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Signaling by purinergic receptors and channels in the pituitary gland

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Abstract

Adenosine 5'-triphosphate is frequently released by cells and acts as an agonist for G protein-coupled P2Y receptors and ligand-gated P2X cationic channels in numerous tissues. The breakdown of ATP by ectonucleotidases not only terminates its extracellular messenger functions, but also provides a pathway for the generation of two additional agonists: adenosine 5'-diphosphate, acting via some P2Y receptors, and adenosine, a native agonist for G protein-coupled adenosine receptors. In the pituitary gland, adenosine 5'-triphosphate is released from the endings of magnocellular hypothalamic neurons and by anterior pituitary cells through pathway(s) that are still not well characterized. This gland also expresses several members of each family of purinergic receptors. P2X and adenosine receptors are co-expressed in the somata and nerve terminals of vasopressin-releasing neurons as well as in some secretory pituitary cells. P2X receptors stimulate electrical activity and modulate InsP₃-dependent calcium release from intracellular stores, whereas adenosine receptors terminate electrical activity. Calcium-mobilizing P2Y receptors are predominantly expressed in non-secretory cells of the anterior and posterior pituitary.

1. Introduction

Purines adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP) and adenosine, and pyrimidines uridine-5'-triphosphate (UTP) and uridine-5'-diphosphate (UDP) are not only energy sources and important intracellular molecules that control various cellular processes, but they are also extracellular messengers. The concept of purinergic signaling was first proposed by G. Burnstock [1]. Since then, the extracellular roles of purines and pyrimidines have been established in numerous tissues, including the pituitary gland. The effects of these messengers are mediated via two main groups of purinergic receptors: G protein-coupled adenosine receptors (ARs), also known as P1 receptors, and ATP, ADP, UTP and/or UDP-activated P2 receptors (P2Rs). ARs are a single family of receptors, whereas P2Rs belong to one of two families: G protein-coupled receptors (P2YRs) and ligand-gated ion channels (P2XRs) [2].

The release of endogenous nucleotides represents a critical component for the activation of these receptors. The roles of ATP as a neurotransmitter or co-transmitter and adenosine as an important neuromodulator are well established in the peripheral and central nervous systems. Cardiac tissue releases adenosine, whereas ATP is secreted by skeletal muscle, adrenal

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chromaffin cells, pancreatic beta cells, mast cells, blood cells, fibroblasts, and endothelial cells in response to mechanical stress, hypoxia, acidosis, osmotic shock or inflammation [3,4]. Several reports have also suggested that pyrimidines are secreted by nonexcitable cells, including endothelial, epithelial, and astrocytoma cells [5]. The duration and distance of ATP's actions are limited by several ectonucleotidases, which ensure that circulating levels of ATP are below that required for the global activation of purinergic receptors. The currently identified ectonucleotidases include members of the ectonucleotide triphosphate diphosphohydrolase family (eNTPDase) and several other subfamilies of enzymes. These enzymes not only hydrolyze extracellular ATP and/or ADP to adenosine 5'-monophosphate (AMP) and adenosine, but also metabolize other nucleotide tri- and diphosphates, including UTP and UDP [5,6].

Released ATP is a native agonist for P2XRs. Molecular, physiological, and pharmacological studies have revealed the existence of seven subunits, denoted as P2X1 through P2X7, as well as several spliced forms of these subunits. Each subunit is proposed to have two transmembrane helices connected by a large extracellular loop, with both N- and C-termini located in the cytoplasm. From the N-termini through the second transmembrane domain, the cloned subunits exhibit a relatively high level of amino acid sequence homology. In contrast, the C-termini vary in length and show no apparent sequence homology, except for the region nearest to transmembrane domain-2 [7]. The functional channels are composed of at least three subunits, which can form ion permeable pores through homo- and heteropolymerization [8,9]. P2XR subtypes differ with respect to their ligand-selectivity profiles, antagonist sensitivity, and cation selectivity. Their activation leads to an increase in the intracellular calcium concentration ($[Ca^{2+}]_i$), with Ca^{2+} influx occurring through the pores of these channels and voltage-gated Ca^{2+} channels, following the initial depolarization of cells by P2XR-generated Na^+ currents [10,11]. These receptor channels are expressed in numerous tissues, including the central nervous system, sensory, motor and sympathetic neurons, chromaffin cells, smooth muscle, testis, colon, gut, bladder, thymus, pancreas, skin, and immune cells [12].

