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Research report

Does conversion of ATP to adenosine terminate ATP-stimulated vasopressin release from hypothalamo-neurohypophyseal explants?

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Abstract

ATP stimulates vasopressin (VP) release from explants of the hypothalamo-neurohypophyseal system (HNS), but the response is not sustained for the duration of exposure to ATP. Since adenosine, a metabolite of ATP, inhibits VP release from neurohypophysial terminals and adenosine receptors (AR) are expressed in supraoptic nucleus (SON) neurons, we postulated that conversion of ATP to adenosine contributed to termination of ATP-stimulated VP release from HNS explants. This was tested using a non-selective AR antagonist, 5-amino-9-chloro-2-(2-furyl)-1, 2, 4-triazolo [1, 5-*c*] quinazoline (CGS-15943). CGS-15943 did not affect basal VP release and did not alter the initial response to ATP. A selective A_1R antagonist, 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX), increased basal VP release at 1 μM, without altering the response to ATP. However, at a higher concentration of DPCPX (10 μM), VP release was enhanced by ATP for an extended period of time. Inhibition of the enzymatic conversion of ATP to adenosine using a combination of a potent ecto-5′-nucleotidase inhibitor, α,β-methylene adenosine 5′-diphosphate (AMP-CP), and a competitive substrate for ecto-5′-nucleotidase (guanosine monophosphate, GMP) did not affect basal VP release. Enzymatic inhibition did slightly prolong the response to ATP, but it was not sustained for the duration of exposure to ATP. We conclude that an endogenous inhibitory influence of adenosine decreases basal VP release from HNS explants and that conversion of exogenously applied ATP to adenosine contributes to termination of ATP-induced stimulation of VP release, but additional mechanisms such as receptor desensitization also limit the response to extended exposure to ATP.

Theme: Endocrinology and autonomic
Topic: Neuroendocrinology: other

Keywords: Supraoptic; Neurohypophysis; Vasopressin; Oxytocin; Adenosine; ATP

1. Introduction

ATP stimulates vasopressin (VP) release from explants of the hypothalamo-neurohypophyseal system (HNS) by activation of purinergic P2 receptors [5]. However, the response to exogenous ATP is transient [5]. Mechanisms that may contribute to the transient nature of the response include desensitization of ATP receptors and metabolism of ATP. The latter possibility is the focus of this study because adenosine, an inhibitory neurotransmitter, is produced by metabolism of ATP.

Adenosine has been shown to function as a neuromodulator in several regions of the CNS [3]. It can be formed in the extracellular space from ATP by the action of a series of ecto-enzymes with ecto-5′-nucleotidase being the last and rate-limiting enzyme in the pathway [2]. It is also released from both neurons and glia via non-vesicular release mechanisms following intracellular metabolism of ATP [6–8]. Once in the extracellular space, adenosine can influence neuronal activity by interacting with a family of G-protein-

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coupled adenosine receptors (A_1, A_2, and A_3) [14]. Most of the CNS effects of adenosine are probably mediated by the A_1 and A_{2a} high affinity receptors. The A_1 receptor is generally coupled to inhibitory G proteins (Gi/Go), while the A_2 receptors are coupled to stimulatory G proteins (Gs). Thus, A_1 receptors inhibit adenylyl cyclase, while A_2 receptors stimulate this enzyme resulting in increased production of cAMP [14]. However, since A_1 receptors have presynaptic effects on both inhibitory and excitatory afferents as well as postsynaptic actions, their activation can result in either increased or decreased neuronal activity. Both presynaptic and postsynaptic actions of adenosine have been demonstrated in the supraoptic nucleus (SON) of the hypothalamus where the magnocellular vasopressin (VP) and oxytocin (OT) neurons are located that project to the neural lobe and release VP and OT into the general circulation. Adenosine can act presynaptically via A_1-type adenosine receptors to inhibit both excitatory and inhibitory afferents [11]. It also has been shown to inhibit voltage-dependent Ca^{++} channels via A_1 receptors in dissociated supraoptic neurons [10]. These observations are consistent with the reports of A_1 mRNA and immunoreactivity in SON [13,15] as well as the detection of all subtypes of adenosine receptors in SON by RT-PCR [10]. These presynaptic and postsynaptic actions have the potential of significantly influencing hormone release from the neural lobe. Voltage-sensitive Ca^{++} channels contribute to facilitation of hormone release by spike broadening and by their contribution to the initiation of phasic firing [1]. Adenosine has also been shown to influence VP and OT release by acting on nerve terminals and pituicytes in the neural lobe [16,20]. It blocks N-type Ca^{++} channels and inhibits VP and OT release from isolated neurohypophysial terminals [20], but it induces stellation of cultured pituicytes [16] suggesting that it may facilitate movement of the hormone into the blood.

