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P2X receptor channels in endocrine glands

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Abstract

The endocrine system is the system of ductless glands and single cells that synthetize hormones and release them directly into the bloodstream. Regulation of endocrine system is very complex and ATP and its degradable products ADP and adenosine contribute to its regulation acting as extracellular messengers for purinergic receptors. These include P2X receptors, a family of ligand-gated ion channels which expression and roles in endocrine tissues are reviewed here. There are seven mammalian purinergic receptor subunits, denoted P2X1 through P2X7, and the majority of these subunits are also expressed in secretory and non-secretory cells of endocrine system. Functional channels have been identified in the neuroendocrine hypothalamus, the posterior and anterior pituitary, the thyroid gland, the adrenals, the endocrine pancreas, the gonads and the placenta. Native channels are capable of promoting calcium influx through its pore in both excitable and non-excitable cells, as well as of increasing electrical activity in excitable cells by membrane depolarization. This leads to generation of calcium transients and stimulation of hormone release. The pattern of expression and action of P2XRs in endocrine system suggests that locally produced ATP amplifies and synchronizes the secretory responses of individual cells.

INTRODUCTION

Purinergic signaling is operative in neuroendocrine and endocrine glands. The cells of these glands release ATP, which acts as an extracellular ligand for two families of membrane receptors, two-transmembrane domain P2X receptor channels (P2XRs) and seven-transmembrane domain P2Y receptors (P2YRs), which are expressed in numerous cells of endocrine glands. Like in other tissues, the duration and extent of ATP actions in endocrine tissues are limited by several ectonucleotidases, which hydrolyze ATP to ADP, AMP, and adenosine. ADP and adenosine also act as extracellular ligands, with ADP as a potent agonist for some P2YRs and adenosine as an agonist for adenosine (P1) receptors. This review focuses on recent findings on the expression and role of P2XRs in control of endocrine system. The gating properties of these channels and their sensitivities to ATP are critical in understanding their physiological role. Briefly, P2X1R and P2X3R rapidly activate and desensitize, P2X2R and P2X4R slowly desensitize, whereas P2X7R does not show an obvious desensitization but exhibit the secondary current growth. Rat P2X5Rs generate low amplitude non-desensitizing currents, and P2X6R does not express well at the plasma membrane. Among receptors, P2X1R and P2X3R have the highest affinity for ATP with an EC_{50} value in a submicromolar concentration range, followed by P2X5R with

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estimated EC_{50} values ranging from submicromolar to low micromolar concentration range. P2X4R and P2X2R are also fully activated by ATP but less sensitive to ATP, with EC_{50} values in a low micromolar concentration range. The P2X7Rs is the least sensitive member of the P2XR family to activation by nucleotides with the EC_{50} value for ATP in a high micromolar concentration range [1]. The action of extracellular ATP in endocrine glands also depends on the co-expression of P2YRs and adenosine receptors; for some details on their expression in endocrine cells see [2, 3].

PARVOCELLULAR HYPOTHALAMIC NEURONS

The hypothalamus is functionally associated with the limbic system and contains a number of small nuclei that are involved in a variety of functions. The paraventricular nucleus lies adjacent to the third ventricle and is composed of magnocellular neurosecretory cells (see hypothalamo-posterior pituitary system) and parvocellular neuroendocrine cells that project to the median eminence where they secrete neurohormones, or 'releasing or inhibiting hormones', into the hypophysial portal system. These include corticotropin-releasing hormone, thyrotropin-releasing hormone, gonadotropin-releasing hormone, growth hormone-releasing hormone, dopamine and somatostatin. The portal system run into the anterior lobe of the pituitary gland, where neurohormones modulate secretory activity of specialized cells. ATP is released by neighboring glial cells [4] and further studies are needed to clarify whether it is also released by neuroendocrine cells and synaptic afferents terminating at these neurons. Several P2XRs appear to be expressed in paraventricular parvocellular neurons [5]. The P2X2R is found in corticotropin-releasing hormone- and thyrotropin-releasing hormone-producing neurons [6]. Double labeling immune-histochemistry revealed the expression of P2X2R, P2X4R, P2X5R and P2X6R on the perikarya of GnRH neurons and P2X2R and P2X6R on their axon terminals, suggesting that P2XRs at both presynaptic and postsynaptic sites could be involved in the regulation of secretion of this neurohormone [7]. The P2X2 and P2X4 subunits are also expressed in gonadotropin-releasing hormone-secreting neurons in olfactory placode cultures from rhesus monkeys. In these neurons, ATP application leads to synchronization of calcium oscillations, which could indicate a role of ATP in pulsatile release of this decapeptide [8]. Electrophysiological experiments also showed that ATP induces inward currents in unidentified neurons of the arcuate nucleus mediated by homomeric and/or heteromeric P2X2Rs [9]. Additional work is needed to clarify the expression pattern and role of P2XRs in control of dopamine, somatostatin, and growth hormone-releasing hormone secretion by neuroendocrine hypothalamus.

