

Purinergic P2Y₁ receptors take centre stage in autocrine stimulation of human beta cells

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Received: 5 September 2014 / Accepted: 10 September 2014 / Published online: 3 October 2014
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Abstract Insulin secretory vesicles contain high concentrations of adenine nucleotides, which are co-released with insulin during exocytosis. There is strong evidence that ATP and ADP serve as autocrine messengers in pancreatic beta cells, but the functional effects and detailed mechanisms of action are under debate. In this issue of *Diabetologia*, Khan and colleagues (DOI: 10.1007/s00125-014-3368-8) present the results of their study of autocrine purinergic signalling in isolated human beta cells. Using a combination of electrophysiological techniques, Ca²⁺ imaging and measurements of insulin secretion, it is demonstrated that voltage-dependent Ca²⁺ influx triggers release of ATP/ADP, which activates purinergic receptors of the G_{q/11}-coupled P2Y₁ isoform. Activation of these receptors leads to membrane depolarisation and phospholipase C-mediated mobilisation of Ca²⁺ from endoplasmic reticulum stores, which amplifies the exocytosis-triggering Ca²⁺ signal. In contrast, there is little evidence for involvement of ionotropic P2X receptors in the autocrine stimulation of human beta cells. This commentary discusses these findings as well as various functional and therapeutic implications of the complex purinergic signalling network in the pancreatic islet.

Keywords ADP · ATP · Ca²⁺ · Insulin secretion · Islets · P2X receptor · P2Y receptor · Phospholipase C

Abbreviation

VNUT Vesicular nucleotide transporter

ATP is a versatile intra- and extracellular messenger in the regulation of insulin secretion from pancreatic beta cells. The

cytoplasmic ATP concentration in beta cells largely reflects the rate of oxidative glucose metabolism, and inhibition of ATP-sensitive K⁺ channels (K_{ATP} channels) mediated by an increased ATP/ADP ratio explains how glucose induces membrane depolarisation, voltage-dependent Ca²⁺ influx and exocytosis of insulin secretory granules [1]. These granules contain millimolar concentrations of ATP and other purine nucleotides [2, 3], a result of vesicular nucleotide transporter (VNUT) activity in the granule membrane [4]. The nucleotides are co-released with insulin during vesicle exocytosis and the local concentration of ATP at the beta cell surface may then reach micromolar levels [5]. The small size of ATP and related nucleotides allows them to escape through the granule fusion pore before insulin is released, and sometimes even without concomitant protein secretion [6]. The purine nucleotides exert auto- and paracrine actions on beta cells, and although there is extensive literature on the subject (reviewed in [7, 8]), the precise receptors and molecular mechanisms involved have not been clarified.

Two classes of purinergic P2 receptors mediate the effects of ATP and ADP: ionotropic P2X receptors and G-protein-coupled P2Y receptors. The P2X receptors constitute a group of seven isoforms that act as ATP-gated cation channels, several of which are expressed in beta cells [7, 8]. Many of the eight P2Y family members are also present in beta cells, with a dominant role of the G_{q/11}-coupled P2Y₁ receptor [7, 9].

Extracellular ATP has been found to amplify glucose-stimulated insulin secretion in both rodent and human studies [7], but inhibitory effects have also been demonstrated, at least in the mouse [10, 11]. A negative effect of ATP is also supported by the observation that glucose-induced insulin secretion is enhanced in P2Y₁-knockout mice [12] and that a P2Y₁ receptor antagonist increases insulin secretion in the perfused rat pancreas [13]. However, recent studies support a stimulatory autocrine effect in human beta cells, although

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these reached different conclusions regarding receptor involvement, favouring either P2X₃ [14] or P2Y₁ [15] receptors.

This issue of *Diabetologia* includes a study in which the late Mathias Braun and colleagues carefully analysed the effects of ATP on membrane potential, membrane currents, Ca²⁺ signalling and exocytosis in isolated human beta cells [16]. Patch-clamp recordings from dispersed islet cells, identified as beta cells by immunocytochemistry, demonstrated that ATP and ADP evoked inward depolarising currents. Similar findings have previously been attributed to activation of P2X receptors [17], but the response could not be reproduced by P2X receptor activation and was instead mimicked and inhibited, respectively, by an agonist and antagonist of the P2Y₁ receptor [16].