ATP, UTP, ADP, and UDP are native agonists for the P2YRs. To date, eight mammalian P2YRs have been identified and are denoted as P2Y₁R, P2Y₂R, P2Y₄R, P2Y₆R, P2Y₁₁R, P2Y₁₂R, P2Y₁₃R, and P2Y₁₄R [13]. The missing numbers represent either non-mammalian versions of these receptors or proteins that have some sequence homology to P2YRs but do not respond to nucleotide application. In contrast to P2XRs and ARs, the genes of all P2YRs, other than P2Y₁₁R, do not contain introns in their coding sequence [12]. Four receptors, P2Y₁R, P2Y₂R, P2Y₄R, and P2Y₆R, signal through the G_{q/11}-dependent pathway, leading to the activation of phospholipase C and the generation of InsP₃ and diacylglycerol. In excitable cells, InsP₃-induced Ca^{2+} mobilization is frequently accompanied with an initial hyperpolarization of the cell membrane and an inhibition of voltage-gated Ca^{2+} influx mediated by Ca^{2+} -controlled K^+ channels. This is subsequently followed by a sustained depolarization of the plasma membrane and the facilitation of voltage-gated Ca^{2+} influx through poorly characterized pathways. Activation of MAP kinase and phospholipase D signaling pathways, both secondary to the activation of protein kinase C, has also been reported for P2YRs, as well as their coupling to G_{i/o} (P2Y₁₃R) and G_s (P2Y₁₁R) signaling pathways [14]. These receptors are expressed in the brain, lymph nodes, bone marrow, placenta, stomach, intestine, adipose tissue, epithelial, endothelial, and immune cells, osteoclasts and osteoblasts, platelets and other cell types [12].

Adenosine is a common agonist for four subtypes that comprise the AR family of G protein-coupled receptors, designated A₁R, A_{2A}R, A_{2B}R, and A₃R. Each of these receptor genes contains an intron within the coding region, and polymorphisms have been observed in the A₁R and A_{2A}R. Activation of these receptors leads to modulation of basal adenylyl cyclase activity. The A₁R and A₃R are negatively coupled to the adenylyl cyclase signaling pathway

through pertussis toxin-sensitive $G_{i/o}$. The $A_{2A}R$ and $A_{2B}R$, on the other hand, stimulate the activity of this enzyme through cholera toxin-sensitive G_s . The $A_{2B}R$ also signals through $G_{q/11}$ -dependent phospholipase C. The intracellular pathways triggered by these receptors include modulation of the activity of voltage-gated Ca^{2+} channels and K^+ channels, as well as activation of the MAP kinase family of signal transduction pathways. The physiological relevance of these receptors in modulation of cardiovascular, immune and central nervous system have been confirmed by transgenic knockout mice [2,12].

All elements required for purinergic signaling are present in the pituitary gland. ATP is released by magnocellular neurons in the posterior pituitary and by anterior pituitary cells. The pituitary gland also contains nucleotide and nucleoside-converting enzymes whose activities account for the formation of ADP and adenosine. The cells of this gland express numerous ARs, P2YRs, and P2XRs. In this paper, we will review the current state of knowledge regarding purinergic signaling in the magnocellular neurons and the pituitary gland. Earlier work in this field has been summarized in several reviews [15-19]. For the role of purinergic signaling in parvocellular hypothalamic nuclei see [20,21].

2. Release and Extracellular Metabolism of ATP

The physiological sources of the extracellular nucleotides required for activation of purinergic receptors in pituitary cells remain largely uncharacterized. In general, neurons, neuroendocrine cells and platelets release ATP by Ca^{2+} -controlled exocytosis of nucleotides stored within synaptic vesicles or dense core granules. The magnocellular neurons of the hypothalamus that control release of vasopressin and oxytocin also contain ATP and the specific pattern of action potentials originating from the cellular bodies of these neurons has been suggested to control the release of ATP [17]. However, inhibition of electrical activity in the magnocellular neurons only partially inhibits secretion, suggesting that afferent signals acting on nerve terminals in the neurohypophysis may also contribute to ATP release [22]. It has also been suggested that extracellular ATP levels could reach 4-40 μM , which are sufficient concentrations to activate the majority of P2Rs [23]. A more recent study confirmed that ATP released from the neurohypophysis during stimulation depolarizes nerve terminals and potentiates vasopressin secretion [24]. In addition to its action on nerve terminals in the posterior pituitary, released ATP probably acts on neurohypophysial astrocytes, also known as pituicytes, but does not affect oxytocin release [23,24].

