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Inhibition by adenine dinucleotides of ATP-induced prostacyclin release by bovine aortic endothelial cells

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Abstract

Adenine dinucleotides are a group of extracellular modulators involved in maintaining blood vessel tone. We have demonstrated previously that Ap_2A and Ap_4A induce the synthesis of both nitric oxide (NO) and prostacyclin (PGI_2) in bovine aortic endothelial cells (BAEC), whereas Ap_3A, Ap_5A, and Ap_6A do not. In this paper, we report that Ap_2A and Ap_4A are partial agonists for ATP in terms of Ca^{2+} mobilization and PGI_2 synthesis. The Ap_4A EC_{50} values for Ca^{2+} mobilization and PGI_2 synthesis were significantly higher than the corresponding values for ATP, while the Ap_4A B_{max} values for Ca^{2+} mobilization and PGI_2 synthesis were significantly lower than those for ATP. Ap_2A and Ap_4A concentration–effect curves for Ca^{2+} mobilization and PGI_2 synthesis demonstrated that Ap_2A and Ap_4A have antagonistic effects at ATP concentrations that induce responses above the maximal amount of Ca^{2+} mobilized or PGI_2 synthesized by these two dinucleotides. On the other hand, Ap_2A and Ap_4A have agonistic effects at ATP concentrations that induce PGI_2 synthesis below the maximal amount of PGI_2 synthesized by these two dinucleotides. We also present evidence that suggests Ap_3A, Ap_5A, and Ap_6A are antagonists for ATP in terms of PGI_2 synthesis. All these data are consistent with the adenine dinucleotides being negative modulators for ATP-induced PGI_2 synthesis.

Keywords: Ap_xAs; Ca^{2+} mobilization; Prostacyclin; Endothelial cells

1. Introduction

Adenine dinucleotides represent a diverse and interesting group of extracellular mediators. These α,ω-adenine dinucleotides contain two adenosine moieties linked via their 5′ positions by a chain of phosphates (Ap_xA; $x = 2-7$). In the vascular system, Ap_xAs are co-stored with ATP in blood platelets and, upon platelet aggregation, are released into the extracellular milieu [1–3]. In the cardiovascular system, Ap_xAs have been shown to act as vasoregulators, and to alter coronary resistance and cardiac electrophysiology [4].

Ap_xAs have been shown to exert various effects on vascular tone depending on the number of phosphate groups in the molecule. Whereas Ap_2A, Ap_3A, and Ap_4A are vasodilators, Ap_5A and Ap_6A exhibit vasoconstrictive properties [5–10]. However, if the endothelium is removed from isolated arteries prior to infusion with Ap_4A, then Ap_4A interacts with VSMC to induce vasoconstriction [6,10]. Other investigators have shown that Ap_xAs ($x = 4-6$) significantly increase the $[Ca^{2+}]_i$ in VSMC by interacting with VSMC purinoceptors [11–14]. These observations suggest that the vasoactive response elicited by Ap_xAs may be determined by whether the dinucleotides interact directly with the endothelium to induce the release of vasoactive mediators or are internalized by the endothelium for trafficking to the basolateral membrane where they are released to interact with VSMC. An important and fundamental question is how are the Ap_xAs, which are released from the blood platelets simultaneously with other mono-

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Abbreviations: 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$; Ap_xA, diadenosine polyphosphates; BAEC, bovine aortic endothelial cells; Bz-ATP, 3′-*O*-benzylol-adenosine 5′-triphosphate; $[Ca^{2+}]_i$, intracellular calcium concentration; EIA, enzyme immunometric assay; FBS, fetal bovine serum; L-Arg, L-arginine; MEM, minimal essential medium; NO, nitric oxide; PGI_2, prostacyclin; VSMC, vascular smooth muscle cells.

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nucleotides, involved in maintaining vascular tone? Normal blood vessel tone appears to be maintained by a balanced constrictor–dilator interaction that is maintained by "cross-talk" among various vasoactive mediators synthesized by the endothelium [15–17]. The endothelium is a critical physiological target for maintaining blood vessel tone.

The biological effects on endothelial cells of adenine mononucleotides, but not the Ap_xAs, have been well characterized. ATP interacts with endothelial P_2 purinoceptors to trigger Ca^{2+} mobilization [18–25], and this Ca^{2+} has been shown to be involved in the release of various vasoactive mediators such as NO and PGI_2 [18,26–28].

