Rate of Diadenosine Polyphosphate Degradation by Various Guinea Pig Tissues

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 127, No. 2, pp. 207-210, February, 1999
Original article submitted April 8, 1998

High-performance liquid chromatography and colorimetry of inorganic phosphorus show that in the presence of muscle tissues from the guinea pig bladder, seminal duct, small intestine, longitudinal cord of the cecum, and right atrium all diadenosine polyphosphates with a common formula AP*n*A (*n*=2-6) are degraded more slowly than ATP. The rate of AP*n*A cleavage is highest in experiments with the small intestinal and right atrial tissues. P2-purine receptor antagonist suramin inhibits degradation of AP3A by right atrial tissue and does not modify degradation of other AP*n*A. Enzymatic stability of endogenous AP*n*A is apparently the principal condition for manifestation of their biological effects.

Key Words: *diadenosine polyphosphates; ectonucleotidases; P2-purine receptors; suramin*

Diadenosine polyphosphates are a family of dinucleotides consisting of two adenosine molecules bound through polyphosphate chain in the 5' position. Diadenosine polyphosphates are usually denoted as AP*n*A, where *n* is the number of phosphoric acid residues (P), usually 1-6. AP*n*A with *n*=4-6 attract the greatest attention, because these substances were isolated from the nervous system and visceral tissues of humans and animals [3,6,9]. AP*n*A released from secretory cells and nerve terminals participate in the regulation of various cell processes via extracellular receptors. AP*n*A are believed to be involved in the regulation of blood pressure and clotting [6,9]. The receptors activated by AP*n*A are heterogeneous. These compounds interact with certain subtypes of P2-purine receptors [5,8]. In the CNS, AP*n*A affect specific dinucleotide receptors different from P2-purine receptors [2].

Extracellular effects of AP*n*A are probably arrested by specific ectonucleotidases cleaving the polyphosphate chain molecules into two parts and yielding adenosine tri-, di-, and monophosphates, which are then catabolized via usual metabolic pathways, i.e. by successive cleavage of phosphoric acid residues [7]. Such ectonucleotidase has been described for the adrenal chromaffine cells [1].

Therefore, if AP*n*A is a mediator, ectonucleotidase is an enzyme limiting their effect. We studied the enzymatic stability of AP*n*A in the presence of tissues most often used in pharmacological studies of P2-purine receptors: the guinea pig urinary bladder, seminal duct, taenia caeci, small intestine, and atrium.

MATERIALS AND METHODS

Experiments were carried out on tissues from male guinea pigs weighing 350-600 g. The animals were euthanazed and dehematized. The bladder, seminal duct, taenia caeci, a fragment of the small intestine, and heart were removed, and muscle preparations were made as for a pharmacological experiment.

The rate of AP*n*A degradation was measured at 37±1°C in a buffer containing 10 mM 4-(2-hydroxyethyl)-1-piperasine ethanesulfonic acid (HEPES), 135 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 10 mM glucose, pH 7.4. Tissue fragments (2-3 mg) were incubated in a 24-well plate in 250 μl buffer containing individual AP*n*A (or ATP) in a concentration of 100 μM for 30 min (10 min for ATP) at constant shaking.

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0007-4888/99/0002-0188$22.00

In the first series, the reaction was stopped by transferring the buffer to 0.9 ml 2.5% sodium dodecyl sulfate. The concentration of formed inorganic phosphorus (P_i) was measured [10] using the corresponding concentrations of K_2HPO_4 reference solution. In the absence of substrates (control) no P_i was detected.

In the second series, AP*n*A were assayed by high-performance liquid chromatography (HPLC). The reaction buffer was immediately frozen in liquid nitrogen. The chromatographic system included a Beckman 114M injector attached to a SA6500 UV/Vis spectrophotometer. AP*n*A were separated on a 25-cm Spherisorb column (Hichrom 0.46 mm internal diameter). The column was washed for 24 h with a mobile phase consisting of 0.2 M KH_2PO_4 with 3% methanol (pH 6.0) at a flow rate of 0.2 ml/min. The separation of AP*n*A (20-μl sample) was carried out at a flow rate of 1.5 ml/min (detection 260 nm wavelength). Operations with AP4A, AP5A, and AP6A were carried out using the same mobile phase as for washing the column, while for AP2A and AP3A the concentration of methanol in the mobile phase was brought to 10% to accelerate the process. The amount of AP*n*A was evaluated by comparing the peaks of experimental samples and standard AP*n*A solutions.

In the reference experiments ATP was used as the reaction substrate. Mean values of at least 3 experiments in 4 parallels were calculated. All reagents were from Sigma.

RESULTS

All studied tissues metabolized AP*n*A, the rate of AP*n*A cleavage being far lower than of ATP. The rate of AP*n*A degradation was the highest in samples containing the cecum and right atrium (25-30% ATP degradation rate, Table 1). The lowest rate of AP*n*A degradation was observed in the presence of the bladder and seminal duct tissues (<5% ATP degradation rate). AP3A displayed the highest enzymatic stability in the presence of the bladder and taenia caeci tissues, while AP2A degraded most slowly in the presence of small intestinal and right atrial tissues. In the seminal duct all AP*n*A degraded at the same rate.

