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Biochemistry

Adenosine triphosphate stimulates inositol phospholipid metabolism and prostacyclin formation in adrenal medullary endothelial cells by means of P_2-purinergic receptors

(inositol trisphosphate/Ca^{2+} mobilization/endothelium-dependent vasodilation)

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ABSTRACT **In the adrenal medulla, chromaffin cells secrete high concentrations of catecholamines, ATP, peptides and other factors that must pass through an endothelial cell barrier to enter the bloodstream. We have measured the effect of several of these chromaffin cell secretory products on cultured bovine adrenal medullary endothelial cells and have found that only ATP stimulates prostacyclin formation. The stimulation of prostacyclin formation by ATP coincides with the metabolism of inositol phospholipids and the accumulation of the putative second messenger inositol trisphosphate. The time course, concentration dependence, and P_2-purinergic receptor specificity were similar for ATP-stimulated prostacyclin formation and ATP-stimulated inositol phospholipid metabolism. Thus, the increase in prostacyclin formation may be secondary to mobilization of intracellular Ca^{2+} by inositol trisphosphate, leading to activation of phospholipase A_2, liberation of arachidonic acid, and the conversion of arachidonic acid to prostacyclin. We propose that the function of ATP, which is often colocalized with cell-specific hormones in secretory cells, may be to regulate blood flow in the adrenal medulla and other endocrine tissues by interacting with adjacent endothelial cells.**

A characteristic of endocrine tissues is the close juxtaposition of endocrine cells to the vascular endothelium. The significance of this arrangement may simply be to allow the rapid entry of hormones into the circulation while maintaining a barrier to the movement of other molecules between the blood and the extracellular space. However, a functional interaction between adrenal chromaffin cells and adjacent endothelial cells with respect to catecholamine metabolism has also been proposed (1, 2).

We have extended the concept of a functional interaction between adrenal chromaffin cells and adjacent endothelial cells to include the control of local blood flow. This extension of the hypothesis follows logically from the observation that chromaffin cells secrete several vasoactive substances, including epinephrine and norepinephrine (3), ATP (4, 5), and peptides (3), which probably bathe the adjacent endothelial cells at high concentrations. Endothelial cells play a critical role not only in controlling the movement of molecules between the blood and the extracellular space but also in regulating local blood flow (6). We have found that ATP, probably acting through P_2-purinergic receptors, stimulates prostacyclin formation in bovine adrenal medullary endothelial cells in culture. We provide evidence that ATP-stimulated prostacyclin formation is secondary to ATP-induced inositol phospholipid metabolism and the formation of the putative second messenger inositol trisphosphate (InsP_3) (7, 8).

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MATERIALS AND METHODS

ATP, GTP, CTP, TTP, and UTP (all sodium salts), α,β-methylene-ATP (lithium salt), Hepes, indomethacin, (9α,11α,13*E*,15*S*)-9,11,15-trihydroxy-6-oxoprosta-13-en-1-oic acid (6-keto-$PGF_{1\alpha}$), phosphatidylinositol 4,5-bisphosphate (PtdInsP_2), phosphatidylinositol 4-monophosphate (PtdIns*P*), phosphatidylinositol (PtdIns), and Cytodex-2 microcarrier beads were obtained from Sigma. Thrombin (bovine) and bradykinin were from Boehringer-Mannheim Biochemicals. 6-keto-[^{3}H]$PGF_{1\alpha}$ (100–200 Ci/mmol; 1 Ci = 37 GBq) was from New England Nuclear. [^{3}H]Inositol (14.2–19.1 Ci/mmol) and ^{3}H-labeled inositol phosphate standards were from Amersham or New England Nuclear. [Leu]enkephalin was from Beckman and dynorphin A-(1–17) was from Bachem (Torrance, CA). 8-(*p*-Sulfophenyl)theophylline was a gift from John Daly (National Institutes of Health).