To characterize the interaction of Ap_xAs with the endothelium, our laboratory has demonstrated that Ap_4A interacts with a heterogeneous population of receptors on BAEC [29]. The first binding site is a high-affinity site that is highly specific for Ap_4A, Ap_3A, and Ap_2A. Nonradiolabeled Ap_4A, Ap_3A, and Ap_2A were effective competitors of radiolabeled Ap_4A binding, while Ap_5A, Ap_6A, and various P_2 purinoceptor agonists and antagonists were not [29]. Competition binding studies also demonstrated that Ap_4A binds with low affinity to a second class of binding sites [29]. Various P_2 purinoceptor agonists and antagonists, along with Ap_xAs ($x = 2-6$), effectively displace radiolabeled Ap_4A from this low-affinity site [29]. We also demonstrated that Ap_4A and Ap_2A induce NO from BAEC, but Ap_3A, Ap_5A, and Ap_6A do not [30]. In addition, we recently demonstrated that Ap_4A and Ap_2A enhance the selective uptake of L-Arg by BAEC and that this L-Arg is delivered to NO synthase for the generation of NO [31]. On the other hand, Ap_3A, Ap_5A, Ap_6A, and Bz-ATP, a P_{2Z} agonist, do not enhance the selective uptake of L-Arg [31]. Ap_4A and Ap_2A also induce Ca^{2+} mobilization and PGI_2 synthesis in BAEC, while Ap_3A, Ap_5A, and Ap_6A induce Ca^{2+} mobilization but not PGI_2 synthesis [32].

In this paper, we report that Ap_4A and Ap_2A induce PGI_2 synthesis by their interaction with low-affinity binding sites. Evidence is also presented that is consistent with Ap_4A and Ap_2A acting as partial agonists to ATP-induced Ca^{2+} mobilization and PGI_2 synthesis. These data suggest that Ap_4A and Ap_2A may act as modulators for ATP-induced PGI_2 synthesis.

2. Materials and methods

2.1. Materials

MEM was purchased from GIBCO. Adenine dinucleotides, ATP, suramin, penicillin, and streptomycin were purchased from the Sigma Chemical Co. Heat-inactivated FBS was purchased from HyClone. The 6-keto $PGF_{1\alpha}$ acetylcholinesterase EIA Kit was purchased from the Cayman Chemical Co. Fluo-3/AM and Pluronic F-127 were purchased from Molecular Probes, Inc. All other reagents were of analytical grade or better.

2.2. Cell culture

BAEC were supplied by Dr. Robert Auerbach of the University of Wisconsin. Cells were grown in medium consisting of MEM, 10% (v/v) FBS, 44 mM $NaHCO_3$, penicillin (100 units/mL), and streptomycin (100 μg/mL). Cell cultures were maintained at 37° in a humidified atmosphere of 95% air−5% CO_2. All experiments were performed with passage 10 cells.

2.3. Fluo-3/AM loading of BAEC

BAEC were cultured in 100-cm^2 petri dishes as previously described [32] until they reached the early growth phase (2.5 to 5.0×10^4 cells/cm^2). We have found that 1.5 mM probenecid effectively inhibits dye leakage from the cytosol (unpublished observations); therefore, all buffers and media used throughout the loading procedure and the fluorescence measurements contained 1.5 mM probenecid. To load the cells with Fluo-3, the plates were incubated with 400 nM Fluo-3/AM in cold MEM, supplemented with 10 μg/mL of BSA and 0.02% Pluronic F-127. Loading was performed for 1 hr in the following sequence: 15 min at room temperature, 15 min at 37°, followed by 30 min at room temperature. Incubations at room temperature were performed with continuous shaking. Plates were subsequently washed with MEM for 15 min at room temperature, and then allowed to recover at 37° for another 15 min in MEM supplemented with 10% FBS. Cells were isolated from plates by trypsinization, resuspended in MEM with 10% FBS, and then washed three times by centrifugation in Krebs-Henseleit buffer [10 mM HEPES (pH 7.4), 120 mM NaCl, 4.6 mM KCl, 1.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1.5 mM NaH_2PO_4, 0.7 mM Na_2HPO_4, and 10 mM glucose]. After resuspension in Krebs-Henseleit buffer, cell density was determined by using a hemacytometer. Cell suspensions with viability values of less than 85%, as determined by trypan blue exclusion, were discarded. Cell density was adjusted to 1.5×10^6 cells/mL with Krebs-Henseleit buffer, and a 2-mL aliquot of cell suspension was transferred to a cuvette for fluorescence measurements.