ANTERIOR PITUITARY GLAND

ATP not only controls the anterior pituitary (AP) function indirectly, by modulating secretion of releasing and inhibitory hypothalamic hormones, but also directly – by activating endogenous P2XRs in endocrine AP cells. AP is composed of several endocrine cell types: corticotrophs releasing corticotropin, thyrotrophs, releasing thyrotropin, gonadotrophs releasing follicle-stimulating hormone and luteinizing hormone, somatotrophs releasing growth hormone, and lactotrophs releasing prolactin. ATP is also released by normal and immortalized pituitary cells [10, 11] and pannexin channels appear to mediate this release [12, 13]. Initial knowledge about the expression and role of P2XRs in AP cells was obtained in experiments using hormone release and calcium measurements in dispersed cells. These experiments, summarized in [14], reveal the presence of functional P2XRs in all secretory cell types and raise the possibility that several subtypes of these channels are expressed in a cell type-specific manner. In more recent studies, molecular biology techniques combined with electrophysiology showed that secretory pituitary cells abundantly express P2X2 and P2X4, with less expression of other subunits [15]. Rat AP cells

express two splice forms of the P2X2 subunit, termed P2X2a and P2X2b [16], whereas mouse pituitary cells express three forms of P2X2 receptor subunit, the full size P2X2a and the shorter forms P2X2b and P2X2e, which are missing 69 and 90 residues, respectively, in their C-termini [17]. The physiological relevance of these splice forms is in formation of functional homomers and heteromers, which desensitize faster than full-size receptors limiting the excessive ion influx. The functional receptors have been identified in pituitary gonadotrophs and somatotrophs, but not other pituitary cell types [16]. In gonadotrophs, their activation leads to plasma membrane depolarization and firing of action potentials along with modulation of the frequency of firing in spontaneously firing cells. This is accompanied by elevation in cytosolic calcium, reflecting both influx of this cation through channel pores and through voltage-gated calcium channels, and gonadotropin release. In these cells, ATP also modulates gonadotropin-releasing hormone-induced calcium/current oscillations and hormone release apparently by refilling of intracellular calcium stores [10, 18]. The biophysical and pharmacological properties of recombinant rat P2X4R cloned from the pituitary gland have also been characterized [19], and the functional receptors have been identified in lactotrophs [20]. Single-cell patch-clamp recordings show that extracellular ATP induced an inward depolarizing current in a majority of lactotrophs, which resembled the current profile generated by recombinant P2X4R. The channels were activated in a concentration-dependent manner, desensitized moderately and were potentiated by ivermectin, a P2X4R-specific allosteric modulator. Activation of these channels leads to stimulation of electrical activity and promotion of voltage-gated and voltage-insensitive calcium influx and prolactin secretion [15].

HYPOTHALAMO-POSTERIOR PITUITARY SYSTEM

In contrast to parvocellular neuroendocrine hypothalamus, the axons of hypothalamic magnocellular paraventricular and supraoptic nuclei neurons transport secretory vesicles from the soma to the posterior pituitary (PP), releasing vasopressin or oxytocin near fenestrated capillaries. The rate and pattern of neuronal firing activity determines the amount of hormone released, and bursting activity differs in oxytocin and vasopressin neurons [21]. Purines have been suggested to play important role(s) in controlling the activity of vasopressin- but not oxytocin-secreting neurons. This conclusion was initially derived from experiments measuring the effects of extracellularly added ATP on vasopressin and oxytocin release from isolated PP terminals [22] and finding that the magnocellular neurons of the hypothalamus also contain ATP and release it in the PP in an action potential-specific manner [22, 23]. It has also been shown that ATP endogenously released from the PP during stimulation is sufficient to depolarize the nerve terminals and potentiate vasopressin secretion [24]. ATP is also released by astrocytes that are positioned in a close proximity of synapses to sense and modulate afferent synaptic activity[4]. These observations suggest that ATP may act on both perikarya and dendrites of vasopressinergic neurons in the hypothalamus and on nerve terminals in the PP. Consistent with this hypothesis, functional P2XRs have been identified in the somata of neurons in the supraoptic nucleus [25] and in isolated PP terminals [22], as indicated by cytosolic calcium measurements. A more recent study showed a role for P2X2Rs in ATP-induced increase in cytosolic calcium and peptide release from isolated rat PP terminals [26]. Electrophysiological experiments confirmed that supraoptic neurons express functional P2X2R, P2X4R and possibly P2X7R, which activation produces depolarization of neuronal somata. On the other hand, presynaptic P2XRs facilitate glutamate and GABA release [27]. Electrophysiological evidence for the existence of P2XR currents in vasopressinergic PP terminals but not in terminals labeled for oxytocin have also been shown [28]. Experiments with knockout mice revealed that sufficient endogenous ATP is released by bursts of action potential to act at P2X2Rs, but not P2X3R or P2X7R [29].