Therefore, all AP*n*A are metabolized in the studied tissues, although to a different degree. However, the method for measuring P_i does not show the tone rate of substrate degradation. For example, complete degradation of the entire polyphosphate chain in AP6A molecule yields 6 H_3PO_4 molecules, and hence, the rate of degradation can be 6-fold overestimated. More accurate measurements were carried out using HPLC in samples containing guinea pig atrium, characterized by the most intense AP*n*A degradation.

As expected, the values were lower than in the first experimental series. AP3A had the highest rate of degradation in this tissue (about 16% of the rate of ATP degradation in the same tissue). Other AP*n*A exhibited similar degradation rates (Table 2).

Bearing in mind that P2-purine receptor antagonist suramin effectively inhibits ectoATPase [11], we studied its effect on AP*n*A degradation. Suramin significantly inhibited degradation of AP3A and ATP but not of other AP*n*A (Table 2). This may be explained by the fact that AP3A is cleaved by ectonucleotidases similar to ectoATPases.

The physiological and pathophysiological role of AP*n*A is still discussed. On the one hand, these substances are present in cells in very low concentrations: in the majority of studied tissues the ATP:AP*n*A ratio is 25:1, and even 100:1 in human platelets [3]. On the other hand, these compounds are characterized by a for longer half-life compared to other endogenous mediators [1,4]. The effects of AP*n*A are mediated via P1- or P2-purine receptors and specific dinucleotide receptors and can be oppositely directed. *In vitro* AP2A, AP3A, and AP4A exert vasodilatory, while AP5A and AP6A vasoconstrictory effects. Consequently, intravenous infusion of AP4A reduces and of AP5A and AP6A elevates blood pressure [7]. Like adenosine, AP*n*A exert cardiodepressive effects which are apparently mediated by type A1 P1-purine receptors [5]. AP*n*A stimulate the contractility of isolated urinary bladder, and this effect is comparable to that of ATP

TABLE 1. The Rate of P_i Release from AP*n*A in the Presence of Various Guinea Pig Tissues, pmol P_i/mg tissue/min ($M \pm m$)

Substrate	Urinary bladder	Seminal duct	Taenia caeci	Small intestine	Right atrium
ATP	348.7±12.8 (100)	364.0±13.1 (100)	303.8±9.8 (100)	579.3±30.1 (100)	595.5±27.2 (100)
AP2A	15.1±6.5 (4.3)	5.1±2.2 (1.4)	21.0±8.0 (6.9)	86.1±5.4 (14.9)	120.5±9.8 (20.2)
AP3A	4.6±1.5 (1.3)	6.2±2.5 (1.7)	14.9±5.3 (4.9)	114.2±12.2 (19.7)	184.0±13.9 (30.9)
AP4A	12.2±6.0 (3.5)	8.5±3.9 (2.3)	32.6±9.6 (10.7)	152.8±14.6 (26.3)	164.5±14.9 (27.6)
AP5A	10.7±5.4 (3.1)	10.6±3.8 (2.9)	20.7±4.7 (6.8)	147.7±10.1 (25.5)	163.5±15.7 (27.4)
AP6A	14.4±6.0 (4.1)	16.1±5.4 (4.4)	22.7±5.4 (7.5)	123.3±11.8 (21.2)	178.7±15.9 (30)

Note. Here and in Table 2: the percentage of ATP degradation rates is given in parentheses.

TABLE 2. Rate of AP*n*A Degradation in Experiments with Guinea Pig Right Atrial Tissue with and without Suramin, pmol AP*n*A/mg tissue/min (*M*±*m*)

Substrate	No suramin	Suramin, 300 μM
ATP	341.5±10.7 (100)	175.5±6.8* (100)
AP2A	11.8±3.9 (3.4)	8.5±4.5 (2.5)
AP3A	52.5±4.3 (15.4)	17.5±2.6* (5.1)
AP4A	13.0±2.0 (3.8)	6.0±3.0 (1.8)
AP5A	18.5±3.9 (5.4)	12.5±2.5 (3.7)
AP6A	13.6±0.9 (3.9)	8.3±1.1 (2.4)

Note. *$p<0.05$ *vs.* samples without suramin.

and mediated by P2X-purine receptors [8]. Such a variety of effects may be explained by modification of the AP*n*A molecule due to enzymatic degradation, depending on the receptor type.

Therefore, AP*n*A are far superior to ATP in their enzymatic stability in all tissues studied. The content of AP*n*A in tissues is relatively low, but unlike ATP which is rapidly destroyed by ectoATPases, AP*n*A are characterized by longer half-life in tissues and a prolonged effect on cell receptors even if released in low amounts, which probably determines the biological role of these molecules.

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