2.4. Fluorescence measurements and $[Ca^{2+}]_i$ determination

Measurements were performed using an SLM 8000C Spectrofluorometer. The cuvette holder was connected to a magnetic stirrer so that cell suspensions were under continuous stirring conditions. The wavelengths used for excitation and emission were 504 and 526 nm, respectively (bandpass 4 nm). Typical fluorescence recordings are shown in Fig. 1. Following agonist stimulation, cells responded by a sharp and transient increase in the fluorescent signal, followed by a slow decay to basal levels. Calcium concentrations were calculated as described [33].

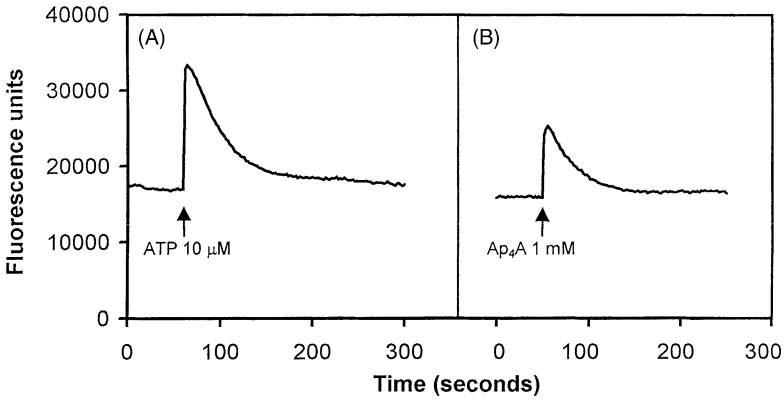

Fig. 1. Typical fluorescence tracings obtained by stimulating BAEC with ATP (A) or Ap_4A (B). BAEC were loaded with Fluo-3/AM, resuspended in Krebs-Henseleit buffer (1.5×10^6 cells/mL), and transferred to the cuvette. After reading the basal level fluorescence, cells were challenged with 10 μM ATP (A) or 1 mM Ap_4A (B). After an initial peak, the fluorescent signal decayed to a level close to basal.

The dissociation constant was assumed to be 400 nM, previously determined at vertebrate ionic strength [33].

2.5. Measurement of 6-keto $PGF_{1\alpha}$ released by BAEC

The accumulation of 6-keto $PGF_{1\alpha}$ was used as an index of PGI_2 release. BAEC were grown in 96-well plates to confluence. Confluent monolayers were incubated overnight at 37° in serum-free MEM. The cells were then washed with Krebs-Henseleit buffer and resuspended in 300 μL of Krebs-Henseleit buffer. To obtain a stable basal level of 6-keto $PGF_{1\alpha}$, the cells were incubated for 30 min at 37°, washed, resuspended in 300 μL of Krebs-Henseleit buffer, and incubated for 30 min at 37°. This procedure was repeated three times. The cells were then resuspended in 300 μL of Krebs-Henseleit buffer containing the adenine dinucleotides. The cells were incubated for 15 min at room temperature, and then aliquots were removed and diluted 100-fold with Krebs-Henseleit buffer for 6-keto $PGF_{1\alpha}$ assays. The amount of 6-keto $PGF_{1\alpha}$ was measured using the acetylcholinesterase immunoassay kit according to the specifications of the manufacturer.

2.6. Statistical analysis

Normally distributed data from a minimum of eight experiments are reported as means ± SEM. Student's two-tailed *t*-test was used to determine the statistical significance of a difference between means. A P value <0.05 was considered to denote significance.

3. Results

3.1. Effect of ATP and Ap_4A on Ca^{2+} mobilization and PGI_2 synthesis in BAEC

Since ATP and Ap_4A induce both Ca^{2+} mobilization and PGI_2 synthesis [18–28,32], we compared the effects of these two nucleotides on both Ca^{2+} mobilization and PGI_2 synthesis (Fig. 2 and Table 1).