P2Y₁ signalling activates phospholipase C and formation of inositol 1,4,5-trisphosphate, which mobilises Ca²⁺ from the endoplasmic reticulum. Indeed, as in mouse beta cells [9], ATP triggered an increase of cytosolic Ca²⁺ in human beta cells, consisting of a distinct peak followed by a sustained plateau. The peak was insensitive to removal of extracellular Ca²⁺, but sensitive to endoplasmic reticulum Ca²⁺ store depletion by thapsigargin or intracellular infusion of the inositol 1,4,5-trisphosphate receptor inhibitor heparin. In contrast, the plateau response required the presence of Ca²⁺ in the extracellular medium and thus reflected Ca²⁺ influx through the plasma membrane.

The expression of P2Y₁ receptors in human beta cells was verified by PCR and immunostaining. Transcripts were also identified for several other P2Y receptor subtypes, including P2Y₂, P2Y₁₁ and P2Y₁₄, but, notably, not for the G_{i/o}-coupled receptor P2Y₁₃, which has been found to inhibit insulin secretion in mouse beta cells [18].

A key question is whether purinergic signalling stimulates or inhibits insulin secretion from human beta cells. To clarify this issue, Khan et al [16] quantified exocytosis by measuring changes in membrane capacitance in response to voltage-clamp depolarisations, which trigger Ca²⁺ influx through voltage-gated plasma membrane channels. Both ATP and ADP strongly potentiated exocytosis under these conditions. ATP also triggered exocytosis in hyperpolarised cells; an effect that depended on intracellular Ca²⁺ mobilisation.

Human beta cells are obviously highly responsive to P2Y₁ agonists, including ATP and ADP, but is there endogenous release of the nucleotides, and is it sufficient to activate the P2Y₁ signalling pathway? By overexpressing P2X₂ receptors [6] in human beta cells, Khan et al [16] demonstrated that the cells release ATP. Accordingly, stimulation of exocytosis by Ca²⁺ infusion into the beta cell via the patch pipette induced transient inward currents, reflecting P2X₂ activation by locally released ATP. Interestingly, these currents became more frequent and pronounced when the cells had been incubated at 10 compared to 1 mmol/l glucose. The increased frequency might be explained by metabolic amplification of exocytosis

by glucose, but the fact that the amplitude increased implies that more ATP was released with each vesicle. The underlying mechanism is unknown, but one interpretation is that glucose stimulates VNUT-mediated ATP transport into the secretory granules.

The functional importance of autocrine purinergic signalling is underlined by the observation that P2Y₁ receptor antagonists lowered cytosolic Ca²⁺ and reduced insulin secretion in human beta cells and islets exposed to a stimulatory glucose concentration [16]. Moreover, when cells were exposed to a series of depolarisations, there was an increase of cytosolic Ca²⁺ and, in a subpopulation of cells, this increase suddenly showed a marked acceleration coinciding with a pronounced increase of exocytosis. This accelerated response was prevented by P2Y₁ inhibition, suggesting that voltage-dependent Ca²⁺ influx triggers exocytosis of ATP (together with insulin) and that the nucleotide mobilises intracellular Ca²⁺ that enhances the exocytosis-triggering Ca²⁺ signal (Fig. 1).

The findings by Khan et al [16] largely confirm the conclusions from a recent study of plasma membrane diacylglycerol signalling that a purinergic P2Y₁ receptor-mediated mechanism mediates autocrine feedback in glucose-stimulated mouse and human beta cells [15]. In contrast, neither of these studies provide support for the previously proposed prominent role of P2X receptors [14]. It is likely that the high concentrations of oxidised ATP and pyridoxal

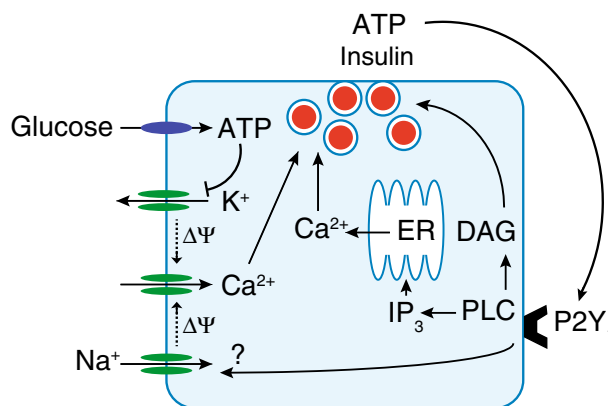


Fig. 1 Schematic drawing of autocrine purinergic signalling in a human beta cell during glucose stimulation. Glucose enters the cell and is metabolised to ATP. Closure of ATP-sensitive K⁺ channels causes membrane depolarisation ($\Delta\Psi$) and opening of voltage-dependent Ca²⁺ channels. Influx of Ca²⁺ triggers exocytosis of granules, with resulting co-release of insulin and ATP. ATP binds to purinergic P2Y₁ receptors, which in turn activate a depolarising current, mediated by an as yet unidentified Na⁺ or non-selective cation channel. P2Y₁ receptors also activate phospholipase C (PLC), which stimulates production of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP₃). While DAG promotes vesicle fusion by incompletely characterised mechanisms, IP₃ mobilises Ca²⁺ from the endoplasmic reticulum (ER). The resulting amplification of the Ca²⁺ signal promotes additional exocytosis of secretory granules

phosphate nucleotide derivatives employed for the P2X receptor inhibition [14] were not specific [19, 20].