We recently showed that ATP is also released by normal and immortalized anterior pituitary cells at resting conditions. Such basal ATP release was enhanced in cells treated with ARL67156, an inhibitor of ectonucleotidases [25]. GnRH-induced stimulation of gonadotropin release was accompanied by elevation in basal ATP release, raising the possibility that ATP is stored in the secretory vesicles of these cells [26]. This is consistent with an earlier study showing calcium-dependence of ATP release [27] and modulation of ATP release by prolactin secretagogues [28]. On the other hand, facilitation of prolactin release did not elevate ATP secretion in perfused pituitary cells, suggesting that it could also be released by another mechanism, possibly from pituicytes. Consistent with our findings, both secretory and non-secretory ATP release pathways were found to be operative in human astrocytes [29]. In other tissues, ABC-binding cassette transporters, hemichannels, and P2X7R, among others have been suggested to participate in non-vesicular ATP release [30]. Interestingly, pituitary cells express functional multidrug resistance proteins [31] and P2X7R [32], which could contribute to ATP release. Finally, we showed that endogenous release of ATP by cultured cells is of sufficient amplitude to desensitize homomeric and heteromeric P2X3Rs, confirming that the measured ATP is biologically active [25]. Certainly, these *in vitro* conditions should not accurately reflect the *in vivo* situation, where the tissue ATP concentration represents a balance between rates of release, hydrolysis, and dilution into intercellular compartments.

The action of ATP as an autocrine/paracrine factor is critically dependent on its rapid metabolism by ectonucleotidases [6]. These enzymes are also expressed on the plasma membrane of pituitary cells and neurosecretory terminals [33]. Extracellularly added ATP is rapidly hydrolyzed by isolated neurohypophysis, indicating that the ectonucleotidases are functional. The group that discovered this also detected accumulation of adenosine when AMP was added to isolated neurohypophysis, suggesting that these enzymes provide a pathway for the activation of ARs in this tissue. This, in turn, is likely to account for the termination of ATP-induced vasopressin release [22]. mRNA transcripts for plasma membrane-located eNTPDases 1, 2 and 3 are expressed in hypothalamic and pituitary tissues, cultured pituitary cells and immortalized lacto-somatotrophs, corticotrophs, gonadotrophs, as well as in GnRH-secreting cells (Fig. 1A and B) [25]. Depending on the cell type and the rates of perfusion, pituitary cells were able to degrade between 30 and 70% of extracellularly added ATP (Fig. 1C). The ectonucleotide cascade not only terminates the extracellular messenger functions of ATP but also provides a pathway for the generation of ADP and adenosine, which in pituitary cells may activate some P2YRs and ARs, respectively (see below). Ecto-5'-nucleotidases (CD73), which generate adenosine from AMP, were found by immunocytochemistry in about 20% of anterior pituitary cells [34].

3. Expression and Signaling by P2XRs

Initial knowledge about the expression and role of P2Rs in the posterior pituitary was obtained in experiments using Ca^{2+} measurements and hormone secretion. These studies revealed that extracellularly added ATP lead to an increase in $[\text{Ca}^{2+}]_i$ and vasopressin secretion, and that these actions were strongly and reversibly inhibited by the P2R blocker suramin (reviewed in [17]). P2XRs have been suggested to mediate the action of extracellular ATP on vasopressin release [35]. Initially, the role of P2X2Rs in ATP-induced increases in $[\text{Ca}^{2+}]_i$ and peptide release from rat isolated neurohypophysial terminals had been suggested [23]. A more recent study indicated that a functional mixture of homomeric P2X2R and P2X3R mediates the majority of ATP's responses in vasopressinergic neurohypophysial terminals. The same study also provided the first electrophysiological evidence for the existence of P2XR currents in vasopressinergic neurohypophysial terminals and showed the lack of expression of P2XRs in terminals labeled for oxytocin [36]. ATP also plays a role in vasopressin release by activating postsynaptic receptors on perikarya or dendrites of supraoptic neurons [37]. At the present time, there is no evidence for the functional role of P2XRs in pituitary cells.

