British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01325.x
www.brjpharmacol.org

COMMENTARY

Many ways to dilate the P2X7 receptor pore

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Keywords
extracellular ATP; purinergic receptors; dye uptake; inflammation; colchicine; pannexin-1; interleukin-1; inflammasome

Received
18 January 2011
Revised
8 February 2011
Accepted
9 February 2011

The P2X7 receptor is associated with two different membrane permeabilities: a small cation conductance which opens within milliseconds, followed by the appearance of a second channel carrying higher molecular weight compounds (including organic dyes) after prolonged agonist stimulation. This activation profile has also been found in cells expressing P2X2 and P2X4 receptors; however, the P2X7 receptor-dependent pathway has the unique ability to activate pro-inflammatory signalling in macrophages. In this issue of the *BJP*, Marques-da-Silva *et al.* demonstrate that colchicine is a potent inhibitor of both P2X7 and P2X2 receptor-dependent dye uptake, without affecting the ion channels. Colchicine also blocked the pro-inflammatory signalling downstream of P2X7 receptor activation, both *in vitro* and *in vivo*. This report suggests that the dye uptake associated with activation of P2X7 receptors is distinct from the P2X7 receptor ion channel and could be a therapeutic target for the treatment of chronic inflammation.

LINKED ARTICLE
This article is a commentary on Marques-da-Silva *et al.*, pp. 912–926 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2011.01254.x

Abbreviations
LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NMDG, N-methyl-D-glucamine; NMM, non-muscle myosin; ROS, reactive oxygen species

Activation of the purinergic P2X7 receptor (nomenclature follows Alexander *et al.* 2009) induces effects rather unusual among ion channels. Brief agonist stimulation induces a non-selective, cation-carrying pore, permitting the permeation of monovalent and divalent cations and leading to depolarization of the plasma membrane (North, 2002), while prolonged and repetitive agonist application promotes increased membrane permeability to larger cations such as N-methyl-D-glucamine (NMDG) [molecular weight (MW) 195]. Membrane permeability increases with time allowing cellular uptake of higher MW fluorescent dyes such as Yo-Pro-1 (MW 629) or ethidium bromide (MW 394). Classically this phenomenon has been attributed to the formation of large cytolytic pores in the plasma membrane leading to cell death (Virginio *et al.*, 1999a; Pelegrin and Surprenant, 2009). There is further evidence that the dilatation of the pore in the plasma membrane during prolonged agonist application is not a unique feature of P2X7 receptors but also occurs in cells expressing P2X2 or P2X4 receptors (Virginio *et al.*, 1999b; Chaumont and Khakh, 2008; Marques-da-Silva *et al.*, 2011).

The time-dependent increase in permeability associated with P2X7 receptor activation is the best studied and has been attributed to two contrasting mechanisms: (i) a conformational change leading to dilation of the integral P2X7 receptor pore which initially forms a channel permeable to small cations; or (ii) the activation of a distinct channel protein permeable to higher MW cations, including fluorescent dyes (North, 2002), where the P2X7 receptor is responsible only for the permeability to small cations and interacts (directly or through second messengers) with the distinct

channel protein. There is evidence for and against these two models and there is no widely accepted hypothesis to explain this phenomenon. The discrepancies could be explained by endogenous channels present in different cell types used (*Xenopus* oocytes, primary cells, cell lines and heterologous expression systems), the orthologous receptor used (human, rat or mouse) or the different protocols applied (electrophysiology, fluorescent microscopy, plate reader or flow cytometry).

The study by Marques-da-Silva *et al*. (2011) in this issue of *BJP* sheds some new light on this confused situation and is important because it demonstrates that colchicine (a well-known disruptor of cytoskeletal microtubules) potently inhibits both P2X2 and P2X7 receptor-dependent dye uptake without affecting receptor channel ionic currents. The effect of colchicine on dye uptake was also independent of cytoskeletal disruption. This work supports the hypothesis of a distinct permeation pathway for high MW dyes which is sensitive to colchicine and shared by both P2X7 and P2X2 receptors. However, the molecular nature of this permeation pathway remains unknown. Recently, P2X7 receptors were found to activate different permeation pathways in different cells and such studies clearly demonstrated separate pathways for cations and anionic dyes in macrophages but not in HEK293 cells (Schachter *et al*., 2008; Cankurtaran-Sayar *et al*., 2009). One of the P2X7 receptor-activated pore pathways has been attributed to the opening of pannexin-1 hemichannels (Pelegrin and Surprenant, 2006; Locovei *et al*., 2007; Iglesias *et al*., 2008) but pannexin-1 seems to be permeable to both cationic dyes and anionic molecules such as ATP, which can also permeate at a high rate (Bao *et al*., 2004; Huang *et al*., 2007; Schenk *et al*., 2008). Pannexin-1 is not involved in the formation of large pores in P2X2 receptors (Chaumont and Khakh, 2008), ruling out the possibility that colchicine was blocking pannexin-1 in the present study of Marques-da-Silva *et al*. (2011). In order to harmonize all previous studies and the two proposed models, a new scheme is needed in which the two current models are merged (Figure 1). This model is based in the recent mathematical modelling of P2X7 receptor gating proposed by Yan *et al*. (2010), which provides evidence