Culture of Bovine Adrenal Medullary Endothelial Cells. Dissociated adrenal medullary cells were prepared by collagenase digestion of bovine adrenal gland as described (9). Endothelial cells were separated from chromaffin cells using a differential plating technique as described (1) with the following modifications. Dissociated cells were initially cultured (5% CO_2/95% air, 37°C) for 3–12 hr in 75-cm^2 Costar culture flasks at a density of 5×10^7 cells per 30 ml of Eagle's minimal essential medium with Earle's salts (EMEM) supplemented with 10% fetal bovine serum (heat-inactivated), glutamine (2 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml). After the unattached cells and medium were removed by aspiration, the remaining adherent cells were washed and then cultured with 30 ml of medium. When the cells reached confluency (5–10 days), they were passaged by trypsinization (0.25% trypsin/0.1% EDTA in phosphate-buffered saline). Cells were seeded either into 75-cm^2 Costar flasks ($1.5–2.0 \times 10^6$ cells per 30 ml of medium) or into 24-well Costar Petri dishes (1×10^5 cells per well per 1.5 ml of medium) and incubated in 5% CO_2/95% air at 37°C. Cells were used between the 2nd and 25th passages. These cultures consisted almost entirely of endothelial cells (1).

Measurement of Prostacyclin Formation. Endothelial cells that had been seeded into 24-well Costar Petri dishes were incubated at 37°C (5% CO_2/95% air) until confluent (4–5 days). The cells were washed with 0.5 ml of supplemented EMEM containing 1% instead of 10% fetal bovine serum and 20 mM Hepes (pH 7.4). Control or test agents dissolved in the

Abbreviations: InsP_3, inositol trisphosphate; InsP_2, inositol bisphosphate; Ins*P*, inositol monophosphate; PtdInsP_2, phosphatidylinositol 4,5-bisphosphate; PtdIns*P*, phosphatidylinositol 4-monophosphate; PtdIns, phosphatidylinositol; 6-keto-$PGF_{1\alpha}$, (9α,11α,13*E*,15*S*)-9,11,15-trihydroxy-6-oxoprosta-13-en-1-oic acid.

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same medium (0.25 ml) were added to each well. At the time points indicated, 0.2-ml samples were removed and frozen on dry ice. The amount of prostacyclin released into aliquots (100 μl) of each sample was determined by measuring the stable prostacyclin metabolite 6-keto-$PGF_{1\alpha}$ with a radioimmunoassay (10) using specific antibodies purchased from L. Levine (Brandeis University). The results were calculated as the amount of 6-keto-$PGF_{1\alpha}$ released into the medium per well and in some cases are expressed as a percentage of the control values (mean ± SEM). The number of cells per well ranged from 4×10^5 to 7×10^5 in different experiments but within an experiment varied <10%. Each figure or table represents the results of one of several experiments.

Measurement of Inositol Phospholipid Hydrolysis and Inositol Phosphate Formation. Cytodex-2 microcarrier beads (60 mg dry weight, equilibrated in Dulbecco's phosphate-buffered saline and sterilized in an autoclave) were added to each 75-cm^2 flask of confluent endothelial cells (4–6 days after passage). [^{3}H]Inositol was added to each flask (3 μCi/ml) and allowed to reach isotopic equilibrium in the cellular inositol phospholipids (which took 72–96 hr; data not shown). Endothelial cells grew over and covered the microcarrier beads within 96 hr. After the 96-hr incubation with [^{3}H]inositol and the microcarrier beads, each flask of endothelial cells was washed with 25 ml of fresh medium containing no [^{3}H]inositol and incubated with an additional 25 ml of medium for 30 min. The cells in each flask were then washed with 25 ml of serum-free medium. Microcarrier beads with adherent endothelial cells were dislodged from the bottom of the flasks by tapping the sides. Microcarrier beads from several flasks were pooled, allowed to settle, and washed with 50 ml of serum-free medium.

