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COMMENTARY

P2Ys go neuronal: modulation of Ca^{2+} and K^+ channels by recombinant receptors

*,[1]Stefan Boehm

[1]Institute of Pharmacology, University of Vienna, Waehringerstrasse 13a, A-1090 Vienna, Austria

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Abbreviations: I_{Ca}, currents through N-type Ca^{2+} channels; SCG, superior cervical ganglion; I_{KM}, currents through M-type K^+ channels; IP_3 inositol trisphosphate; PLC, phospholipase C

The function of a neuron critically depends on the opening and closure of Ca^{2+} and K^+ channels. Voltage-activated Ca^{2+} channels mediate transmembrane Ca^{2+} entry in response to membrane depolarization and thereby contribute to transmitter release, whereas K^+ channels help to keep the membrane polarized and thus control neuronal excitability. Sympathetic neurons express N-type Ca^{2+} channels and M-type K^+ channels, which both are characterized by their propensity to be inhibited *via* G protein-coupled receptors. A plethora of transmitters (such as acetylcholine, adenosine, noradrenaline, prostaglandin E_2, somatostatin, substance P, vasoactive intestinal polypeptide) act *via* appropriate receptors to control the gating of one or both of these ion channels in rat superior cervical ganglion (SCG) neurons (Hille, 1994). Filippov *et al.* (2002) have now added adenine and uridine nucleotides to this growing list of neuromodulators.

Extracellular nucleotides exert their effects *via* ionotropic P2X and metabotropic P2Y receptors. At least seven different P2Y receptors have been identified in mammalian species ($P2Y_{1,2,4,6,11,12,13}$; Ralevic & Burnstock, 1998; Communi *et al.*, 2001; Hollopeter *et al.*, 2001; Zhang *et al.*, 2001). They are all characterized by a common structural profile with seven putative transmembrane domains typical of G protein-coupled receptors, but display considerable heterogeneity in amino acid sequences. $P2Y_{1,11,12}$ and $_{13}$ are activated by adenine nucleotides, whereas $P2Y_6$ is activated by uridine nucleotides. $P2Y_2$ and $_4$ receptors, in contrast, are sensitive to both adenine and uridine nucleotides. In heterologous expression systems, all P2Y receptor subtypes, with the exception of $P2Y_{12}$, couple to phospholipase C (PLC) and mediate increases in inositol trisphosphate (IP_3; Ralevic & Burnstock, 1998; Communi *et al.*, 2001). $P2Y_{12}$ and $P2Y_{13}$ mediate an inhibition of adenylyl cyclase (Hollopeter *et al.*, 2001; Communi *et al.*, 2001).

Transcripts for $P2Y_{1,2,6}$ and $_{13}$ are widely distributed in a variety of tissues including the nervous system. $P2Y_4$ and $_{11}$, in contrast show a restricted expression pattern that excludes neuronal tissues (Ralevic & Burnstock, 1998; Communi *et al.*, 2001). Finally, $P2Y_{12}$ was reported to be restricted to blood platelets and to the brain (Hollopeter *et al.*, 2001; Zhang *et al.*, 2001). Hence, most of the known P2Y receptors have been detected in neuronal tissues. Nevertheless, in contrast to P2X receptors which may mediate and/or modulate synaptic transmission (Khakh, 2001), functions of P2Y receptors in neurons remained largely unknown. By using rat SCG neurons as an expression system, Alexander Filippov, Eric Barnard, David Brown and collaborators (Filippov *et al.*, 2002; Simon *et al.*, 2002) demonstrate that all P2Y receptor subtypes (with the exception of $P2Y_{11}$ and $_{13}$, which have not yet been investigated) are capable of coupling to neuronal ion channels. Nevertheless, these receptors do not simply act in one common manner to mediate one typical neuronal effect of nucleotides. They rather employ the entire repertoire provided by SCG neurons to regulate currents through N-type Ca^{2+} channels (I_{Ca}) and/or M-type K^+ channels (I_{KM}).