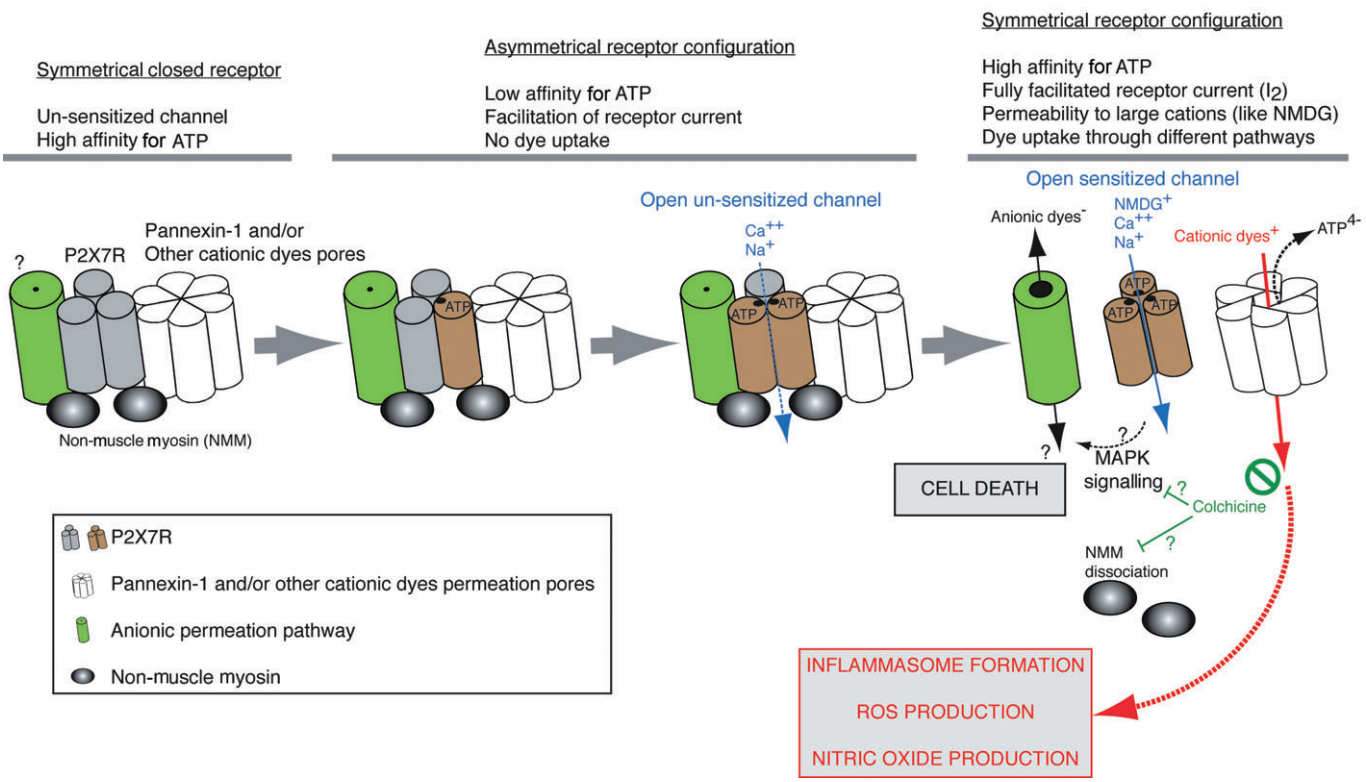

Figure 1

Schematic representation of mechanisms underlying dilatation of the P2X7 receptor-associated pore. In resting conditions, P2X7 receptor is a symmetrical trimer, the pore is closed and the channel is associated with non-muscle myosin (NMM) and probably with the different closed dye uptake channels, such as pannexin-1. The first ATP molecule binds to the symmetrical receptor with high affinity causing a conformational change resulting in an asymmetrical receptor, distorted in such a way as to reduce the affinity of the remaining ATP binding sites. Binding of the second ATP molecule further disrupts the receptor decreasing the affinity for the third molecule of ATP, but the channel opens in a low conductance state. Binding of the third ATP molecule restores receptor symmetry allowing the pore to dilate to a high conductance state. This new conformation may cause the dissociation of NMM from the P2X7 receptor, allowing dye uptake through the opening of different pores (adapted from Pelegrin and Surprenant, 2009 and Yan *et al*., 2010). Pannexin-1 is permeable to cationic dyes and the anionic ATP molecule. Colchicine blocks cationic dye uptake and further pro-inflammatory cascades without affecting cell death. We do not know whether colchicine is also able to block anionic dye uptake, NMM dissociation or mitogen-activated protein kinase (MAPK) signalling promoted dye uptake. P2X7R, P2X7 receptor; NMM, non muscle myosin; NMDG, N-methyl-D-glucamine; ROS, reactive oxygen species.