What underlies the depolarising action of ATP if not P2X receptors? In mouse beta cells, P2Y₁-receptor activation has been linked to closure of K_{ATP} channels, potentially via activation of phospholipase A₂ [11]. In contrast, several observations by Khan et al [16] indicate that the ATP-regulated membrane currents in human beta cells are carried by Na⁺ rather than K⁺. This could indicate involvement of a store-operated channel activated by intracellular Ca²⁺ mobilisation. However, the ATP-induced currents were insensitive both to thapsigargin and to gadolinium ions, which are typical modulators of store-operated channels. Further studies are needed to clarify the molecular identity of this conductance. Members of the transient receptor potential family of proteins constitute promising candidates [21, 22].

Although the work by Khan et al [16] provides valuable insights into purinergic signalling in isolated beta cells, additional aspects come into play when considering the more complex situation in the intact pancreatic islet. The endocrine cells are not the only source of purine nucleotides as ATP is also released from nerve endings [23]. In mouse islets, transient and self-regenerating release of ATP from beta cells serves as a complement to gap junctions for synchronising cytosolic Ca²⁺ oscillations between beta cells to generate pulsatile insulin secretion [9, 23]. Similarly, neurally derived ATP may help to synchronise oscillations between islets in different parts of the pancreas to generate insulin pulses in the circulation [13, 23]. In both rodent [24, 25] and human [26] islets, pulses of insulin occur in phase with pulses of somatostatin but in opposite phase with peaks of glucagon. It is possible that ATP is also important for communication between beta and delta cells, which are not coupled by gap junctions.

It should also be pointed out that ATP in the extracellular space of the islet is rapidly degraded to adenosine by ectonucleotidases [27], and adenosine can in turn suppress insulin secretion via purinergic P1 receptors on the beta cells [28, 29]. The work by Khan et al [16] indicates many similarities between mouse and human beta cells, but there may be species differences that add to the difficulties involved in clarifying the functional complexity of the purinergic signalling network in the pancreatic islet. Future challenges include a better understanding of the precise distribution and function of the many different purinergic receptors expressed in human beta cells. Beta cells are known to exhibit marked heterogeneity in their functional responsiveness to glucose [30]. Khan et al [16] also observed heterogeneity in purinergic signalling, indicating that there might be subpopulations of beta cells with different sets of receptors.

A physiological consequence of the positive feedback mechanism is that beta cells could respond faster and with more insulin secretion to a rather modest change in blood

glucose concentration. An important question is whether purinergic signalling is altered in diabetes. Previous studies have reported alterations in P2X₇ receptor expression in obese and diabetic patients [31], and both P2X and P2Y receptors have been implicated in the regulation of beta cell mass and viability (reviewed in [8]). The study by Khan et al [16] includes the intriguing observation that intracellular Ca²⁺ mobilisation in response to ATP tended to be reduced in islets from a donor with type 2 diabetes. This might indicate a link between deficient P2Y₁ signalling and impaired insulin secretion, but the finding needs to be reproduced before firm conclusions can be drawn. Since purinergic receptors directly affect beta cell function and insulin secretion, they have attracted interest as therapeutic targets in diabetes. Promising effects of P2Y receptor agonists have indeed been reported in experimental studies (reviewed in [7, 8]) but the progress has been relatively slow [32]. Challenges include the wide distribution of purinergic receptors in different tissues, as well as the diversity of receptor subtypes even in a single cell type, such as the beta cell. Moreover, hetero-oligomerisation of receptor subtypes may modulate their function and ligand selectivity [7]. Continued functional investigations of individual beta cells and islets, such as that conducted by Khan et al [16], will increase our understanding of human islet physiology and pathophysiology and provide an important basis for drug development.

Funding The work in the author's laboratory is supported by funding from the European Foundation for the Study of Diabetes/Merck Sharp & Dohme (EFSD/MSD), the Novo Nordisk Foundation, the Swedish Diabetes Association, the Swedish Research Council and the Family Erfnors Foundation.

Duality of interest The author declares that there is no duality of interest associated with this manuscript.

Contribution statement The author was the sole contributor to this paper.

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