Aliquots (100 or 200 μl) of settled microcarrier beads (corresponding to $2–6 \times 10^4$ beads, $1–3 \times 10^6$ cells, and 0.2–0.6 mg of protein) were put in glass test tubes and the same volume of a control or test solution (pH 7.4) was added. After the time periods indicated in the figures, 1 ml (100 μl of beads) or 2 ml (200 μl of beads) of chloroform/methanol, 1:2 (vol/vol), was added and the [^{3}H]inositol phosphates were extracted, separated on anion-exchange columns, and assayed for radioactivity as described (11). Authentic [^{3}H]-inositol phosphate standards were used to verify the elution profile of sample inositol phosphates. The identity of the various inositol phosphates was subsequently verified by high-performance liquid chromatography using the procedure of Batty *et al.* (ref. 12; unpublished results). In some experiments, the remaining organic (lower) phase was dried under nitrogen and the [^{3}H]inositol phospholipids were separated on thin-layer chromatography plates and assayed for radioactivity as described (11). In experiments in which the inositol phospholipids were not analyzed, the lower organic phase (which included the interface) was dried under nitrogen and redissolved in 0.1 M NaOH, and total ^{3}H radioactivity was determined using liquid scintillation spectrophotometry.

To standardize the results, the ^{3}H radioactivity (cpm) in each compound was divided by total ^{3}H cpm measured in the aliquot. The results are expressed as a percentage of total cpm or as a percentage of the control values (mean ± SEM). Control cpm were determined for each time point or condition. Each figure or table represents the results of one of several experiments.

RESULTS

ATP-Induced Prostacyclin (6-keto-$PGF_{1\alpha}$) Formation. Endothelial cells of different types release prostacyclin in response to specific stimuli (13, 14). Of interest was whether components from chromaffin cell secretory granules could stimulate prostacyclin formation in bovine adrenal medullary endothelial cells in culture. Whereas epinephrine (10–50 μM), norepinephrine (10–50 μM), [Leu]enkephalin (1 μM), and dynorphin A-(1–17) (1 μM) had no effect on prostacyclin formation (not shown), ATP (100 μM) stimulated rapid and large increases in the formation of the stable prostacyclin metabolite 6-keto-$PGF_{1\alpha}$ in cultured endothelial cells (Fig. 1). Significant increases in 6-keto-$PGF_{1\alpha}$ over basal values occurred within 15 sec and peaked between 1 and 2 min after ATP addition. Prostacyclin formation apparently ceased after 2 min since 6-keto-$PGF_{1\alpha}$ levels remained nearly constant for at least 10 min following ATP addition (Fig. 1).

The concentration dependence of ATP-induced 6-keto-$PGF_{1\alpha}$ formation showed that low concentrations (25–100 μM) produced significant ($P \leq 0.01$) increases in prostacyclin formation (Fig. 2). The effect of ATP on prostacyclin formation peaked between 100 and 500 μM ATP, whereas higher ATP concentrations had no additional effect on 6-keto-$PGF_{1\alpha}$ levels (Fig. 2).

Nucleotide Specificity of Prostacyclin Formation. To establish the nature of the receptor mediating the effects of ATP on prostacyclin formation in endothelial cells, other adenine nucleotides were tested. Both ATP and ADP (each 0.5 mM) produced similar large increases in 6-keto-$PGF_{1\alpha}$ formation 2 min after their addition, from 218 ± 13 pg per well (2-min control, $n = 19$) to 503 ± 38 and 655 ± 63 pg per well ($n = 5$), respectively. However, neither AMP nor adenosine (0.5 mM) significantly affected basal 6-keto-$PGF_{1\alpha}$ levels [250 ± 30 and 198 ± 13 pg per well ($n = 5$), respectively]. These results are consistent with the notion that P_2-purinergic rather than adenosine receptors mediated the effects of ATP on prostacyclin formation. Moreover, the adenosine receptor antagonist 8-(*p*-sulfophenyl)theophylline (100 μM), when added 2 min before ATP (0.5 mM), did not inhibit ATP-stimulated prostacyclin formation (485 ± 150 pg per well, $n = 5$).

