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COMMENTARY

Novel $P2X_7$ receptor antagonists ease the pain

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In recent months, a series of chemically diverse antagonists has been identified for the ATP-gated $P2X_7$ receptor. In particular, two classes of highly-selective competitive $P2X_7$ antagonists have been developed by Michael Jarvis and his colleagues at Abbott Laboratories. These di-substituted *tetrazole* and *cyanoguanidine* derivatives are outstanding for a number of reasons (not least their stability, selectivity, potency and, of course, reversibility); most exciting is their near equal potency at human and rodent $P2X_7$ isoforms. Armed with drugs such as A740003 and newer A438079, Jarvis and colleagues have explored the role of $P2X_7$ receptors in the onset and persistence of chronic pain in animal models. Their findings - and applicability to the human condition - are reviewed in this current issue of *British Journal of Pharmacology*. This accompanying Commentary describes the progress made by Jarvis and others in developing novel $P2X_7$ antagonists for pain relief.
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Abbreviations: A317491, 5-({(3-phenoxybenzyl)((1*S*)-1,2,3,4-tetrahydro-1-naphthalenyl)amino}carbonyl)-1,2,4-benzenetricarboxylic acid; A438079, 3-(5-(2,3-dichlorophenyl)-1*H*-tetrazol-1-yl) methyl pyridine; A740003, *N*-(1-{((cyanoimino)(5-quinolinylamino)methyl)amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide

In equal measures, the $P2X_7$ receptor is viewed as the darling and devil of the adenosine triphosphate (ATP) receptor family. Scientists have been beguiled by the molecular complexity of this unique ATP-gated ion channel, including its rapid conversion from an ion-channel (8–11 Å diameter) to an enlarged pore (30–50 Å diameter), accompanied by the influx of permeants of large molecular weight (such as ethidium, propidium and Yo-Pro-1 ions) (see Egan *et al.*, 2006), its ability to initiate morphological changes such as cell swelling and membrane is blebbing (see North, 2002) and its downstream activation of intracellular caspases and maturation of releasable interleukin-1β (IL-1β) (see Ferrari *et al.*, 2006). Scientists have also been frustrated by the lack of pharmacological tools to control these functional properties and, hence, have been frustrated by an inability to study the role of $P2X_7$ receptors in inflammation and pain in a systematic way.

Since its isolation, the $P2X_7$ receptor has been considered to be the molecular embodiment of a cell-permeabilizing ATP receptor found first by UCL scientists in rat mast cells (Cockcroft and Gomperts, 1979). This ATP receptor was later classified as the P2Z receptor and was also identified as present in immune cells (Gordon, 1986). A gradual adjustment in thinking has led to the realization that at least two separate pathways (and accessory pores) can be linked molecularly to the activation of $P2X_7$ receptors (Pelegrin and Surprenant, 2007). The P2Z receptor appears to be much more than a simple homomeric $P2X_7$ receptor. This emerging viewpoint may explain why the permeant size pass for P2Z receptors differs between macrophages (<900 Da) and lymphocytes (<400 Da). The precise molecular nature of P2Z receptors still requires further evaluation, but the field is now much closer to resolving this question.

Early studies of the ATP^{4-}-activated P2Z receptor showed that excess Mg^{2+} could reseal the membrane of rat mast cells (Di Virgilio and Gomperts, 1983). Mg^{2+}-dependent reduction in permeability was also reported for murine macrophage P2Z receptors expressed in oocytes (Nuttle and Dubyak, 1994), human lymphocyte P2Z receptors expressed in oocytes (King and Wiley, unpublished data) and rat $P2X_7$ receptors expressed in HEK293 cells (Virginio *et al.*, 1997). Excess Mg^{2+} is not an effective tool for studies *in vivo* and also has limited applicability to studies *in vitro*. However, it was the first available tool, and its insurmountable actions were indicative of more than a reduction of extracellular levels of free ATP^{4-} ions. Others have shown that inorganic cations (Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}, for example) can inhibit $P2X_7$ receptors, but only some of these exert an insurmountable antagonism (see Virginio *et al.*, 1997). The most striking finding in recent months is the discovery of an oxide of the metal vanadium (decavanadate $H_2V_{10}O_{28}^{4-}$) as a competitive antagonist at human, rat and mouse $P2X_7$ isoforms (Michel *et al.*, 2006). The complex method of preparing decavanadate solutions and its potency at $P2X_2$ receptors may detract from its future and frequent use *in vivo*.