Single-cell $[\text{Ca}^{2+}]_i$ measurements, along with studies on hormone secretion in cells stimulated with various agonists in the presence and absence of P2XR inhibitors were also instrumental in the initial characterization of purinergic receptors in anterior pituitary cells [26,28,32,38, 39]. These experiments revealed that functional P2XRs are operative in all secretory cell types and raised the possibility that several subtypes of these channels could be expressed in a cell type-specific manner. However, this method is of limited use for the identification of the receptor subtypes expressed, especially in characterizing the rapidly desensitizing homomeric and heteromeric P2XRs, and their roles in electrical activity [40].

In more recent studies, we combined molecular biology techniques with electrophysiology to obtain additional data on expression and roles of these channels in pituitary cells. With the use of specific rat P2X primers and sequence analysis, P2X2, P2X3, P2X4, and P2X7 mRNA transcripts were detected in a mixed population of anterior pituitary cells (Fig. 2A, lane 1). Immortalized GH₃ pituitary cells also expressed transcripts for P2X3, P2X4, and P2X7 subunits, but not the P2X2 subunit (Fig. 2A, lane 4). The mRNA levels of P2XRs in anterior pituitary cells were also examined by *in situ* hybridization. Figure 2A, top left panel, shows the position of the anterior and posterior pituitary. In parallel to RT-PCR analysis, the mRNA hybrids of the P2X2, P2X3, P2X4, and P2X7 subunits were also present in the anterior pituitary.

The P2X4 mRNA subunit was more pronounced in the anterior than in the posterior pituitary, whereas the expression of the P2X3 subunit was relatively low in both areas. These mRNA expression patterns were consistent with immunohistochemical studies in the rat hypothalamo-neurohypophyseal system at the electron microscope level. Immunoreactivity to P2X2Rs was localized in paraventricular and supraoptic nuclei, as well as in the posterior pituitary – in pituicytes and a subpopulation of neurosecretory axons [41]. Immunocytochemical labeling in vasopressinergic terminals also suggested the existence of P2X2, P2X3, P2X4, and P2X7 subunits [36].

Abundant PCR products of two different sizes, approximately 1.6 and 1.4 kb long, were consistently amplified by the P2X₂ primers in mixed rat pituitary cells (Fig. 1A). Earlier experiments revealed that the primary P2X₂ gene transcript in rats undergoes extensive alternative splicing resulting in modified mRNA sequences. One of the spliced subunits, termed P2X2b, lacks a series of 69 C-terminal amino acids and creates a functional homomeric channel, which desensitizes more rapidly than the full-size receptor, termed P2X2a [42,43]. This form of the subunit was also detected in other tissues [44-46]. It has also been suggested that electrostatic charges of six amino acid side chains located close to the proximal splicing site play a critical role in controlling the rate of receptor desensitization [42].

Recently, we also analyzed the expression of P2X2Rs in mouse pituitary cells and investigated the functional significance of the N- and C-termini specific structures of these receptors in terms of current, calcium signaling, and inter-subunit interactions in living cells. This study showed that the mouse pituitary expresses three forms of P2X₂ subunits: the full size P2X2a and the spliced forms P2X2b and P2X2e, which are missing 69 and 90 residues in their C-termini, respectively. Furthermore, all three mouse forms have an additional 13 N-terminal residues not present in the rat pituitary gland. Electrophysiological experiments revealed that the rate of homomeric P2X2eR desensitization was comparable to the rates of rapidly desensitizing P2X1R and P2X3R. The rate of P2X2bR desensitization, however, was faster than P2X2aR but slower than P2X2eR (Fig. 3). These deletions in the C-terminal, in turn, effectively reduced the peak amplitude and duration of calcium signals [47]. The ion-permeating pores of P2XRs are formed by three subunits, and because the same primary P2X₂ transcript can yield three different splicing variants, it is probable that a single pituitary cell expresses a series of homo- and heteromeric P2X2Rs. The structure of the N-terminal tail also influences the duration of P2X2R-mediated channel signaling in the continuous presence of an agonist. Specifically, deletion of the first 13 amino acids at the N-terminus of the mouse P2X2R resulted in accelerated desensitization rates of all C-terminal splicing isoforms [47].