THYROID GLAND

The thyroid consists of large cavities, the thyroid follicles, which contain a single layer of epithelial cells, called the thyroxine and triiodothyronine-secreting thyroid follicular cells. Dispersed between the thyroid follicles are the calcitonin-secreting parafollicular cell or C-cells, which contribute to the control of the body's calcium homeostasis. Anterior pituitary controls thyroid function by thyroid-stimulating hormone, which stimulates the exocytosis and endocytosis of thyroglobulin primarily via its receptors coupled to adenylyl cyclase signaling pathway and cross-coupled to phospholipase C signaling pathway. It also appears that extracellular ATP contributes to regulation of thyroid function. The thyroid is innervated by sympathetic, parasympathetic and sensory afferents and ATP could be released from the reach vegetative innervations of the thyroid gland, from capillary endothelial cells, or from the thyrocytes themselves [30]. In cultured thyrocytes and thyroid cell lines, extracellular ATP stimulates thyroglobulin secretion, and it appears that both calcium-mobilizing P2YRs and calcium-conducting P2XRs contribute to this action. The mRNA transcripts for P2X3, P2X4, and P2X5 subunits are present in thyroid FRTL-5 cells, and at least the P2X5R is functionally expressed in these cells [31]. Immunohistochemical analysis confirmed the expression P2X3R, P2X4R, and P2X5R in follicular cells and no P2XR immunostaining on parafollicular cells [32]. In the FRTL thyrocyte cell lines, ATP generates an inward current and stimulates membrane internalization presumably by activating endogenous P2X7R [30, 33]. The P2X7R mRNA transcripts and protein expression are present in thyroid papillary carcinoma, further suggesting that enhanced function of these receptors might be a feature of thyroid cancer [34].

ADRENAL GLAND

The adrenal glands are composed of two distinct structures: cortex and medulla. The adrenal cortex comprises three zones producing and secreting distinct hormones: zona glomerulosa, the main site for production of mineralocorticoids, zona fasciculata, responsible for producing glucocorticoids, and zona reticularis producing androgens. ATP is released from zona glomerulosa region of the adrenal gland and contributes to the control of steroidogenesis [35, 36]. Cortex cells express connexin43, which is known to contribute to ATP release as a hemichannel [37]. Immunohistochemical studies revealed the expression of several P2XR subtypes in cortical cells [38, 39]. RNA blot analysis indicated significant levels of P2X4R mRNA in the cortex of the adrenal gland [40]. Further srtudies are needed to identify the role of these channels in adrenal cortex function. The chromaffin cells of medulla produce catecholamines norepinephrine and epinephrine. ATP is co-stored and co-released with catecholamines from chromaffin cells [41] and contributes to the control of catecholamine release [42, 43]. A small number of chromaffin cells show positive immunoreaction for P2X1R, P2X2R, P2X4R, P2X5R and P2X7R in a species- and age-specific manner [38, 39]. Other show positive immunoreaction for P2X1R and P2X2R in chromaffin and PC12 cells [44] and P2X4R subtype in medulla [40] and PC12 cells [45]. Activation of endogenous P2XRs in chromaffin cells causes elevation in cytosolic calcium, reflecting predominantly calcium influx via voltage-gated calcium channels, and catecholamine release [46, 47].

OVARY

The gonadal and endocrine functions of ovaries are controlled by luteinizing hormone and follicle-stimulating hormone of AP. The endocrine function is mediated by granulosa cells, which surround the oocyte in the preovulatory follicle and convert androgens (coming from the thecal cells) to estradiol by aromatase during the follicular phase of the menstrual cycle. However, after ovulation the granulosa cells turn into luteal cells that produce progesterone.

Granulosa cells express connexin43 hemichannels, which could provide a pathway for ATP release [48] and both cell types also express functional adenosine receptors and P2YRs [49–51]. The mRNA transcripts for P2X1R and P2X2R are also found in ovarian tissues and immunohistochemical analysis revealed the presence of P2X2R in granulosa cells as well as in the smooth muscle of perifollicular rings and blood vessels [52]. Further studies are needed to clarify the physiological role of P2XRs in endocrine functions of ovary.

