PURINERGIC RECEPTOR TYPES in the HYPOTHALAMIC-NEUROHYPOPHYSIAL SYSTEM

José R. Lemos¹, Edward E. Custer¹, and Sonia Ortiz-Miranda²

¹MaPS, Univ. Mass. Med. School, Worcester, MA 01605

²Neurobiology Depts. & Prog. Neurosci., Univ. Mass. Med. School, Worcester, MA 01605

Abstract

Many different types of purinergic receptors are present in the Hypothalamic-Neurohypophyseal System (HNS), which synthesizes and releases vasopressin and oxytocin. The specific location of purinergic receptor subtypes has important functional repercussions for neuronal activity and synaptic output. Yet, until the advent of receptor KOs, this had been hindered by the low selectivity of the available pharmacological tools. The HNS offers an excellent opportunity to differentiate the functional properties of these purinergic receptors in cell bodies vs. terminals of the same physiological system. P2X2, P2X3, P2X4 and P2X7 receptors are present in vasopressin terminals while oxytocin terminals exclusively express the P2X7 subtype. The latter is not functional in the cell bodies of the HNS. These purinergic receptor subtypes are permeable to sodium vs. calcium in varying amounts and this could play an important role in the release of vasopressin vs. oxytocin during bursting activity. Endogenous ATP and its metabolite, adenosine, have autocrine and paracrine modulatory effects on the release of these neuropeptides during physiological stimulation. Finally, we hypothesize that during such action potential bursts, ATP potentiates the release of vasopressin but not of oxytocin, and that adenosine, via A1 receptors, inhibits the release of both neuropeptides.

Keywords

Vasopressin (AVP); Oxytocin (OT); P2X receptors; ATP; Adenosine; A receptors

Introduction

The use of pharmacological inhibitors has suggested a key role for purinergic receptors in synaptic plasticity and in many neuronal diseases (11). Until recently, the specific receptors involved were not well understood, however, because such studies relied exclusively on the use of pharmacological agents that cannot truly discriminate between purinergic receptors (21). Purinergic receptor knockouts (rKO) have provided a way to overcome these pharmacological limitations (3, 6). Such knockouts have been vital in elucidating the role of

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Corresponding Author: - sonia.ortiz-miranda@umassmed.edu.
DR JOSE LEMOS (Orcid ID : 0000-0002-9234-0680)
DR SONIA ORTIZ-MIRANDA (Orcid ID : 0000-0002-6657-3691)

adenosine triphosphate (ATP) receptors in pain mechanisms of sensory neurons (4) and in establishing which types of purinergic receptors are modulating neuropeptide release (6, 18).

Adenosine Triphosphate

The importance of ATP as a neurotransmitter is demonstrated by its extensive involvement in multiple processes throughout the nervous system; including modulation of long-term potentiation and depression, pain transduction, bladder control, modulation of vascular tone, and control of the gastrointestinal system (2, 14,13). ATP may differentially affect plasticity depending upon which type of purinergic receptor it activates and whether that receptor is located pre- or post-synaptically (14, 24, 45, 31, 13). Thus, the ATP receptor subtype and its localization at central nervous system (CNS) synapses is critical, but for the most part, still unknown.

Extracellular ATP communicates physiological signals through the activation of P2X ligand-gated cation channels (ionotropic) and P2Y G-protein-coupled (metabotropic) receptors. Of the seven P2X receptor subunits (P2X1–P2X7), a great deal of evidence supports a major role for P2X2 and P2X3 subunits in mediating the primary effects of ATP on sensory neurons (2, 28). The P2Y receptor has important functions in glia of the neurohypophysis (43) and in modulation of microglial cell activation (39). ATP increases intracellular calcium in supraoptic neurons by activation of both P2X and P2Y purinergic receptors (38). However, the relative contribution of these receptor subtypes to different functions of ATP is still poorly understood (3).