To compare the effect of ATP and Ap_4A on Ca^{2+} mobilization, we measured $[Ca^{2+}]_i$ transients in response to various concentrations of ATP and Ap_4A. As shown in Fig. 2A, both ATP and Ap_4A induced concentration-dependent Ca^{2+} mobilization. However, the concentration–effect curve for Ap_4A relative to ATP was shifted to the right. As shown in Table 1, the change in $[Ca^{2+}]_i$ versus nucleotide concentration analyzed by nonlinear least-squares generated EC_{50} values of 0.034 and 64.9 μM for ATP and Ap_4A, respectively, and $\Delta[Ca^{2+}]_{max}$ values of 165 and 100 nM, respectively. Suramin (500 μM), a nonselective P_2 purinoceptor antagonist, completely abolished Ap_4A-induced Ca^{2+} mobilization (data not shown). These data suggest that Ap_4A interacts with P_2 purinoceptors to mobilize Ca^{2+}.

To compare the effects of ATP and Ap_4A on PGI_2 synthesis, BAEC were incubated for 15 min in the presence of various concentrations of ATP and Ap_4A (Fig. 2B). As was the case with Ca^{2+} mobilization, the Ap_4A concentration–effect curve for PGI_2 synthesis was shifted to the right relative to ATP. The change in amount of PGI_2 synthesized versus nucleotide concentration analyzed by

Table 1
Kinetic parameters of ATP- and Ap_4A- induced Ca^{2+} mobilization and PGI_2 synthesis

Agonist	Calcium mobilization		PGI_2 release	
	EC_{50} (μM)	$\Delta[Ca^{2+}]_{max}$ (nM)	EC_{50} (μM)	Δ[6-keto $PGF_{1\alpha}]_{max}$ (pg/10^5 cells)
ATP	0.034 ± 0.01	165 ± 0.7	0.60 ± 0.06	2987 ± 57
Ap_4A	64.90 ± 1.57	100 ± 0.6	60.1 ± 3.00	1324 ± 20

Experiments were performed as described in the legend of Fig. 2. Values are means ± SEM, N ≥ 8.

