP [105] and ATP [106] in bullfrog sympathetic neurons. Although the receptors responsible for these effects remained unknown at that time, the authors could subsequently show that I_M inhibition by nucleotides involved a G protein [107, 108]. Only a few years later, after the cloning of the first G protein-coupled nucleotide receptors, Filippov et al. [99] could demonstrate that

activation of the PLC-linked $P2Y_2$ receptor in NG108-15 neuroblastoma $\times$ glioma hybrid cells led to an inhibition of native I_M. Subsequently, several other groups could also observe an inhibition of I_M mediated by native P2Y receptors. In rat superior cervical ganglion neurons, a uridine nucleotide preferring receptor, most likely $P2Y_6$, was reported to mediate an inhibition of I_M [109]. The signalling cascade underlying this inhibition included activation of PLC, generation of IP_3, and release of Ca^{2+} from intracellular stores [110]. In rat thoracolumbar sympathetic neurons [111], UTP and UDP reduced I_M in cultures isolated from 9- to 12-day-old rats, but not in cultures prepared from newborn animals. More recently, Meng et al. [112] could show that the nucleotide induced inhibition of I_M in bullfrog sympathetic neurons is mediated by a P2Y receptor, most likely $P2Y_4$, as suggested by the observed rank order of agonist potencies.

In addition to I_M modulation, several other neuronal potassium channels were also found to be modulated by nucleotides. In a series of papers published in the mid nineties, Ikeuchi and Nishizaki described an outwardly rectifying potassium current activated by nucleotides. The current was found in various regions of the rat brain, including striatal neurons [113], inferior colliculus neurons [114], superior colliculus neurons [115], cerebellar neurons [116] and in hippocampal neurons [117]. Interestingly, although the current that was induced seemed to be the same in all these studies, the signal transduction mechanisms, as well as the receptor subtypes involved in channel activation seemed to differ between the various regions of the brain that were investigated. Responses that were induced by ATP in striatal neurons and by adenosine in superior colliculus and hippocampal neurons involved a diffusible second messenger and protein kinase C, as could be shown by single channel recordings from cell-attached patches and by using the protein kinase C inhibitor GF109203X, respectively. By contrast, the actions of ADP in inferior colliculus neurons were membrane delimited, presumably based on a direct interaction of βγ subunits of a PTX-insensitive G protein with the channel protein, as could be shown by single channel recordings from outside-out patches.

In rat hippocampal neurons, ATP was also reported to inhibit a voltage-gated K^+ channel [118]. UTP was as potent as ATP, and ADP and α,β-methylene ATP also inhibited the outward current. More recently, another voltage-gated potassium channel, a fast delayed inward rectifier, in *Xenopus* spinal neurons was found to be inhibited by adenine and uridine nucleotides [119]. Since a much larger proportion of the neurons responded to ADP, but not to ATP or UTP, the authors speculated about the presence of two different P2Y receptor subtypes, one mediating the effects of the triphosphates and the other mediating the effects of ADP.

Recombinant receptors

The potassium channels that have been studied most extensively with respect to their modulation by heterolo-

gously expressed P2Y receptors are the KCNQ channels and the GIRKs. Among the receptors that were investigated, the $G_{q/11}$ coupled receptors $P2Y_{1,2,4}$ and $P2Y_6$, when expressed in rat superior cervical ganglion neurons, all inhibited the I_M in a PTX-resistant manner [100, 101, 103, 104]. However, when the $P2Y_{12}$ receptor was expressed in these neurons, no inhibition of I_M by nucleotides could be observed [14]. When rat GIRK1 and GIRK2 (Kir3.1 and 3.2 subunits) were co-expressed, 2MeSADP and 2MeSATP, two potent $P2Y_{12}$ receptor agonists, evoked a K^+ current through GIRK channels. This is in accordance with the general concept that $G_{i/o}$ coupled receptors mediate an activation of GIRK channels. A more unexpected finding was that the $G_{q/11}$-linked $P2Y_1$ receptor, when co-expressed with Kir3.1 and 3.2 subunits instead of $P2Y_{12}$, also produced a PTX-sensitive activation of GIRK. This further supports the idea that a single P2Y receptor may couple to multiple G proteins. However, with $P2Y_1$, the fast activation was followed by a slower, but almost complete inactivation of the current in the continued presence of the agonist, an effect that has previously been observed when rat $P2Y_2$ receptors were expressed together with GIRK1/4 in *Xenopus* oocytes [120]. Similar results were also obtained with the mouse $P2Y_2$ receptor, but not with human $P2Y_6$, when expressed together with GIRK channels in *Xenopus* oocytes [121].