that the P2X7 receptor intrinsic pore can dilate over the time in physiological conditions, with gating fitting a Markov state model. In this model, the binding of one ATP molecule to one subunit of the P2X7 receptor trimer causes an asymmetry within the receptor which consequently discourages the binding of a second ATP molecule. Binding of the second ATP molecule thus requires a higher concentration and leads to the opening of a low-conductance pore permeable to small cations. Finally, binding of the third ATP molecule, which requires more energy due to further asymmetrical distortion of the receptor conformation, fully activates the receptor. This model explains the run-up or facilitation observed for activation of P2X7 receptors (Roger *et al.*, 2010) and the two components of the P2X7 receptor-associated current (Yan *et al.*, 2010) (authors showing only one component of P2X7 receptor currents have facilitated the receptor before recording i.e. Pelegrin and Surprenant, 2006). In this fully facilitated, symmetrical state, the P2X7 receptor channel is also permeable to large cations such as NMDG (Jiang *et al.*, 2005) and activates the different dye uptake pathways depending on the cell type in which it is expressed (Figure 1). The opening of such pathways could be favoured by the dissociation of P2X7 receptors from the interacting protein non-muscle myosin (NMM), this dissociation somehow facilitates the uptake of high MW dyes (Gu *et al.*, 2009). At this stage, the high intracellular concentration of Ca^{++} can also activate second messengers such as mitogen-activated protein kinase (MAPK) p38, which are also involved in activating some dye uptake pathways (Donnelly-Roberts *et al.*, 2004; Faria *et al.*, 2005). The dissociation of ATP from the fully facilitated receptor induces a closure of the receptor and probably restoration of the original complex with NMM binding and closure of the dye uptake pathway. However, while the receptor lies in a symmetrical conformation subsequent ATP can quickly activate the fully facilitated receptor with a parallel dye uptake (Yan *et al.*, 2010). This close relationship between P2X7 receptor activation and the dye uptake is why virtually all pharmaceutical companies have used high throughput screening on P2X7 receptors, using dye uptake assays as a direct evidence of channel activation when developing selective P2X7 receptor antagonists (Pelegrin, 2008; Donnelly-Roberts *et al.*, 2009). Moreover, the dye uptake assay has been suggested as a high throughput screening method to identify the response of blood monocytes to bacterial lipopolysaccharide (LPS) and to detect P2X7 receptor polymorphisms (Denlinger *et al.*, 2005).

In addition to this mechanistic insight, the study by Marques-da-Silva *et al.* (2011) also contributes to one important aspect of the P2X7 receptor-associated permeabilization, its pathophysiological function. One of the most studied functions of the P2X7 receptor-associated pore is apoptotic cell death (Virginio *et al.*, 1999a; MacKenzie *et al.*, 2005). More recently, it has been shown that the pannexin-1 permeabilization pathway is important for the activation of the inflammasome and caspase-1, releasing IL-1β in different cell types such as neurons, astrocytes, monocytes and macrophages (Pelegrin and Surprenant, 2006; 2009; Marina-García *et al.*, 2008; Silverman *et al.*, 2009). However, there is evidence that the P2X7 receptor-induced dye uptake pathway activated by MAPK does not affect IL-1β release (Donnelly-Roberts *et al.*, 2004), supporting the idea of different permeation pathways with diverse pathophysiological functions. Marques-da-Silva *et al.* (2011) found that blocking P2X7 receptor-induced dye uptake in mouse macrophage with colchicine reduced IL-1β release and also decreased the production of nitrites and reactive oxygen species (ROS), following P2X7 receptor activation, without affecting cell death. More importantly, they observed a reduced release of inflammatory mediators *in vivo* using a mouse model of peritonitis induced by LPS and ATP. In this model, colchicine was able to significantly reduce IL-1β, ROS, nitrites and IFNγ production and also to decrease fever.

In conclusion, the P2X7 receptor is of great interest as a drug target to treat chronic inflammation (Pelegrin, 2008). Despite all the accumulated knowledge about P2X7 receptor-associated dye uptake, we still have a lot to learn about this phenomenon. We need to identify the molecules constituting the different permeabilization pathways and to understand which physiological metabolites pass through it. This could provide information regarding signal transduction between dye uptake and activation of the inflammasome and other pro-inflammatory signalling cascades triggered by P2X7 receptor activation. This knowledge may allow us to design new potential therapeutic agents against chronic inflammation, that target P2X7 receptor-associated dye uptake pathways.

Acknowledgements

The author is indebted to Dr Samuel J. Fountain for critical revision of the manuscript. Research work in the author's laboratory is supported by the *PN I+D+I 2008–2011, Instituto de Salud Carlos III-FEDER* (grant number EMER07/049 and PS09/00120), *Fundación Séneca* (grant number 11922/PI/09) and *British Council* (project number 09.021).

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