Since activation of adenosine receptors by endogenous adenosine has been demonstrated in SON and ATP release is known to occur from nerve terminals in the neural lobe [4,11,19,21] and since we have shown that endogenous release of GABA and glutamate is determinants of basal hormone release from explants of the hypothalamo-neurohypophyseal system (HNS) [17,18], we postulated that endogenous adenosine might also be a determinant of basal release. In addition, since hormone release in response to exogenous ATP is transient in HNS explants [5], we postulated that conversion of ATP to adenosine might inhibit neuronal activity leading to termination of the ATP-induced increase in hormone release. Therefore, experiments were performed to assess the effect of adenosine receptor antagonists as well as inhibitors of enzymatic conversion of ATP to adenosine on basal and ATP-stimulated VP release from HNS explants.

2. Materials and methods

2.1. Perifusion of HNS explants

Explants of the HNS were obtained from decapitated male Sprague–Dawley rats (125–150 g). They were dissected from the base of the hypothalamus and included the VP neurons of the supraoptic nuclei (SON) with their axonal projections extending through the median eminence and terminating in the neural lobe (NL). They also included the organum vasculosum of the lamina terminalis (OVLT), and the arcuate nuclei, but not paraventricular nuclei (PVN). Explants were perifused individually in closed culture chambers (0.5 ml) at 37 °C with oxygenated culture medium at a rate of 2.0 ml/h. The medium consisted of F12 nutrient mixture (Sigma) fortified with 20% fetal bovine serum, 1 mg/ml glucose, 50 μU/ml penicillin, and 50 μg/ml streptomycin. Bacitracin at 0.28 mM was added to the medium to prevent degradation of VP. The explants were equilibrated for 4 h and then maintained under different treatment conditions for additional 4 to 5 h. Drug delivery to the explants occurred at approximately 40 min after its addition to the perifusate (e.g. at 4.6 h). Outflow was collected individually at 20-min intervals using a refrigerated fraction collector maintained at 4 °C.

2.2. Experimental design

Experiment 1: Explants were maintained in control medium or treated with ATP (100 μM), CGS15943 (1 μM; 5-amino-9-chloro-2-(2-furyl)-1, 2, 4-triazolo [1, 5-*c*] quinazoline, a non-selective antagonist of adenosine receptors), or a combination of ATP and CGS15943. ATP was prepared in medium and added to the medium immediately prior to perifusion. CGS was solubilized in DMSO. The same amount of DMSO was added to the perifusion medium of the time controls and ATP groups.

Experiment 2: Explants were maintained in control medium or treated with ATP (100 μM), DPCPX (1 μM; 8-cyclopentyl-1, 3-dipropylxanthine, a selective A_1R antagonist), or a combination of ATP and DPCPX (1 μM). In a subsequent experiment, explants were maintained either in control medium or treated with DPCPX (10 μM) or a combination of ATP and DPCPX (10 μM). DPCPX was administered 1 h prior to ATP in the latter experiment. DPCPX was dissolved in ethanol, and the same amount of ethanol was added to the perifusion medium of the other groups without DPCPX.

Experiment 3: Explants were maintained in control medium or treated with an inhibitor of ecto-5′-nucleotidase, α,β-methylene adenosine 5′-diphosphate (AMP-CP; 250 μM) and guanosine monophosphate (GMP; 2 mM), a competitive substrate for ecto-5′-nucleotidase, ATP (100 μM), or a combination of ATP and AMP-CP plus GMP.