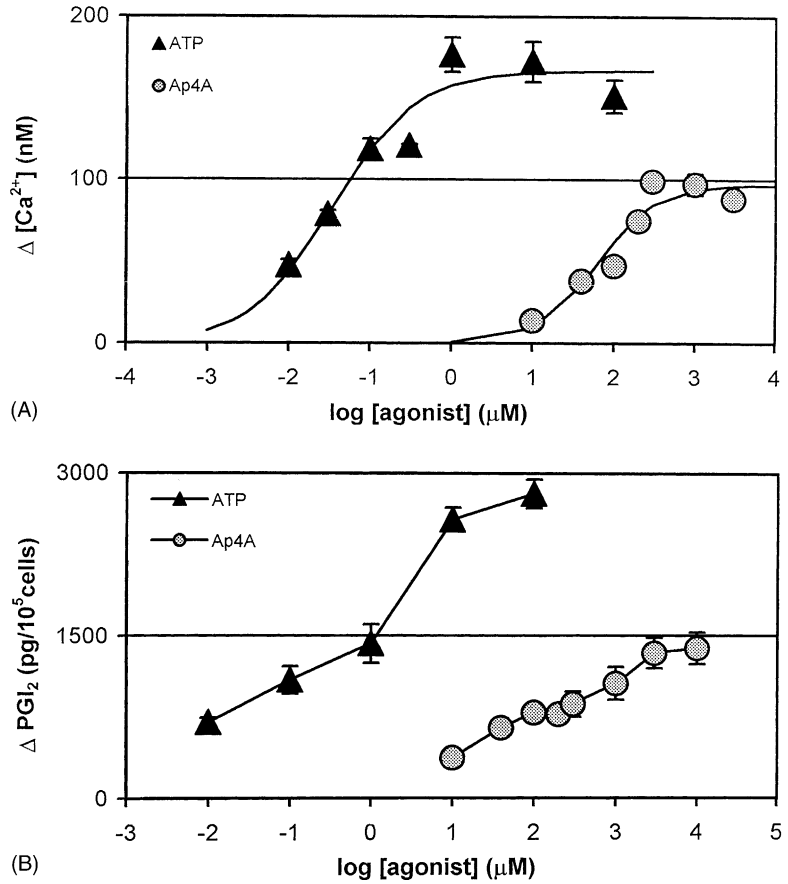

Fig. 2. Effect of various concentrations of ATP and Ap_4A on Ca^{2+} mobilization and PGI_2 synthesis in BAEC. (A) Fluo-3-loaded cells were suspended in Krebs-Henseleit buffer. After reading the baseline, the cells were challenged with the indicated concentrations of ATP or Ap_4A. Calcium concentrations were determined as described under Section 2. Basal $[Ca^{2+}]_i$ values were subtracted from ATP- and Ap_4A-induced peak $[Ca^{2+}]_i$ values. Data (means ± SEM) are from an average of at least eight different experiments. (B) BAEC were grown in gelatin-coated 96-well plates, and PGI_2 synthesis studies were performed as described under Section 2. BAEC were incubated at room temperature for 15 min in the presence of the indicated concentrations of ATP or Ap_4A. Basal PGI_2 values were subtracted from Ap_4A-induced PGI_2 synthesis values. Data (means ± SEM) are averages of three different experiments performed in triplicate.

nonlinear least-squares generated EC_{50} values of 0.6 and 60.1 μM for ATP and Ap_4A, respectively, and Δ[6-keto $PGF_{1\alpha}]_{max}$ values of 2987 and 1324 pg/10^5 cells, respectively. Both suramin (500 μM) and another nonspecific P_2 purinoceptor antagonist, Reactive Blue (10 μM), inhibited Ap_4A-induced PGI_2 synthesis (data not shown). These data support the notion that Ap_4A interacts with P_2 purinoceptors to induce the synthesis of PGI_2.

3.2. *Effect of various concentrations of Ap_4A on ATP-induced Ca^{2+} mobilization in BAEC*

To determine whether Ap_4A interacts with P_2 purinoceptors, the effect of Ap_4A on ATP-induced Ca^{2+} mobilization was examined. We measured $[Ca^{2+}]_i$ transients in response to a fixed amount of ATP and various concentrations of Ap_4A (Fig. 3). In the presence of 1 μM ATP (a concentration that induces maximal $[Ca^{2+}]_i$ response), Ap_4A had antagonistic effects, decreasing the ATP response to the level of the maximal Ap_4A response (100 nM). The fact that the inhibition was not complete suggests that Ap_4A may act as a partial agonist for the ATP response.

3.3. *Effect of various concentrations of Ap_4A on ATP-induced PGI_2 synthesis by BAEC*

We determined the effect of Ap_4A on ATP-induced PGI_2 synthesis. BAEC were incubated for 15 min in the presence or absence of fixed amounts of ATP and various concentrations of Ap_4A. Fig. 4 shows a series of concentration–effect curves for Ap_4A in the absence and in the presence of several different fixed concentrations of ATP. In the absence of ATP, Ap_4A (1 mM) produced 48% of the maximal response generated by ATP. The effect of Ap_4A in the presence of 0.1 and 1.0 μM ATP (ATP concentrations that produce responses less than the maximal response of Ap_4A, i.e. 48%) was additive to those of ATP up to the maximal response of Ap_4A. In contrast, Ap_4A in the presence of 10 and 60 μM ATP (ATP concentrations that produce responses higher than the maximal response of Ap_4A) had antagonistic effects, decreasing the response of ATP to the level of the maximal response of Ap_4A. The increase in concentrations of Ap_4A required to antagonize the effects of increasingly higher concentrations of ATP is indicative of the competitive nature of the

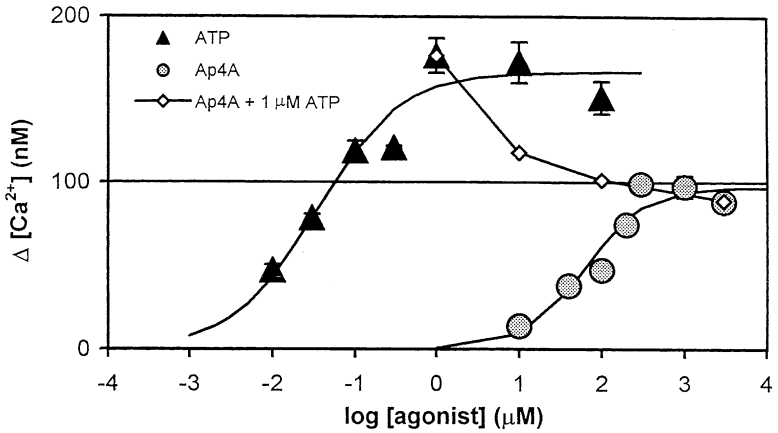

Fig. 3. Effect of various concentrations of Ap_4A on ATP-induced Ca^{2+} mobilization in BAEC. Experiments were performed as described in the legend of Fig. 2A using 1 μM ATP and the indicated concentrations of Ap_4A. Basal $[Ca^{2+}]_i$ values were subtracted from the agonist-induced peak $[Ca^{2+}]_i$ values. Data (means ± SEM) were obtained from an average of at least eight different experiments.

effect. These data also support the notion that Ap_4A is indeed a partial agonist for ATP [34].