TESTIS

The testis consists of seminiferous tubules containing germ and Sertoli cells and interstitial spaces between these tubules containing androgen-producing Leydig cells and other cell types. The gonadal and endocrine functions of testis are also controlled by luteinizing hormone and follicle-stimulating hormone of AP. ATP acts as a modulator of testicular cells, including Sertoli cells [53] and spermatogonia [54]. The Sertoli cells release ATP endogenously through a still not clarified mechanism [55]. Leydig cells express pannexin channels, which could account for ATP release [56]. In rat Sertoli cells ATP elevates cytosolic calcium and estradiol secretion, probably by activating both P2XRs and P2YRs [57–59]. In rat and mouse Leydig cells, ATP also increases cytosolic calcium and testosterone secretion [60, 61], indicating its modulatory role in androgen production through activation of P2XRs. Immunopositive P2X1R, P2X2R, P2X3R, P2X5R and P2X7R cells are identified in testis; P2X2R, P2X3R, and P2X5R are expressed differentially in various germ cell types, whereas Sertoli cells express P2X2R, P2X3R and P2X7R [62]. Functional P2X2Rs are also identified in mouse Leydig cells using whole-cell current measurements and specific agonist and antagonists [63]. Western blot experiments also revealed that mouse Leydig cells express P2X2R, P2X4R, P2X6R and P2X7R and functionality of these receptors is confirmed by electrophysiological measurements of the whole-cell current [64].

PLACENTA

The placenta is also an endocrine organ, secreting chorionic gonadotropin, placental lactogen, estrogens and progesterone and expressing several P2YRs. We were unable to find published information about ATP release in placenta. However, the mRNA transcripts for P2X1, P2X4, P2X5, P2X6, and P2X7 subunits are present in human placenta vessels and these receptors contribute to the humoral regulation of placental blood flow [65]. These mRNAs are also present in human placental cytotrophoblast cells, and the western blot analysis confirmed the presence of P2X4R and P2X7R proteins in these cells, which assemble functional receptors as indicated by cytosolic calcium measurements [66]. The potential role of these channels in advancing gestation [67] and restoring placental cell homeostasis after preeclampsia [68] has also been proposed, as well as the role of P2X7R in regulation of phospholipase D in trophoblasts [69].

ENDOCRINE PANCREAS

The pancreas is a gland with both endocrine and exocrine functions. The part of the pancreas with endocrine function is made up of cell clusters called islets of Langerhans. Four main cell types exist in the islets: α-cells secrete glucagon, β-cells secrete insulin, δ-cells secrete somatostatin, and γ-cells, secrete pancreatic polypeptide. The major source of extracellular ATP in β-cells is apparently the ATP released from insulin secretory vesicles containing milimolar concentrations of ATP and ADP [70–72] together with insulin granule markers IAPP [73] and serotonin [74]. Application of glucose releases ATP from single pancreatic β-cells [75] with a local extracellular ATP concentration exceeding 25 μM[76]. It also appears that vesicular-nucleotide transporter mediates ATP release from these cells [77]. This is consistent with the hypothesis that ATP represents a positive feedback signal amplifying the glucose-induced insulin release [3]. The islets express P2X1R, P2X4R, and P2X7R in α- and β-

cells [78, 79]. The functional expression of P2X4R in β-cells is indicated by facilitatory effect of ivermectin, a specific allosteric regulator of these channels, on insulin secretion [80]. Others found that P2X7Rs regulate interleukin-1 secretion, which in turn regulates β-cells mass and function [81]. There is also electrophysiological evidence that mouse β-cells express rapidly desensitizing P2X1R and P2X3R [82]. The role of P2X3R in calcium signaling and insulin release in human β-cells has also been shown [83].

CONCLUSION

It is widely accepted that ATP is co-secreted with other neurohormones by hypothalamic magnocellular neurons in PP and adrenergic and noradrenergic neurons in adrenal medulla. ATP is also released by hypothalamic astrocytes and other cells in endocrine glands, but the mechanism of its release has not been characterized in details. The released ATP activates both P2YRs and P2XRs, expressed in all endocrine glands, but it is also degraded to adenosine, leading to subsequent activation of adenosine receptors expressed in some secretory cell types. It appears that sequential activation of these three families of receptors is complementary, causing a transient stimulation of hormones, thus acting as an autocrine/paracrine amplifier of secretory response. P2XRs have a dual role in this amplification. These receptors are calcium-conducting channels, thus also providing a pathway for increase in intracellular calcium concentration in a receptor-specific manner for both non-excitable and excitable endocrine cells. In excitable endocrine cells, their activation also leads to inward depolarizing currents, which trigger electrical activity and facilitation of voltage-gated calcium influx. Extracellular ATP through P2XR activation may also potentiate synaptic efficacy in magnocellular neurons of the hypothalamus and synchronize the secretory activity of individual cells within the endocrine glands by generating intercellular calcium waves.

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