Single-cell patch-clamp analysis in gonadotrophs from embryonic, neonatal, and adult rats revealed that ATP application in all gonadotrophs generates a depolarizing and non-oscillatory current (Fig. 4B), in contrast to GnRH that triggered an oscillatory hyperpolarizing current (Fig. 4A). This clearly indicates that Ca²⁺-mobilizing P2YRs are not expressed in this particular cell type. The biophysical and pharmacological properties of ATP-induced depolarizing current (kinetics of activation, deactivation, desensitization, and resensitization) in gonadotrophs from adult and neonatal animals were comparable with those observed in cells expressing recombinant P2X2R. Sensitivity of ATP-evoked current to inhibition by pyridoxal 5-phosphate 6-azophenyl-2',4'-disulfonic acid, reactive blue 2, and suramin, as well as the lack of effect of ivermectin, further confirmed this conclusion. ATP-induced rapid depolarization of gonadotrophs leads to the initiation of firing in quiescent cells, an increase in the frequency of action potentials in spontaneously active cells (Fig. 4C), and a transient stimulation of LH release. ATP also influenced GnRH-induced currents and membrane potential oscillations as well as LH release in an extracellular Ca²⁺-dependent manner. These InsP₃-dependent oscillations were facilitated, slowed, or stopped, depending on the ATP concentration, the time of its application, and the level of Ca²⁺ content in intracellular stores. These results indicate

that in gonadotrophs P2X2Rs could operate as pacemaking channels and modulators of GnRH-controlled electrical activity and secretion [48].

Approximately 90% of identified lactotrophs, from neonatal to adult, also responded to ATP application by generating inward depolarizing currents. Although the average peak amplitude of currents in response to supramaximal ATP concentrations was several-fold higher in gonadotrophs than in lactotrophs, the profiles of currents were highly comparable in the two cells types. Ivermectin, the specific P2X4R allosteric modulator, affected the ATP-induced current in lactotrophs but not in gonadotrophs. In the presence of ivermectin, there was large, roughly 6-fold increase in the maximum amplitude of current. Ivermectin also significantly increased the sensitivity of channels for ATP, delayed the deactivation of receptors, and enhanced ATP-induced PRL release. These results indicate that lactotrophs express homomeric and/or heteromeric P2X4Rs, which could operate as pacemaking channels [49].

4. Expression and Signaling by P2YRs

Since pituicytes are intimately associated with neurosecretory terminals, the released ATP from neuronal endings could affect their function in neurohypophysis. In the majority of pituicytes in primary culture, ATP triggers a rapid extracellular calcium-independent rise in $[Ca^{2+}]_i$, which is abolished in cells when phospholipase C and the endoplasmic reticulum calcium pump are blocked, indicating the functional operation of calcium-mobilizing P2YRs [50]. The stimulatory action of ATP is followed by potassium efflux [51]. The identification of the P2YR mediating the action of ATP in pituicytes has not been completed. The pharmacological profile of the calcium response in these cells suggests that ADP, UTP, and UDP are not endogenous agonists for these receptors [50].

Sheep and rat anterior pituitary cells also express P2YRs and their activation by ATP is associated with an elevation in $[Ca^{2+}]_i$ due to $InsP_3$ -dependent release of Ca^{2+} from intracellular stores [52,53]. Molecular cloning and the functional characterization of rat P2YRs in the pituitary revealed the expression of P2Y₂R with a pharmacological profile resembling that of the sheep pituitary [54]. For this type of receptor, ATP and UTP are equipotent agonists. The presence of UTP-sensitive P2YRs has been suggested in rat pituitary folliculo-stellate cells in primary culture [55]. The expression of these receptors has also been suggested in gonadotrophs [56] and lactotrophs [57], but our laboratory was unable to confirm these findings [26,58].