3.4. Effect of various concentrations of Ap_2A on ATP-induced PGI_2 synthesis by BAEC

Since Ap_2A also induces PGI_2 synthesis in BAEC [32], we determined the effect of Ap_2A on ATP-induced PGI_2 synthesis. BAEC were incubated for 15 min in the presence of fixed amounts of ATP and various concentrations of Ap_2A (Fig. 5). In the absence of ATP, 800 μM Ap_2A produced 62% of the maximal response of ATP. At 1 μM ATP, the effect of Ap_2A was additive, but at 10 and 60 μM ATP the effect of Ap_2A was antagonistic. These data are consistent with Ap_2A acting as a partial agonist for ATP.

3.5. Effect of other Ap_xAs on ATP-induced PGI_2 synthesis by BAEC

Ap_3A, Ap_5A, and Ap_6A do not induce the release of PGI_2 from BAEC [32]. To determine whether these dinu-

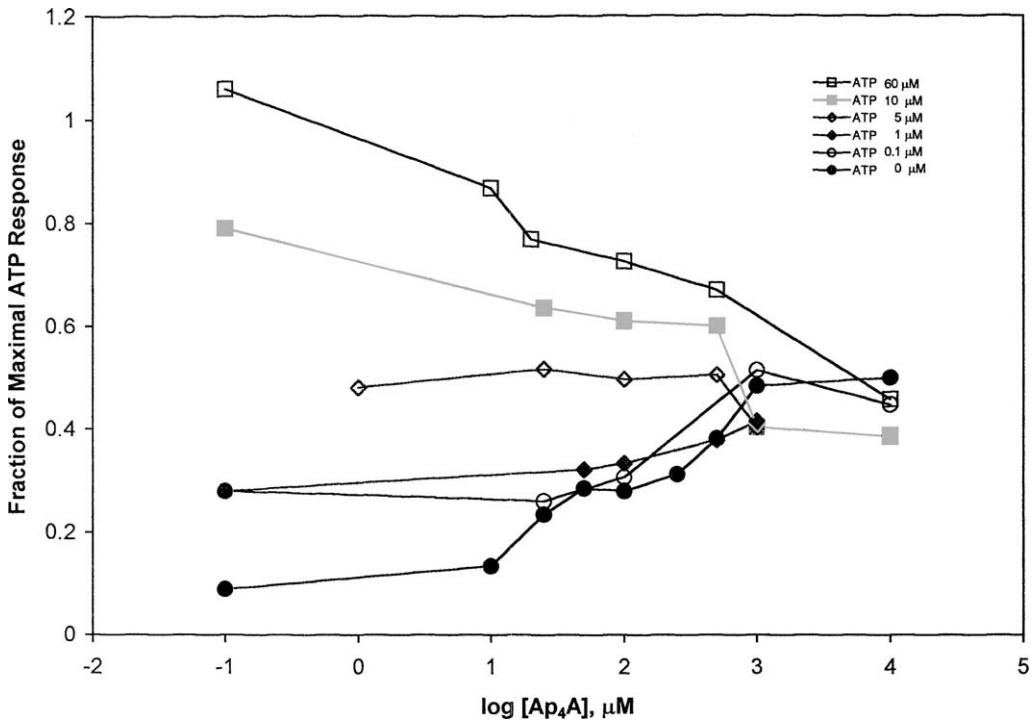

Fig. 4. Effect of various concentrations of Ap_4A on ATP-induced PGI_2 synthesis by BAEC. Experiments were performed as described in the legend of Fig. 2B using fixed concentrations of ATP and the indicated concentrations of Ap_4A. Basal PGI_2 values were subtracted from agonist-induced PGI_2 synthesis values. Data (means ± SEM) are averages of at least three different experiments performed in triplicate.