Regulation of ligand-gated ion channels by P2Y receptors

Native receptors

In neurons, nucleotides can act not only on voltage-gated, but also on ligand-gated ion channels. In layer V pyramidal neurons of the rat prefrontal cortex, adenine and uridine nucleotides, presumably acting on $P2Y_2$ receptors, were found to enhance currents elicited by *N*-methyl-D-aspartate (NMDA) [122]. By contrast, ADP-βS, a potent $P2Y_1$ receptor agonist, inhibited currents through NMDA receptors [123] in the same cells. Since this inhibition was antagonized by PPADS and MRS2179, an involvement of the $P2Y_1$ receptor seemed most likely. In addition, ATP was found to directly inhibit NMDA receptors independently of G proteins and, therefore, not by activation of P2Y receptors. This latter inhibition was revealed to involve a direct binding of the nucleotide to the glutamate-binding pocket of the NR2B subunit of NMDA receptors [124].

Another ligand-gated ion channel that was found to be modulated by nucleotides is the vanilloid receptor 1 (VR1). In rat dorsal root ganglion neurons, capsaicin-evoked currents through VR1 were enhanced by the application of nucleotides [125]. This nucleotide-induced potentiation was abolished by application of calphostin C, a potent protein kinase C inhibitor, and was mimicked by phorbol esters, thus suggesting that a P2Y receptor linked to protein kinase C via $G_{q/11}$ proteins was involved. Considering the observed rank order of agonist potencies, the $P2Y_1$ receptor

was considered to be the most likely candidate. However, more recently the same group could show that the ATP-induced potentiation can also be observed in dorsal root ganglion neurons of $P2Y_1$-deficient mice [126]. Moreover, in *in situ* hybridizations rat dorsal root ganglion neurons were found to co-express VR1 and $P2Y_2$ mRNA, but not $P2Y_1$ mRNA. UTP was reported to be as potent an agonist as ATP, and suramin (which blocks $P2Y_2$ but not $P2Y_4$) abolished the potentiation by UTP. Taken together, these results indicated that it was rather the $P2Y_2$ than the $P2Y_1$ receptor that mediated the effects of nucleotides on VR_1 in mouse and rat DRG neurons.

Recombinant receptors

In addition to the above effects obtained with native P2Y receptors in primary neuronal cell cultures, akin results were also obtained when P2Y receptors were heterologously expressed in *Xenopus* oocytes. When co-expressed together with the $P2X_1$ receptor, activation of both, $P2Y_1$ and $P2Y_2$, caused a significant potentiation of the α,β-MeATP-induced current through $P2X_1$ [127].

Functional consequences of neuronal ion channel regulation via P2Y receptors

The effects of P2Y receptor activation on neuronal ion channels that have been observed most consistently and frequently are (1) inhibition of voltage-activated Ca^{2+} currents, (2) activation of Ca^{2+}-dependent K^+ channels or GIRKs, and (3) inhibition of I_M. Voltage-gated Ca^{2+} channels, in particular N- and P/Q-type channels, are located at presynaptic nerve terminals and link invading action potentials to transmembrane Ca^{2+} influx and ensuing vesicle exocytosis. Accordingly, the modulation of these ion channels via G protein-coupled receptors will lead to changes in transmitter release [128]. The kinetics of presynaptic action potentials are shaped by voltage-activated and Ca^{2+}-dependent K^+ channels, and modulation of these channels via G protein-coupled receptors can also lead to changes in vesicle exocytosis [129, 130]. In contrast to these K^+ channels, inwardly rectifying K^+ channels including GIRKs are hardly found at presynaptic nerve terminals and are generally not believed to directly contribute to transmitter release [129, 131]. With respect to KCNQ channels, which mediate I_M, contrasting results have been obtained. In peripheral sympathetic neurons, no evidence could be obtained for a role of these ion channels in presynaptic transmitter release [132, 133]. However, in central synaptosomes, modulators of these ion channels were found to affect the release of various transmitters [134]. Hence, the regulation of Ca^{2+} channels and Ca^{2+}-dependent K^+ channels via P2Y receptors will preferentially lead to changes in synaptic transmission via a presynaptic modulation of transmitter release, whereas the control of GIRKs and KCNQ channels will rather cause changes in the postsynaptic excitability. The modulation of ligand-gated ion channels may lead to pre- as well as

postsynaptic effects depending on where the regulated ionotropic receptors are located [135]. In fact, ATP and other nucleotides acting at P2Y receptors may cause both, pre- and postsynaptic effects in the central as well as peripheral nervous system.

