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EDITORIAL

Intracellular expression of purinoceptors

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Purinoceptors, receptors for nucleosides and nucleotides, have been identified on the plasma membranes of many cell types [1]. The early hints and recent evidence for localization of purinoceptors on intracellular sites, including lysosomes [2–4], mitochondria [5, 6] and in nuclei where they open ion channels and appear to influence mRNA activity [7, 8], offers up a whole new aspect of purinergic signalling.

Purinergic signalling, ATP acting as an extracellular signalling molecule, was proposed in 1972 [9]. Separate families of purinergic receptors were recognised, named P1 receptors for adenosine and P2 receptors for ATP and ADP [10]. Two subtypes of P2 receptors were shown in 1985, based on pharmacology [11] and in the early 1990s P1, P2X and P2Y receptor subtypes were cloned and characterised: four subtypes of P1 receptors (A_1, A_{2A}, A_{2B} and A_3), seven subtypes of P2X ion channel receptors (P2X1-7) and eight subtypes of P2Y G protein-coupled receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$) [1, 12].

Cloning of receptors made it possible to generate polyclonal antisera for immunohistochemical studies of their expression and distribution [13]. Many papers using this technique were published [14–17]. As expected, the receptors were located on the plasma membranes of cells, but sometimes there was also intracellular immunostaining. This was often dismissed by referees as artefacts. However, later studies have revealed that intracellular localization of purinoceptors is genuine. For example, it was suggested that uptake of P2X1 receptors into smooth muscle cells of the rat vas deferens was responsible for desensitization ([18] and see [19]). $P2Y_2$ receptor internalization via the clathrin-mediated pathway was observed in HEK293 cells using receptors tagged with green fluorescent protein (GFP), to be colocalized with endosomes and lysosomes [2]. GFP was also used to show internalization of $P2Y_1$ receptors in HEK-293 cells [20, 21]. Ser352 and Ser354 in the carboxyl terminus of human $P2Y_1$ receptors were shown to be needed for internalization in MDCK cells [22]. P2X3 receptors transfected into HEK-293 cells and expressed endogenously in dorsal root ganglion sensory neurons undergo rapid constitutive endocytosis, targeting the late endosomal/lysosomal system [23]. The role of internalised P2X7 receptors on lysosomes in macrophages in the killing of mycobacteria is discussed in a review [24]. A Rab5-dependent pathway was described for internalisation of P2X4 receptors in HEK-293 cells [25]. The internalised P2X4 receptors are located on lamellar bodies, lysosomes, vesicles and vacuoles in HEK-293 cells, hippocampal neurons and alveolar type II cells [3, 4]. P2X4 receptor channel activity was directly measured in intact lysosomes in HEK-239 cells [26]. Both ATP and P2X4 receptors were present in lysosomes and the lysosomal P2X4 receptors were activated by ATP at the luminal side in a pH-dependent manner. The lysosomal P2X4-mediated responses were potentiated by ivermectin, but were insensitive to suramin and PPADS, as for the plasma membrane P2X4 receptors. Mitochondrial calcium transport was shown to be regulated by $P2Y_1$- and $P2Y_2$-like mitochondrial receptors from rat liver cells [5]. ATP, acting on the nuclear envelope, was reported to open ion channels in both *Xenopus* oocytes [27, 28] and patch-clamped isolated mouse liver nuclei [29]. It was later reported that P2X7 receptors were expressed in the outer nuclear membranes of rat

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hippocampal inhibitory neurons [7]. P2X7 receptor immunoreactivity was also shown on the nuclear membrane of guinea pig visceral smooth muscle cells [30]. Studies of the expression of P2X-like receptors in amoeba showed that most of the immunostaining was on the membranes of intracellular vacuoles, required for osmoregulation, rather than on the plasma membrane [31–33], encouraging further investigations of intracellular localization of P2 receptors in mammals.

A recent paper showed immunostaining of P2X6 receptors within the nucleus of cultured hippocampal neurons [8]. It was shown that once inside the nucleus, the P2X6 receptor interacts with the splicing factor 3A1, which results in a reduction of the mRNA splicing activity, which is relevant in the ageing process.

These findings open up a whole new aspect of purinergic signalling and new studies will be of much interest.

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