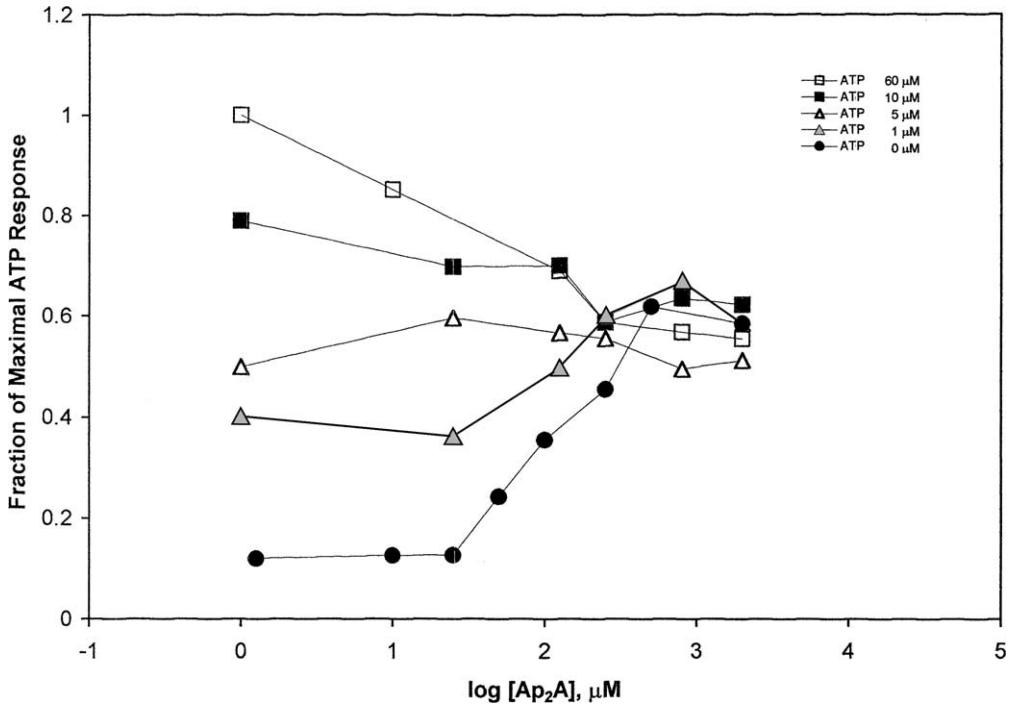

Fig. 5. Effect of various concentrations of Ap_2A on ATP-induced PGI_2 synthesis by BAEC. Experiments were performed as described in the legend of Fig. 2B using fixed concentrations of ATP and the indicated concentrations of Ap_2A. Basal PGI_2 values were subtracted from agonist-induced PGI_2 synthesis values. Data (means ± SEM) are averages of at least three different experiments performed in triplicate.

cleotides act as antagonists for ATP-induced PGI_2 synthesis, we measured ATP-induced PGI_2 synthesis in the presence and absence of the dinucleotides. BAEC were incubated for 15 min in the presence or absence of 500 μM Ap_xA (a concentration that elicited maximal Ap_2A and Ap_4A responses) plus 60 μM ATP (a concentration that elicited maximal ATP response). As shown in Table 2, all three dinucleotides inhibited ATP-induced PGI_2 synthesis. Ap_5A and Ap_6A inhibited 43% of the maximal ATP response, while Ap_3A inhibited 27%. These data are consistent with Ap_3A, Ap_5A, and Ap_6A having an antagonistic effect on ATP-induced PGI_2 synthesis.

Table 2
The effect of Ap_xAs on ATP-induced PGI_2 synthesis by BAEC

Nucleotide	6-keto $PGF_{1\alpha}$ (pg/10^5 cells)	*P* value	Relative percent
60 μM ATP	2650 ± 160		100
60 μM ATP plus 500 μM Ap_3A	1940 ± 180	<0.05	73
60 μM ATP plus 500 μM Ap_5A	1520 ± 190	<0.05	57
60 μM ATP plus 500 μM Ap_6A	1510 ± 180	<0.05	57

Experiments were performed as described in the legend of Fig. 2B. Basal PGI_2 were subtracted from nucleotide-induced PGI_2 synthesis values. Data (means ± SEM) were obtained from an average of three different experiments performed in triplicate.