In the cortex and hippocampus, nucleotides were found to inhibit the release of acetylcholine and noradrenaline via presynaptic sites of action. With respect to the release of dopamine, serotonin and glutamate, inhibitory as well as facilitatory presynaptic effects have been described for nucleotides in a variety of brain regions. The release of the inhibitory transmitters GABA and glycine, however, is rather enhanced than reduced by nucleotides. The precise receptor subtypes involved in these presynaptic effects remained mostly unknown, and only the facilitatory effects were evidenced to be mediated by P2X receptors. Moreover, a clear-cut role of presynaptic Ca^{2+} or K^+ channels in the modulatory effects of nucleotides was not established, as reviewed by Cunha and Ribeiro [136]. In hippocampal neurons, a presynaptic P2Y receptor mediated an ATP-dependent inhibition of excitatory, but not of inhibitory, synaptic transmission via PTX-sensitive G proteins, and an inhibition of voltage-activated Ca^{2+} currents. However, the receptor subtype(s) involved were not further characterized [137]. In the medial habenula, a presynaptic $P2Y_4$-like receptor was shown to enhance glutamate release, whereas a presumed $P2Y_2$-like receptor mediated an inhibition [138]. Whether these presynaptic P2Y receptors affected transmitter release through a modulation of Ca^{2+} or K^+ channels remained unfortunately unknown. In the prefrontal and parietal cortex, activation of a $P2Y_1$-like receptor reduced glutamatergic transmission, but only the component involving NMDA receptors. Furthermore, the receptor mediated an inhibition of depolarizations caused by *N*-methyl-D-aspartate, but not of those induced by alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid [123]. Thus, P2Y receptor activation may also interfere with synaptic transmission via a modulation of postsynaptic ionotropic receptors.

In the periphery, in autonomic and motor neurons, release of acteylcholine or noradrenaline was also reported to be controlled by presynaptic P2X and P2Y receptors [136]. The system that has been studied in greatest detail is the sympathetic nervous system, since ATP and noradrenaline have long been known as cotransmitters there. Early evidence for presynaptic nucleotide receptors in sympathetic neurons has been obtained 20 years ago [139]. More recently, several nucleotides were found to reduce, whereas P2 receptor antagonists were found to enhance, noradrenaline release from the mouse vas deferens as indication of inhibitory presynaptic P2Y autoreceptors [140]. In numerous other sympathetically innervated tissues, nucleotides were also reported to inhibit transmitter release [141, 142]. For a long period of time, the P2Y receptor subtypes involved in these effects remained unknown, but recent evidence suggests that $P2Y_{12}$ and/or $P2Y_{13}$ receptors mediate autoinhibition of transmitter release from sympathetic neurons [143]. In

PC12 cells which are ontogenetically related to sympathetic neurons, $P2Y_{12}$ receptors mediate an inhibition of voltage-activated Ca^{2+} channels (see above and [91–93]). Most recently, this effect was also observed in primary cultures of rat superior cervical ganglion neurons and it was demonstrated to mediate autoinhibition of transmitter release [96]. Hence, sympatho-effector transmission is regulated by an inhibition of voltage-activated Ca^{2+} channels via presynaptic $P2Y_{12}$ receptors. In sensory neurons, in contrast, $P2Y_1$ receptors mediate an inhibition of voltage-activated Ca^{2+} channels [144], and this effect apparently leads to reduced synaptic transmission in pain pathways and to analgesic effects [95].

However, P2Y receptor activation mediates not only inhibitory, but also excitatory effects in sensory neurons. $P2Y_1$ receptor activation, in particular, was suggested to facilitate touch-induced impulse generation in sensory neurons [145], but the underlying mechanisms remained unknown. In this context, the interaction with VR1 receptors might be a relevant mechanism (see above). In autonomic neurons, $P2Y_1$ receptors were also involved in excitatory postsynaptic effects: Excitatory postsynaptic potentials that were blocked by a $P2Y_1$ receptor antagonist were observed in the enteric nervous system. Direct activation of these receptors caused a Na^+-dependent increase in membrane conductance, but the precise ionic mechanisms were not further investigated [146]. In postganglionic sympathetic neurons, P2Y receptors were also found to mediate excitatory postsynaptic effects. UTP depolarizes sympathetic ganglia and triggers transmitter release from dissociated sympathetic neurons via receptors different from those activated by ATP [147, 148]. The secretagogue action of UTP was mimicked equipotently by UDP [111, 148], and both nucleotides also caused an inhibition of I_M [109, 111]. The receptor involved was evidenced to be $P2Y_6$. However, the stimulatory effect did not only involve the inhibition of KCNQ channels, but also an activation of protein kinase C [149].

Conclusion

The modulation of neuronal ion channels via G protein-coupled receptors is a major means of adapting the responsiveness of a neuron to changes in environmental conditions [1]. Such modulatory effects have been described for virtually all known neurotransmitters and nucleotides are no exception. Within the last 15 years, 8 different G protein-coupled nucleotide receptors have been characterized, and numerous examples of ion channels that are regulated via one or more of these receptors are described here. Thus, aside of their functions as fast synaptic transmitters acting at P2X receptors [150], nucleotides also fulfil roles as neuromodulators, as do many other classical transmitter, such as glutamate, GABA, acetylcholine, and serotonin. Thus, this summary of ion channels regulated via P2Y receptors adds ATP and related nucleotides to this list of pluripotent neurotransmitters.

Acknowledgements

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