Two major types of signalling pathways have been identified in the G protein-dependent modulation of these ion channels: (i) A membrane-delimited pathway mediates a voltage-dependent inhibition of I_{Ca}; this effect occurs only between receptors and channels localized at the same side (either inside or outside) of a cell-attached recording pipette (Figure 1) indicating that no diffusible second messenger is involved. This inhibition of I_{Ca} is in most cases abolished by pertussis toxin (Hille, 1994), and it is based on a direct interaction between G protein $\beta\gamma$ subunits and Ca^{2+} channels (Zamponi & Snutch, 1998). (ii) Pathways involving the synthesis of diffusible second messengers lead to a voltage-independent reduction of I_{Ca}, on one hand, and to an inhibition of I_{KM}, on the other hand (Hille, 1994; Figure 1). These effects involve α subunits of the $G_{q/11}$ protein family (Kammermeier *et al.*, 2000; Haley *et al.*, 2000), and the inhibition of I_{KM} was shown to be mediated by PLC (Suh & Hille, 2002) and IP_3-dependent increases in intracellular Ca^{2+} (Selyanko & Brown, 1996).

Amongst the P2Y receptors that have been investigated by Filippov *et al.* 2002; Simon *et al.* (2002), $P2Y_{12}$ couples to members of the $G_{i/o}$ protein family, whereas $P2Y_{1,2,4}$ and $_6$ are linked to $G_{q/11}$ proteins (Ralevic & Burnstock, 1998; Hollopeter *et al.*, 2001). In accordance with these preferences in G protein coupling, the $P2Y_{12}$ receptor inhibited only I_{Ca} (Simon *et al.*, 2002), whereas the other four P2Y receptors inhibited both I_{Ca} and I_{KM}. The inhibition of I_{Ca} by $P2Y_{12}$ was voltage-dependent and abolished by pertussis toxin and

*Author for correspondence; E-mail: Stefan.Boehm@univie.ac.at

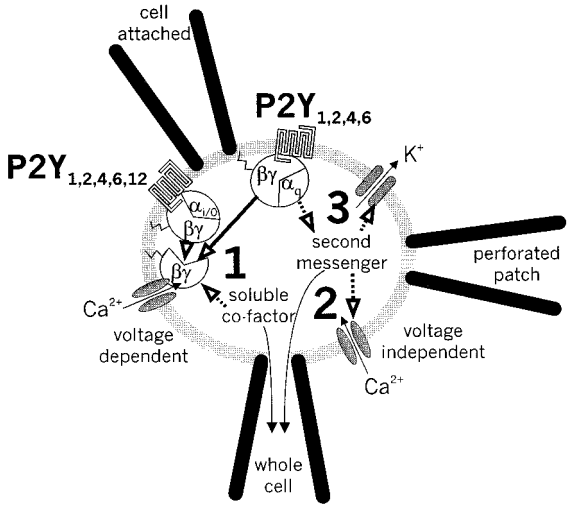

Figure 1 Mechanisms underlying the regulation of ion channels by P2Y receptors heterologously expressed in sympathetic neurons under different patch-clamp conditions. P2Y nucleotide receptors may use at least three different pathways to modulate the functions of N-type Ca^{2+} and/or M-type K^+ channels in rat superior cervical ganglion neurons (Filippov *et al*., 2002). (1) When P2Y receptors linked to heterotrimeric pertussis toxin-sensitive $G_{i/o}$ proteins are activated, $\beta\gamma$ subunits are liberated and directly interact with pore-forming Ca^{2+} channel proteins. This pathway does not cross the barrier provided by a cell-attached recording pipette and therefore does not include a diffusible second messenger (Hille, 1994). However, Filippov *et al*. (2002) now demonstrate that this mechanism may also require a soluble co-factor in addition to the membrane-associated G proteins which is lost during whole cell recordings. The inhibition of Ca^{2+} currents *via* this pathway is attenuated by large depolarizations and is thus voltage-dependent. P2Y receptors may also mediate such a voltage dependent inhibition of Ca^{2+} currents via pertussis toxin-insensitive G proteins (e.g. Filippov *et al*., 1999) (2) Activation of P2Y receptors linked to pertussis toxin-insensitive $G_{q/11}$ proteins leads to the synthesis of diffusible second messengers which mediate a voltage-independent inhibition of Ca^{2+} currents. Thus, this mechanism appears most pronounced when determined in the perforated-patch mode (Filippov *et al*., 1999). (3) In addition, P2Y receptors linked to pertussis toxin-insensitive $G_{q/11}$ proteins mediate an inhibition of M-type K^+ currents. This effect also involves diffusible second messenger systems and has therefore been determined in the perforated-patch configuration (Filippov *et al*., 2002).

