$P2X_1$ receptors and the endothelium

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Adenosine triphosphate (ATP) is now established as a principle vaso-active mediator in the vasculature. Its actions on arteries are complex, and are mediated by the P2X and P2Y receptor families. It is generally accepted that ATP induces a bi-phasic response in arteries, inducing contraction via the P2X and P2Y receptors on the smooth muscle cells, and vasodilation via the actions of P2Y receptors located on the endothelium. However, a number of recent studies have placed $P2X_1$ receptors on the endothelium of some arteries. The use of a specific $P2X_1$ receptor ligand, α, β methylene ATP has demonstrated that $P2X_1$ receptors also have a bi-functional role. The actions of ATP on $P2X_1$ receptors is therefore dependant on its location, inducing contraction when located on the smooth muscle cells, and dilation when expressed on the endothelium, comparable to that of P2Y receptors.

Key words: $P2X_1$ receptor - endothelium - smooth muscle cells - adenosine triphosphate

Adenosine triphosphate (ATP) is recognised as an important mediator of vasomotor tone in most vascular beds (Kunapuli & Daniel 1998). It is released into general circulation by a variety of cellular types, including platelets, vascular and cardiac cells in response to common receptor-mediated ligands (Yang et al. 1994), physical forces and after ischemia-reperfusion (Burnstock 1999). ATP induces complex reactions, and may either stimulate specific receptors or may be metabolised by a group of ecto-enzymes (Zimmermann 2000) to products including ADP, AMP, and adenosine, which are also potent mediators in the cardiovascular system.

ATP and other purines act via the purinergic (P2) receptors located throughout the blood vessels to induce both vasodilation and contraction, depending on the receptor subtype activated. Traditionally it was thought that activation of P2Y receptors located on the endothelium induced vasodilation of the artery via the release of NO and prostacyclin (Ralevic & Burnstock 1991, Boeynaems et al. 2000) or endothelium derived hyperpolarising factor (EDHF) (Stanford et al. 2001, Malmsjo et al. 2002). On the other hand, activation of P2Y and P2X receptors located on the smooth muscle cells induce contraction (Ralevic & Burnstock 1988); the pharmacology of which has been fully characterised (Ralevic & Burnstock 1991, North 2002).

The complexity of the effects of ATP on vessels is best described in the perfused intact mesenteric arterial bed of the rat. At low doses ATP induces a transient dilation mediated by the activation of P2Y receptors and the consequent co-release of NO and prostacyclin. At higher doses dilation induced by ATP consists of two discernible phases, the transient phase followed by a sustained phase, which is mediated by the release of EDHF (Stanford et al. 2001, Harrington & Mitchell 2004). The emergence of the second and sustained phase of endothelial dependent dilation induced by ATP coincided with the emergence of the typical $P2X_1$ mediated vasoconstrictor response.

A dilatory role for P2X receptors was first suggested by Ralevic, although this phenomenon was attributed to a functional rebound effect of contraction and suggested to be endothelium independent (Ralevic 2002). However, this study was conducted in the whole rat mesenteric bed, where it is technically difficult to remove the endothelium. An alternative method would be to use the isometric wire myograph, where the physical disruption and removal of the entire endothelium can be ensured. Using this method, we have demonstrated that vasodilation induced by the selective and highly stable P2X ligand, α, β methylene ATP (Burnstock & Kennedy 1985) is endothelial cell dependant (Harrington & Mitchell 2004). Furthermore, we established that vasodilatation was induced predominately by the release of endothelium derived hyperpolarising factor (EDHF).

α, β methylene ATP is a selective ligand for both $P2X_1$ and $P2X_3$ subunits (Virginio et al. 1998, Surprenant et al. 2000, North 2002). However, there is mounting evidence to suggest that $P2X_1$ is the receptor responsible for endothelium dependant vasodilation. Firstly, $P2X_1$ immunoreactivity is localised on the endothelial as well as the smooth muscle cell layer of blood vessels (Hansen et al. 1999, Yamamoto et al. 2000, Glass et al. 2002, Ray et al. 2002, Harrington & Mitchell 2004). Secondly, the vasoconstrictor actions of α, β methylene ATP in mesenteric vessels is ascribed solely to activation of $P2X_1$ receptors (Lewis & Evans 2000). Most significantly, we have recently found that first order mesenteric arteries from the $P2X_1^{-/-}$ mouse do not respond to α, β methylene ATP (Harrington & Mitchell, unpublished data). It also interesting to note that renal arteries contract without subsequent vasodilation in response to α, β methylene ATP, which express $P2X_1$ receptors on the smooth muscle cells, and not on the endothelium (Knight et al. 2003).

While it is possible that α, β methylene ATP is not a P2X specific agonist, there is no body of data that suggests that α, β methylene ATP is anything other than a specific P2X ligand (Simon et al. 1995). There are some isolated reports suggesting that α, β methylene ATP can

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act weakly on $P2Y_{11}$ receptors, which are not expressed on the endothelium and do not affect vasomotor responses. Where the $P2Y_{11}$ receptors have been studied their role on leukocytes appear to be important in the immunological responses (van der et al. 2000, Conigrave et al. 2001).

In conclusion, activation of $P2X_1$ receptors located on the endothelium induce vasodilation in resistance arteries via the release EDHF. P2X receptors may have a bifunctional role in arteries, causing constriction when activated on smooth muscle and dilation when activated on the endothelium. This would be comparable to the variable functions of P2Y receptors, which mediate relaxation when located on the endothelium and contraction when situated on the smooth muscle cells (Ralevic & Burnstock 1998).

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