Interestingly, nucleoside triphosphates other than ATP produced similar increases in prostacyclin formation. For example, GTP, TTP, and UTP (0.5 mM) increased 6-keto-$PGF_{1\alpha}$ levels after 2 min to 538 ± 25, 518 ± 75, and 582 ± 13 pg per well ($n = 5$), respectively. The sensitivity of P_2-purinergic receptors to nucleotides other than ATP has been demonstrated in other cell types (see *Discussion*) and may reflect the existence of a specific subclass of P_2-purinergic receptors.

The stable analog of ATP α,β-methylene ATP (0.5 mM) also stimulated 6-keto-$PGF_{1\alpha}$ formation (to 403 ± 25 pg per well at 2 min, $n = 5$), albeit to a lesser degree than ATP, suggesting that ATP hydrolysis was not necessary for the effect of ATP on 6-keto-$PGF_{1\alpha}$ accumulation.

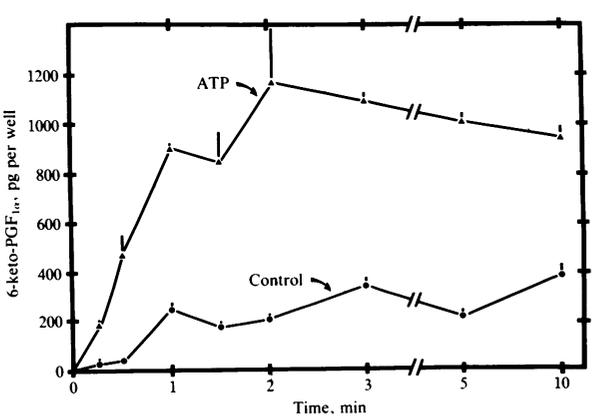

FIG. 1. Time course of ATP-induced prostacyclin formation. The amount of prostacyclin formed was determined by measuring the accumulation of the stable breakdown product of prostacyclin, 6-keto-$PGF_{1\alpha}$ (pg per well) ($n = 4$). ●, Control; ▲, 100 μM ATP.

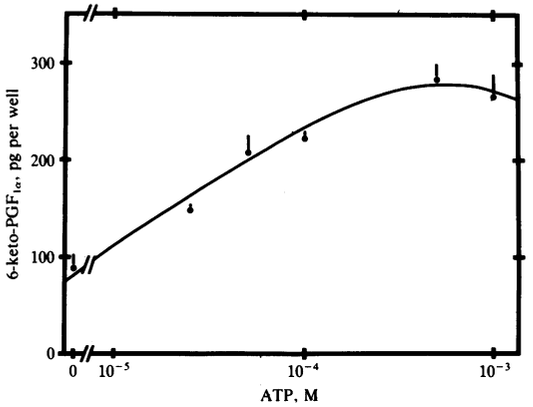

FIG. 2. Concentration dependence of ATP-induced prostacyclin (6-keto-PGF$_{1\alpha}$) formation 2 min after ATP addition (n = 4).

Other Agents that Affected Prostacyclin Formation. Both basal and ATP-stimulated prostacyclin formation were inhibited by the prior addition of 10 μM indomethacin [to 4% ± 1% and 7% ± 1% of control, respectively (n = 6)], indicating that prostacyclin formation occurred through the cyclooxygenase pathway. Two additional stimuli that have been shown to have effects on endothelial cells are thrombin and bradykinin (6, 15). Thus, it was not surprising to find that thrombin (2 units/ml) and bradykinin (1 μM) (both at 2 min) stimulated prostacyclin formation [to 219% ± 43% and 381% ± 12% of control, respectively (n = 4)].