4. Discussion

The work presented in this paper arose from the observations that Ap_2A and Ap_4A mobilize Ca^{2+} and induce the synthesis of PGI_2 in BAEC [32]. Our long-term goal is to characterize the interaction of Ap_xAs with endothelial cells, a critical physiological target. We have demonstrated previously that Ap_4A interacts with a heterogeneous population of receptors on BAEC [29]. Ap_4A binds with high affinity to a receptor that induces the release of NO [30,31], and it binds with low affinity to a second class of binding sites [29]. Competition binding studies using radiolabeled Ap_4A and nonradiolabeled P_2 purinoceptor agonists and antagonists are consistent with the Ap_4A low-affinity binding sites being P_2 purinoceptors [29]. The results reported herein are consistent with Ap_2A and Ap_4A acting as partial agonists for ATP-induced Ca^{2+} mobilization and PGI_2 synthesis from P_2 purinoceptors.

The EC_{50} values obtained for Ap_4A for Ca^{2+} mobilization and PGI_2 synthesis are significantly higher than the corresponding values obtained for ATP (Table 1). These data suggest that, when compared with ATP, Ap_4A has a low affinity for the receptor system that induces Ca^{2+} and PGI_2 synthesis. Furthermore, the ability of suramin/Reactive Blue to inhibit Ap_4A-induced Ca^{2+} mobilization and PGI_2 synthesis (data not shown) was consistent with Ap_4A inducing the synthesis of PGI_2 by interacting with one or more P_2 purinoceptor subtypes. Because of a lack of a generally accepted classification and of specific agonists

and antagonists, it is hard to identify the purinoceptor subtypes involved in the responses to ATP and Ap_xAs. However, in view of the fact that BAEC seem to be devoid of P_{2x}-purinergic receptors, which within the cardiovascular system are preferentially located on cardiomyocytes and VSMC [35], it is conceivable that ATP and the Ap_xAs interact with P_{2y} receptor subtypes. These receptors are found on the endothelium and mediate vasodilation through a G-protein-coupled system, unlike the P_{2x} receptors, which are ligand-operated cation channels and mediate vasoconstriction on smooth muscle cells [35,36].

The B_{max} values obtained for Ap_4A for Ca^{2+} mobilization and PGI_2 synthesis are 1.6- and 2.2-fold lower, respectively, than the corresponding values obtained for ATP (Table 1). These data suggest that Ap_4A induces Ca^{2+} mobilization and PGI_2 synthesis from a different receptor system than ATP or that Ap_4A interacts with the same receptor system as ATP and is a partial agonist to ATP. Partial agonists by virtue of their occupation of a large number of receptors competitively block the effects of full agonists only at high concentrations of the full agonists. Since the effects of the partial agonist are the result of the interactions with the same site as the natural ligand, antagonism from partial agonists should be observed at the same concentrations that produce the agonist effect. Therefore, the agonistic response to a ligand that occurs at concentrations different than those that produce antagonism cannot be ascribed to interaction of the ligand with a single receptor. At ATP concentrations that induced PGI_2 synthesis above the maximal response elicited by Ap_4A, Ap_4A had an antagonistic effect up to the maximal Ap_4A response (Fig. 4). On the other hand, at ATP concentrations that induced PGI_2 synthesis below the maximal response of Ap_4A, Ap_4A had an agonistic effect up to the level of the maximal Ap_4A response. Similar concentration–effect curve experiments were performed using Ap_2A and ATP (Fig. 5); the results were essentially the same as seen with Ap_4A. These data are consistent with Ap_2A and Ap_4A being partial agonists for ATP. Alternatively, the agonistic effect seen in the presence of Ap_4A may be due to a small ATP contamination (<1%). In this case, Ap_4A would act as an antagonist instead of a partial agonist. Nevertheless, our data clearly demonstrate that Ap_4A inhibits ATP-induced Ca^{2+} mobilization and PGI_2 synthesis. Furthermore, we also demonstrated that Ap_3A, Ap_5A, and Ap_6A inhibit ATP-induced PGI_2 synthesis (Table 2). These data are consistent with Ap_xAs inhibiting ATP-induced PGI_2 synthesis from P_2 purinoceptors. Other investigators, using clones of brain capillary endothelial P_{2y} purinoceptors, have demonstrated that Ap_4A and Ap_5A also inhibit ADP-induced increases in $[Ca^{2+}]_i$ [37]. In addition, Ap_xAs have been shown to induce Ca^{2+} mobilization in different tissues via their interactions with various P_2 purinoceptor subtypes [6,11–15,32,37–42]. Thus, a fundamental and important question that needs to be addressed is whether the major physiological function for the interaction of Ap_xAs with P_2 purinoceptors is to be a modulator of ATP signal transduction responses by acting as partial agonists or antagonists.

Acknowledgments

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