In addition to P2Y₂R, RT-PCR analysis revealed the presence of transcripts for G_q-coupled calcium-mobilizing P2Y₁R, P2Y₄R, and P2Y₆R, as well as G_i-coupled P2Y₁₂R, in mixed anterior pituitary cells (Fig. 5A). Unlike UTP and UDP, in the absence of extracellular Ca^{2+} , ATP and ADP triggered calcium signaling in about 40% of lactotrophs and prolactin release in mixed populations of secretory pituitary cells. A fraction of other unidentified cells also responded to ATP/ADP application with a rise in $[Ca^{2+}]_i$ (Fig. 5B-D). The ligand-selectivity profile of calcium mobilization-dependent signaling and the blockade of these responses by pyridoxal 5-phosphate 6-azophenyl-2',4'-disulphonic acid indicated that P2Y₁Rs could be responsible for extracellular Ca^{2+} -independent stimulation of prolactin release, but further pharmacological characterization of these receptors is required [58].

5. Expression and Signaling by ARs

Extracellular ATP has a profound effect on the three-dimensional structure of pituicytes; it changes them from a flat shape to a stellate morphology. However, this action of ATP is not mediated by P2Rs, but rather requires both its hydrolysis by ectonucleotidases and the activation of ARs [59]. The action of adenosine in the posterior pituitary is not restricted to astroglial cells, but also activates ARs expressed in neurohypophysial terminals, leading to the

inhibition of N-type calcium channels. In turn, this attenuates high K^+ -induced release of vasopressin and oxytocin by activating the A_1R subtype [60]. Conversion of ATP to adenosine also terminates the stimulatory action of endogenously released and exogenously added ATP on vasopressin release from hypothalamo-neurohypophysial explants [61,62]. These results indicate the dual control of the stimulatory action of ATP on neuropeptide release by ectonucleotidases and the subsequent activation of A_1Rs .

The expression and role of A_1Rs in the anterior pituitary gland has been reviewed [18,19]. Briefly, functional receptors in immortalized pituitary cells were first described in 1985 [63]. Later, they were also identified in pituitary lactotrophs [64,65]. Pharmacological, electrophysiological, and secretory data indicated the negative coupling of these receptors to the adenylyl cyclase signaling pathway through pertussis toxin-sensitive G proteins, causing the abolition of spontaneous electrical activity, Ca^{2+} signaling, and hormone release. Cessation of spontaneous electrical activity by adenosine probably occurs indirectly, by the activation of voltage-gated K^+ channels, and/or directly by the inhibition of voltage-gated Ca^{2+} channels. Pituitary cells also express $A_{2A}R$, $A_{2B}R$, and A_3R [66-68]. The $A_{2B}Rs$ are expressed in folliculo-stellate cells, stimulate IL-6 secretion [69], and trigger the formation of gap junctions [34]. A_3Rs could contribute to the antiproliferative activity of adenosine in pituitary tumor cells [68].

6. Concluding Remarks

Once secreted by pituitary cells, the extracellular messenger actions of ATP are controlled by ecto-ATPases and ecto-5'-nucleotidase, which degrade ATP to ADP, AMP, and adenosine. While the capacity of these enzymes to hydrolyze ATP and generate adenosine from extracellular AMP is well established, further work is required to clarify the mechanism of ATP release by posterior and anterior pituitary cells. ATP, ADP, and adenosine are native agonists for pituitary cells and have dual actions on Ca^{2+} signaling: stimulatory and inhibitory, depending on the receptor subtype expressed. ATP activates $P2XRs$, which are expressed in all secretory anterior pituitary cells, as well as in nerve terminals in the posterior pituitary. There is molecular evidence for the expression of $P2X_2$, $P2X_3$, $P2X_4$, and $P2X_7$ subunits and immunocytochemical evidence for the expression of the $P2X_6$ subunit. Electrophysiological studies have established the expression of $P2X_2Rs$ in vasopressinergic neurohypophysial terminals and gonadotrophs, as well as the expression of $P2X_4R$ in lactotrophs. These receptors facilitate the firing of action potentials, elevate $[Ca^{2+}]_i$, and stimulate hormone release. The identification of the $P2XR$ subtype(s) expressed in somatotrophs, thyrotrophs, corticotrophs, and pituicytes necessitates further investigation. Molecular and physiological evidence for the expression of several Ca^{2+} -mobilizing $P2YRs$ has also been obtained. In contrast to $P2XRs$, the expression of these receptors is more limited in the anterior pituitary gland. A fraction of lactotrophs probably expresses $P2Y_1R$, whereas folliculo-stellate cells express $P2Y_2Rs$. Other secretory anterior pituitary cells also express $P2YRs$, but their identification will require additional studies. ADP is a potent agonist for the activation of $P2Y_1R$ in anterior pituitary cells. Posterior and anterior pituitary cells also express ARs . Among the members of this family of receptors, the best characterized are the A_1Rs . These receptors are coupled to the $G_{i/o}$ signaling pathway and effectively attenuate electrical activity through multiple mechanisms. Such organization of the purinergic pathway in the pituitary gland provides an effective but transient mechanism for the action of ATP as an agonist. The details of this mechanism include the prolonged activation of $P2Y_1R$ by ADP as well as termination of the stimulatory actions of $P2XRs$ not only by hydrolysis of ATP, but also by the generation of adenosine and activation of A_1Rs . Future work on the mechanism of purinergic release and the distribution of specific purinergic signaling elements should help us unravel the spatial and temporal aspects of ATP's actions in the pituitary gland.