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Turning to drugs other than modulatory inorganic ions, amiloride and its analogues were identified as antagonists of P2Z receptors (Wiley *et al*., 1990). However, amiloride derivatives are unsuitable for all studies except those on isolated immune cells, because of their high affinity for non-purinergic ion channels such as the epithelial sodium channel ENaC. Attention then turned to exploiting very low-efficacy ATP derivatives as weak antagonists, and those tested on P2Z receptors on rat mast cells revealed a range of weak partial agonists and one (2-MeSATP) with overt inhibitory activity (Tatham *et al*., 1988). Similar studies revealed that oxidized ATP (oATP) also possessed inhibitory activity at P2Z receptors (Murgia *et al*., 1993). However, 2-MeSATP and oATP ultimately showed little promise in the study of $P2X_7$ receptors *in vivo*, mainly due to their lack of selectivity. The same can also be said of general P2 receptor antagonists – such as suramin, Reactive Blue 2 and PPADS – that, in various studies, have been shown to exert inhibitory activity at P2Z receptors and recombinant $P2X_7$ receptors. Incidentally, Jarvis and colleagues have extensively studied the inhibitory actions of a wide range of established P2 receptor antagonists at human and rodent $P2X_7$ isoforms and this work was recently presented in poster form at a Society for Neuroscience meeting (Namovic *et al*., 2006).

Strong interest was expressed in Ca^{2+}/calmodulin-dependent protein kinase II (CamKII) inhibitors, including calmodazolium and KN62, as potent inhibitors of $P2X_7$ receptors. Here, the first inklings surfaced for species- and use-dependent blocking activities for these inhibitors – with overt differences at human, rat and mouse $P2X_7$ isoforms. The molecular basis for such differences has yet to be resolved, and a recent review of the pharmacology of these compounds appears elsewhere (Gever *et al*., 2006). Similar problems surfaced for the polysulphonic dye, Coomassie Brilliant Blue G (BBG), which showed some selectivity for the rat $P2X_7$ receptor (Jiang *et al*., 2000). However, the lowered potency of BBG at the human $P2X_7$ receptor and similar potency at $P2X_2$ and $P2X_4$ receptor subtypes have limited its promise for use *in vivo*.

The involvement of Jarvis and colleagues in the search for selective $P2X_7$ receptors came at a relatively late stage. This group (and others based in pharmaceutical companies) had concentrated foremost on a role for native $P2X_3$ and $P2X_{2/3}$ receptors in the sensitization of spinal sensory neurons in experimental models of persistent pain. This work led to the discovery of 5-({(3-phenoxybenzyl)((1*S*)-1,2,3,4-tetrahydro-1-naphthalenyl)amino}carbonyl)-1,2,4-benzenetricarboxylic acid (A317491), a structurally novel and highly potent antagonist for $P2X_3$ and $P2X_{2/3}$ receptors (Jarvis *et al*., 2002). This antagonist was outstanding in terms of its novel chemistry, as it is structurally unrelated to any of the known P2 receptor antagonists, and also stood out in terms of its high potency and selectivity. Having cut their teeth on identifying and producing novel $P2X_3$ receptor antagonists, it was a natural step for Jarvis and colleagues to search for antagonists of other P2X receptor subtypes, including the hardest target, $P2X_7$.

A series of reports has commented on the presence of $P2X_7$-like immunoreactivity in nerve endings in central nuclei for second-order sensory neurons. There have been some problems with these findings, since positive immunoreactivity (albeit at reduced levels) still persists in gene-deleted $P2X_7^{-/-}$ mice (see Anderson and Nedergaard, 2006). However, the presence of $P2X_7$ in microglia is widely acknowledged (as is the presence of microglia close to sensory neurons and axons), and $P2X_7$ receptor activation, in turn, does release IL-1β from microglia to initiate inflammation processes (Chakfe *et al*., 2002). In fact, functional $P2X_7$ receptors and COX-2 production are significantly upregulated in a number of human neurological disorders, including multiple sclerosis (see Yiangou *et al*., 2006). In this vein, Jarvis and colleagues identified $P2X_7$ receptors in non-neuronal cells in rat spinal ganglia (Zhang *et al*., 2005). With their chemistry, screening procedures and functional assays already fine-tuned, Jarvis and colleagues needed only a short period of time to produce the highly potent $P2X_7$ antagonist *N*-(1-{((cyanoimino)(5-quinolinylamino)methyl)amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide (A740003) (see Honore *et al*., 2006), which was followed thereafter by 3-((5-(2,3-dichlorophenyl)-1*H*-tetrazol-1-yl) methyl pyridine (A438079) (Nelson *et al*., 2006).

In the accompanying review by Donnelly-Roberts and Jarvis (2007), the authors describe the pharmacological properties of their lead $P2X_7$ receptor antagonists and their utility in humans as potent analgesics of chronic pain. They provide a clear and balanced account of $P2X_7$ receptor pharmacology and also provide an unbiased comparison of their drugs with other lead $P2X_7$ receptor antagonists from industry rivals. The work summarized in this review represents a major step towards developing a new generation of analgesics for the relief of chronic pain. This review is not only timely, but also marks out a field that should be followed closely in the next few years.

References

Anderson CM, Nedergaard M (2006). Emerging challenges of assigning $P2X_7$ receptor function and immunoreactivity in neurons. *Trends Neurosci* **29**: 257–262.