Adenosine

Adenosine, which is a product of ATP hydrolysis, is produced by ecto-ATPases (41) localized to the outer surface of plasma membranes (Fig. 1). This purine interacts with a family of four (A1, A2a, A2b, & A3) adenosine receptors (11, 29). The A1 receptor, in particular, is highly and widely expressed in the brain (10). Adenosine-mediated effects that occur via the A1 receptor include depression of neurotransmission, sleep induction, antinociception, and ethanol-induced motor incoordination (36, 29). Adenosine also inhibits voltage-dependent Ca^{2+} currents in rat dissociated supraoptic neurones via A1 receptors (27). Adenosine receptors are major targets of caffeine, the most commonly consumed drug in the world. There is growing evidence that they could also be promising therapeutic targets for many conditions, including cerebral and cardiac ischemias, sleep disorders, immune and inflammatory disorders and cancer (11). Activation of adenosine A2a receptors alters postsynaptic currents and depolarizes neurons of the supraoptic nucleus (34). The A2b receptor also mediates adenosine inhibition of taurine efflux from pituitary cells in neurohypophysis (32). The A3-receptor subtype, however, have no relevant effect on hypothalamic activity (32).

Hypothalamic-Neurohypophyseal System

In the HNS, two neuropeptides, AVP and OT, are synthesized in magnocellular neurons located in the hypothalamus and released at peripheral sites in the neurohypophysis (23).

Both hormones may also be central neurotransmitters and have been implicated in sexual behavior (16), stress, learning, and memory processes (9). Purinergic receptors are present (17, 37) throughout the HNS (25). Local regulation of vasopressin and oxytocin secretion by extracellular ATP has been shown in the isolated posterior lobe of the rat neurohypophysis (40, 42). In the intact HNS however, purinergic and adrenergic agonists seem to synergize in stimulating vasopressin and oxytocin release (16).

Purinergic receptors subtypes

Our laboratory has identified the presence of P2X2, P2X3, P2X4 and P2X7 receptors in AVP terminals while OT terminals exclusively express the P2X7 subtype. ATP stimulation of identified (8) rat AVP-neurohypophysial (NH) terminals (NHT) generate currents with an EC_{50} of $\sim 10 \mu M$ (17, 20). In contrast, dose-response data show that OT terminals have a much lower sensitivity (EC_{50} of $>100 \mu M$) to ATP (5) than AVP terminals. OT-NH terminals demonstrate increased responses to the P2X7R selective agonist Benzyl-ATP (BzATP) when compared to equivalent doses of ATP (6). In agreement with the electrophysiological results, P2X2 receptors specifically regulate AVP release (19), while OT release appears to be regulated by P2X7 receptors (5). In contrast, activation of adenosine receptors inhibits release of both neuropeptides (44).

We have also examined P2X7R localization (5), by using a stereo-specific ecto-P2X7 antibody. While the SON and PVN contain its mRNA (37) and show P2X7R immunoreactivity (IR), the receptor is absent from membranes of magnocellular neurons. In contrast, AVP- and OT-terminals demonstrate P2X7R-IR suggesting that this receptor subtype is only targeted to the plasma membrane of secretory terminals (Fig. 1 & ref. 17). Together these pharmacological and immunohistochemical results are consistent with the presence of a functional P2X7R in both types of HNS terminals. We believe that this helps clear up a major P2X7R controversy; *i.e.* whether P2X7Rs are actually functional in neurons. Thus, our data establishes that these receptors are only functional at terminals and not at somata of neurons (5, 17, 20). This is, to our knowledge, the first direct evidence of a P2X7R function in neurons.

Receptor ion selectivity

The P2X receptor subtypes are known to be cation selective (28). We (7) have recently shown that both sodium (Na^+) and calcium (Ca^{2+}) are permeable through purinergic receptors in NH terminals and that the overall magnitude of the ATP-current is inversely related to the amount of extracellular calcium ($[Ca^{2+}]_o$). Increasing levels of $[Ca^{2+}]_o$ decrease the ATP-current. Moreover, neuropeptide release exhibit a biphasic response to exogenous ATP, where the transient release appears to be through P2X2 and/or P2X3 receptors and is dependent on extracellular calcium. The sustained release, on the other hand, seems to be mediated by P2X7 receptors and is dependent on extracellular Na^+ . Thus, these receptors might be localized not only to different terminal types but also to different functional areas of the same terminals (see Fig. 1). P2X2 and/or P2X3 receptors could be co-localized with release sites and thus Ca^{2+} entry through them can directly cause release. In contrast, P2X7 receptors could be localized away from release sites, so that only Na^+ entry

through them can cause release (7). Since, extracellular Ca^{2+} is depleted during bursting stimulation of these NH terminals (1), these differences could play an important role during such physiological activity (22).