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Fig. 1.

Expression and activity of ectonucleotidase in hypothalamic and pituitary tissues, pituitary cells, and immortalized hypothalamic (GT1-1) and pituitary (At-T20, αT3-1, and GH₃) cells. *A and B*, Expression of ectonucleotidase eNTPDase 1-3 transcripts in hypothalamic and pituitary tissues and cells. In both panels, DNA markers are shown in the last right lanes. In this and following figures, for negative controls, PCR was conducted performing first-stand cDNA synthesis without RT (-). *C*, Cell-type specificity of extracellular ATP metabolism. Chambers without and with cells (5×10^6 per column) were perfused with Krebs-Ringer buffer at flow rate of 0.8 ml/min. Samples were collected every minute and immediately tested for ATP concentrations. *Gray areas* indicate the duration of ATP application and its concentrations in the medium. *Traces* show measured ATP concentrations in effluents.

Fig. 2. Expression of P2XR mRNAs in the pituitary gland. *A*, Detection of mRNA transcripts for P2XRs in anterior pituitary cells (line 1) and a GH₃ cell line (4). Lines 2 and 5 indicate positive controls and lines 3 and 6 negative controls. *B*, Detection of mRNA transcripts in the rat pituitary by *in situ* hybridization. *PP*, posterior pituitary, *AP*, anterior pituitary.

Fig. 3. Electrophysiological characterization of P2X2Rs cloned from mouse anterior pituitary cells. *Top panel*, Schematic representation of splice forms of P2X2R in pituitary cells. Horizontal dotted lines indicate missing sequences. Black areas indicate transmembrane domains (TM1 and TM2). *Bottom panels*, Patterns of ATP-induced current profiles in GT1 neurons (*left*) and HEK293 cells (*right*) expressing recombinant P2X2Rs. Green traces, P2X2e currents; red traces, P2X2bR currents; blue traces, P2X2aR current. Horizontal bars indicate duration of ATP application.

Fig. 4. ATP-induced current and pacemaking activity in identified rat pituitary gonadotrophs. *A*, GnRH-induced outward oscillatory calcium-activated SK type potassium current. HP, holding potential. *B*, ATP-induced non-oscillatory inward current. *Gray trace*, experimental record; *black solid line*, the monoexponential fitting curve for the decay of current in the presence of ATP (desensitization). *C*, Concentration-dependent effects of ATP on the frequency of action potentials and the level and duration of afterhyperpolarization in a spontaneously active cell. Horizontal bars indicate duration of agonist application.

Fig. 5. Characterization of P2YRs expressed in rat anterior pituitary cells. *A*, Detection of P2YR mRNA transcripts in a mixed population of anterior pituitary cells. *B-D*, ATP (10 μ M)-induced calcium mobilization from endoplasmic reticulum in pituitary cells bathed in calcium-deficient medium. Numerators indicate number of cells responding to ATP and TRH (*B*, lactotrophs), TRH only (*C*, lactotrophs), and ATP only (*D*, unidentified pituitary cells). Arrows indicate moments of agonist applications, which were present in medium to the end of recordings.