Chakfe Y, Sequin R, Antel JP, Morissette CP, Malo D, Henderson D *et al*. (2002). ADP and AMP induce interleukin-1β release from microglial cells through activation of ATP-primed $P2X_7$ receptor channels. *J Neurosci* **22**: 3061–3069.

Cockcroft S, Gomperts BD (1979). ATP induces nucleotide permeability in rat mast cells. *Nature* **279**: 541–542.

Di Virgilio F, Gomperts BD (1983). Cytosol Mg^{2+} modulates Ca^{2+} ionophore induced secretion from rabbit neutrophils. *FEBS Lett* **163**: 315–318.

Donnelly-Roberts DL, Jarvis MF (2007). Discovery of $P2X_7$ receptor selective antagonists offers new insights into $P2X_7$ receptor function and indicates a role in chronic pain states. *Br J Pharmacol* **151**: 571–580 (this issue).

Egan TM, Samways DS, Li Z (2006). Biophysics of P2X receptors. *Pflügers Arch* **452**: 501–512.

Ferrari D, Pizzirani C, Adinolfi E, Lemolo RM, Curti A, Idzko M *et al*. (2006). The $P2X_7$ receptor: a key player in IL-1 processing and release. *J Immunol* **176**: 3877–3883.

Gever J, Cockayne DA, Dillon MP, Burnstock G, Ford AP (2006). Pharmacology of P2X channels. *Pflügers Arch* **452**: 513–537.

Gordon JL (1986). Extracellular ATP: effects, sources and fate. *Biochem J* **233**: 309–319.

Honore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, Mikusa JP *et al*. (2006). A-740003 [*N*-(1-{[(cyanoimino)(5-quinoli-

nylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective $P2X_7$ receptor antagonist, dose-dependently reduces neuropathic pain in the rat. *J Pharmacol Exp Ther* **319**: 1376–1385.

Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, Brennan TJ *et al*. (2002). A-317491, a novel potent and selective non-nucleotide antagonist of $P2X_3$ and $P2X_{2/3}$ receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc Natl Acad Sci USA* **99**: 17179–17184.

Jiang HL, Mackenzie AB, North RA, Surprenant A (2000). Brilliant blue G selectively blocks ATP-gated rat $P2X_7$ receptors. *Mol Pharmacol* **58**: 82–88.

Murgia M, Hanau S, Pizzo P, Rippa M, Di Virgilio F (1993). Oxidized ATP: an irreversible inhibitor of the macrophage purinergic P2Z receptor. *J Biol Chem* **268**: 8199–8203.

Michel AD, Xing M, Thompson KM, Jones CA, Humphrey PP (2006). Decavanadate, a P2X receptor antagonist, and its use to study ligand interactions with $P2X_7$ receptors. *Eur J Pharmacol* **534**: 19–29.

Namovic MT, Han P, Jarvis MF, Donnelly-Roberts DL (2006). C-terminus P451L mutation does not alter the function of mouse recombinant $P2X_7$ receptors: a pharmacological comparison of mouse, rat, and human $P2X_7$ receptors. *Soc Neurosci Abst* **958**: 4.

Nelson DW, Gregg RJ, Kort ME, Perez-Medrano A, Voight EA, Wang Y *et al*. (2006). Structure-activity relationship studies on a series of novel, substituted 1-benzyl-5-phenyltetrazole $P2X_7$ antagonists. *J Med Chem* **49**: 3659–3666.

North RA (2002). Molecular physiology of P2X receptors. *Physiol Rev* **82**: 1013–1067.

Nuttle LC, Dubyak GR (1994). Differential activation of cation channels and non-selective pores by macrophage P2z purinergic receptors expressed in *Xenopus* oocytes. *J Biol Chem* **269**: 13988–13996.

Pelegrin P, Surprenant A (2007). Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. *J Biol Chem* **282**: 2386–2394.

Tatham PE, Cusack NJ, Gomperts BD (1988). Characterisation of the ATP^{4-} receptor that mediates permeabilisation of rat mast cells. *Eur J Pharmacol* **147**: 13–21.

Virginio C, Church D, North RA, Surprenant A (1997). Effects of divalent cations, protons and calmidazolium at the rat P2X7 receptor. *Neuropharmacol* **36**: 1285–1294.

Wiley JS, Jamieson JP, Mayger W, Cragoe Jr EJ, Jopson M (1990). Extracellular ATP stimulates an amiloride-sensitive sodium influx in human lymphocytes. *Arch Biochem Biophys* **280**: 263–268.

Yiangou Y, Facer P, Durrenberger P, Chessell IP, Naylor A, Bountra C *et al*. (2006). COX-2, CB_2 and $P2X_7$-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurol* **6**: 12 (1–14).

Zhang XF, Han P, Faltynek CR, Jarvis MF, Shieh CC (2005). Functional expression of $P2X_7$ receptors in non-neuronal cells of rat dorsal root ganglia. *Brain Res* **1052**: 63–70.