Endogenous Purines

ATP is concentrated in the secretory granules of the NH (33) and is co-released with the neuropeptides (42, 40, 18, 19). Although there is possible regulation by pannexin channels of ATP-induced currents in vasopressin neurons from the rat supraoptic nucleus, there appears to be no release of ATP via connexin channels (30).

Endogenous ATP, and its metabolite adenosine, have autocrine and paracrine modulatory effects on the release of AVP vs. OT (22) during physiological bursting patterns of stimulation (35). Activity-dependent feedback modulation of spike patterning of supraoptic nucleus neurons by endogenous adenosine has been shown to be via A1R (1). This means that similar or even greater levels of endogenous adenosine should be achieved in NH during a burst stimulation (19). Our group (20), as well as others (43, 39), have shown that ATP is released in sufficient quantities to affect purinergic receptors in the HNS and subsequent release of the peptides (18).

Importantly, treatment of neurohypophyses from WT mice with suramin/PPADS (pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid) significantly reduced electrically stimulated AVP release. A similar inhibition by these P2X receptor antagonists was observed in electrically stimulated neurohypophyses from P2X3 and P2X7 rKO mice but not from P2X2/P2X3 rKO mice, confirming that the endogenous ATP facilitation of electrically stimulated AVP release is mediated primarily by the activation of the P2X2 receptor (5). An enhancement of OT release is not observed under these conditions. The role of P2X4 receptors is questionable since none of the blockers used in this or other (20) studies affect its function. P2X3 and P2X4 receptors could, instead, have modulatory effects at the level of the HNS somata.

Finally, using loose-patch clamp on the intact neurohypophysis, the A1 antagonist 8-cyclopentyltheophylline (CPT) increased voltage-dependent currents in wild type but not in adenosine receptor A1KO mice. In **agreement**, pretreatment with CPT potentiated electrically-stimulated AVP and OT release from wild type mice by approximately 20%, but had no effect on A1KO mice (18).

Model

As a result of this data, we hypothesize (see Fig. 1 in ref. 25) that during the initial period of a physiological AVP-like burst, co-released ATP has a positive feedback effect via P2X2 receptors by increasing $[\text{Ca}^{2+}]_i$ and subsequent AVP release. In the late portion of the burst, the hydrolysis by ecto-nucleotidases of ATP to adenosine leads, via activation of A1 receptors, to the inhibition of N-type calcium channels. Since this receptor sub-type is present in both types of NH terminals, the release of both neuropeptides could be reduced by the concurrent decrease in calcium influx. However, since there are no ecto-nucleotidases on OT terminals (Fig. 1), there should be no effects by adenosine on its release. Furthermore,

during a physiological OT-like burst, co-released ATP probably does not have a positive feedback effect on OT release via increases in $[Na^+]_i$, because those terminals only express the lower-affinity P2X7 receptor. Interburst silent periods are necessary for the clearance of the accumulated purines (22, 26).

Conclusions

The HNS provides an excellent example of a physiological system where the specific expression and localization of purinergic receptor subtypes modulates neuronal activity and has profound implications on how synaptic output is regulated. The HNS shows differences in functional purinergic receptors not only between cell bodies and their terminals (17), but also between and within terminal types. Furthermore, it has allowed resolution of the functional P2X7 receptor controversy in neurons (5). The use of specific knockouts for purinergic receptors is an important tool that has allowed delineation of their functions at specific cellular compartments of these CNS neurons.

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Fig. 1. Purinergic modulation of AVP vs. OT release from terminals in the HNS

Upon depolarization calcium channels (L-, N-, R- or P/Q-type) open and calcium influx leads to release of AVP and OT. ATP is co-released with the neuropeptides at concentrations that are sufficient to activate P2X2, P2X3 & P2X4 receptors causing an influx of calcium and thus increasing $[Ca^{2+}]_i$ and subsequent AVP release. ATP levels, however, are probably insufficient to activate P2X7 receptor and OT release via increasing $[Na^+]_i$. Hydrolysis of ATP to adenosine by ecto-nucleotidases on only AVP terminals leads to A1R G-protein mediated inhibition of N-type Ca-channels and of release of OT and AVP from NH terminals. Names in red are those with most important functions in each type of terminal. (modified from ref.26)