thus appeared to involve only the membrane-delimited pathway. In contrast, the inhibition of I_{Ca} by $P2Y_{1,2,4,}$ and $_6$ included the voltage-dependent and pertussis toxin-sensitive membrane-delimited pathway as well as voltage-independent and/or pertussis toxin-insensitive components (Figure 1). The second messengers involved in these latter effects were not investigated. However, the inhibition of I_{KM} by P2Y receptors endogenously expressed in SCG neurons is mediated by PLC and IP_3-dependent increases in intracellular Ca^{2+} (Bofill-Cardona *et al*., 2000). Hence, it appears reasonable to assume that the same signalling cascade is also used by heterologously expressed P2Y receptors.

In addition to assigning distinct neuronal signalling pathways to certain P2Y receptor subtypes, the most recent report by Filippov *et al*. (2002) reveals a novel component involved in the membrane-delimited regulation of neuronal Ca^{2+} channels: the pertussis toxin-sensitive and voltage-dependent inhibition of I_{Ca} *via* $P2Y_4$ receptors was almost lost in whole-cell patch-clamp recordings, but maintained in perforated-patch recordings. In the whole-cell configuration, soluble constituents of the cytosol are washed out *via* the recording pipette, which does not happen during perforated-patch recordings. Therefore, these findings show that even the membrane-delimited, pertussis toxin-sensitive pathway may require some soluble co-factor in addition to the membrane-associated G proteins (Figure 1). Beforehand, Filippov *et al*. (1999) had found that the $P2Y_6$ receptor-mediated inhibition of I_{Ca} was more pronounced in perforated-patch (73%) as compared to whole-cell (53%) recordings. In that case, however, the effect observed in the perforated-patch configuration was hardly altered by pertussis toxin. Taken together, these results indicate that the receptor-dependent modulation of I_{Ca} should be investigated in the perforated-patch rather than the whole-cell variant of the patch-clamp technique to avoid a loss of certain signal transduction components. Unfortunately, most of our current knowledge concerning the regulation of neuronal Ca^{2+} channels *via* G proteins stems from whole-cell experiments.

In whole sympathetic ganglia, the inhibition of M-type K^+ channels is involved in slow ganglionic excitation and leads to depolarization and increased action potential discharge, whereas the blockade of N-type Ca^{2+} channels underlies the presynaptic inhibition of sympathetic transmitter release *via* auto-and heteroreceptors. Feedback inhibition of transmitter release from sympathetic neurons *via* nucleotide receptors is a well established phenomenon, and preliminary evidence suggests that this effect is mediated by a $P2Y_{12}$-like receptor-dependent inhibition of neuronal Ca^{2+} channels (Kubista *et al*., 2002; Boehm & Kubista, 2002). Thus, depending on the subcellular distribution of P2Y receptors in neurons, they may be involved in synaptic transmission (when present at the somatodendritic region) and in the presynaptic regulation of transmitter release (when present at axon terminals). ATP is stored and released together with classical neurotransmitters at many synapses. The results provided by Filippov *et al*. (2002) demonstrate that the nucleotide may choose not only from a large family of ionotropic and metabotropic receptors, but also from a variety of associated, presynaptic and postsynaptic, excitatory and inhibitory signalling cascades, respectively. Thus, ATP may be as versatile a neurotransmitter as, for instance, acetylcholine or glutamate. It will be interesting to discover which synapses in the peripheral and central nervous system are equipped with particular subtypes of endogenous P2Y receptors.

This commentary is dedicated to Alexander Selyanko who worked side-by-side with Alexander Filippov and David Brown to decisively contribute to the current knowledge about M-type K^+ channels and who died untimely at the age of 48. Work in the author's laboratory is supported by grants from the Austrian Science Fund (FWF; P13920, P14951) and from the Jubiläumsfonds of the Österreichische Nationalbank (8377).

References

BOEHM, S., & KUBISTA, H. (2002). Fine tuning of sympathetic transmitter release via ionotropic and metabotropic presynaptic receptors. *Pharmacol. Rev.,* **54,** 43 – 99.

BOFILL-CARDONA, E., VARTIAN, N., NANOFF, C., FREISSMUTH, M. & BOEHM, S. (2000). Two different signaling mechanisms involved in the excitation of rat sympathetic neurons by uridine nucleotides. *Mol. Pharmacol.,* **57,** 1165 – 1172.

COMMUNI, D., GONZALES, N.S., DETHEUX, M., BREZILLON, S., LANNOY, V., PARMENTIER, M. & BOEYNAEMS, J.M. (2001). Identification of a novel human ADP receptor coupled to Gi. *J. Biol. Chem.,* **276,** 41479 – 41485.

FILIPPOV, A.K., SIMON, J.A., BARNARD, E.A. & BROWN, D.A. (2002). Coupling of the nucleotide P2Y4 receptor to neuronal ion channels. *Br. J. Pharmacol.,* in press.

FILIPPOV, A.K., WEBB, T.E., BARNARD, E.A. & BROWN, D.A. (1999). Dual coupling of heterologously-expressed rat $P2Y_6$ nucleotide receptors to N-type Ca^{2+} and M-type K^+ currents in rat sympathetic neurons. *Br. J. Pharmacol.,* **126,** 1009 – 1017.

HALEY, J.E., DELMAS, P., OFFERMANNS, S., ABOGADIE, F.C., SIMON, M.I., BUCKLEY, N.J. & BROWN, D.A. (2000). Muscarinic inhibition of calcium current and M current in Galpha q-deficient mice. *J. Neurosci.,* **20,** 3973 – 3979.

HILLE, B. (1994). Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci.,* **17,** 531 – 536.

HOLLOPETER, G., JANTZEN, H.M., VINCENT, D., ENGLAND, L., RAMAKRISHNAN, V., YANG, R.B., NURDEN, A., JULIUS, D. & CONLEY, P.B. (2001). Identification of the plateleted ADP receptor targeted by antithrombotic drugs. *Nature,* **409,** 202 – 207.

KAMMERMEIER, P.J., RUIZ-VELASCO, V. & IKEDA, S.R. (2000). A voltage-independent calcium current inhibitory pathway activated by muscarinic agonists in rat sympathetic neurons requires both $G\alpha_{q/11}$ and $G\beta\gamma$. *J. Neurosci.,* **20,** 5623 – 5629.

KHAKH, B.S. (2001). Molecular physiology of P2X receptors and ATP signalling at synapses. *Nat. Rev. Neurosci.,* **2,** 165 – 174.

KUBISTA, H., LECHNER, S.G., WOLF, A.M. & BOEHM, S. (2002). Attenuation of the P2Y-receptor mediated control of neuronal Ca^{2+} channels in PC12 cells by antithrombotic drugs. *Br. J. Pharmacol.,* in press.

RALEVIC, V. & BURNSTOCK, G., (1998). Receptors for purines and pyrimidines. *Pharmacol. Rev.,* **50,** 413 – 492.

SELYANKO, A.A. & BROWN, D.A. (1996). Intracellular calcium directly inhibits potassium M channels in excised membrane patches from rat sympathetic neurons. *Neuron,* **16,** 151 – 162.

SIMON, J., FILIPPOV, A.K., GORANSSON, S., WONG, Y.H., FRELIN, C., MICHEL, A.D., BROWN, D.A. & BARNARD, E.A. (2002). Characterization and channel coupling of the $P2Y_{12}$ nucleotide receptor of brain capillary endothelial cells. *J. Biol. Chem.,* **277,** 31390 – 31400.

SUH, B.C. & HILLE, B. (2002). Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-biphosphate synthesis. *Neuron.,* **35,** 507 – 520.

ZAMPONI, G.W. & SNUTCH, T.P. (1998). Modulation of voltage-dependent calcium channels by G proteins. *Curr. Opin. Neurobiol.,* **8,** 351 – 356.

ZHANG, F.L., LUO, L., GUSTAFSON, E., LACHOWICZ, J., SMITH, M., QIAO, X., LIU, Y.H., CHEN, G., PRAMANIK, B., LAZ, T.M., PALMER, K., BAYNE, M. & MONSMA JR, F.J. (2001). ADP is the cognate ligand for the orphan G protein-coupled receptor SP1999. *J. Biol. Chem.,* **276,** 8608 – 8615.

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