ATP-Induced Inositol Phosphate Formation. Since it was possible that ATP-induced prostacyclin formation was secondary to an increase in intracellular Ca^{2+} levels mediated by inositol phospholipid metabolism (7, 8), ATP was tested for its effects on inositol phosphate production in adrenal medullary endothelial cells prelabeled to isotopic equilibrium with [^{3}H]inositol. Fig. 3 shows that ATP (1 mM) stimulated the rapid formation of InsP_3, inositol bisphosphate (InsP_2), and inositol monophosphate (Ins$P$) with a time course similar to ATP-stimulated prostacyclin formation. ATP-stimulated

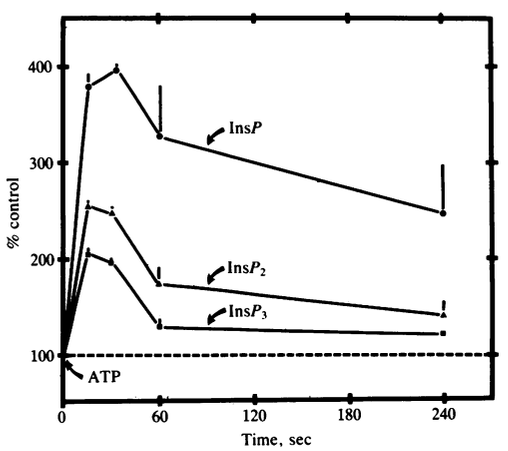

FIG. 3. Time course of ATP-induced inositol phosphate accumulation. Endothelial cells grown on microcarrier beads were prelabeled to isotopic equilibrium with [^{3}H]inositol. The radioactivities (cpm) of InsP_3 (■), InsP_2 (▲), and Ins$P$ (●) were determined for both unstimulated and ATP-stimulated (1 mM ATP) cells at each time point. The results were calculated as the ratio of the cpm in each inositol phosphate to total cpm in each sample and are expressed as a percentage of the control values (mean ± SEM, n = 3). Shown is one of several experiments, each of which yielded similar results. Examples of control cpm (15 sec) were InsP_3 (578 ± 43), InsP_2 (825 ± 26), Ins$P$ (1558 ± 36), and total (217,952 ± 1294); n = 3. Control ratios did not change during the time course.

InsP_3 and InsP_2 formation peaked at 200–250% of control levels within 15 sec, whereas Ins$P$ levels reached almost 400% of control levels within 30 sec after ATP addition. Thereafter, inositol phosphate levels began to decline but remained above control values for at least 4 min (Fig. 3). ATP-stimulated inositol phosphate accumulation did not require the influx of extracellular Ca^{2+} since the addition of 5 mM EGTA 15 sec before ATP (which reduced extracellular Ca^{2+} from 1.8 mM to about 10 nM) had little effect on inositol phosphate levels (data not shown). It is possible that the accumulation of the inositol phosphates reflected the direct activation of the enzyme phospholipase C by ATP.

This suggestion is further supported by the observation that ATP (1 mM) produced rapid decreases in two inositol phospholipids, PtdInsP_2 and PtdIns$P$, in endothelial cells prelabeled to isotopic equilibrium with [^{3}H]inositol (Fig. 4). The effect was time dependent in as much as PtdInsP_2 and PtdIns$P$ levels declined to 70–80% of control levels within 60 sec after ATP addition but returned to control levels within 4 min. By contrast, no significant changes in PtdIns levels were measured during the 4-min incubation with ATP.

The observation that Ins$P$ levels increased 4-fold whereas PtdIns levels remained unchanged may be explained by the rapid conversion of InsP_3 and InsP_2 to Ins$P$ by phosphomonoesterases (16) without any direct action of phospholipase C on PtdIns. Alternatively, since basal Ins$P$ cpm represented only about 4% of the cpm in PtdIns (see legends to Figs. 3 and 4) and since PtdIns is probably rapidly resynthesized following its hydrolysis (17), changes in PtdIns levels may have been too small to be detected. Furthermore, the rate of PtdInsP_2 and PtdIns$P$ hydrolysis rather than their actual levels correlated most closely with the extent of InsP_3 and InsP_2 production, suggesting that InsP_3 and InsP_2 were rapidly degraded.

The concentration dependence of ATP-induced increases in the inositol phosphates at 15 sec is shown in Fig. 5. At 15 sec after ATP addition, the concentration dependence of ATP-induced InsP_3 and InsP_2 accumulation was biphasic (Fig. 5) and at ATP concentrations ≤1 mM it resembled the concentration dependence of ATP-induced prostacyclin formation (Fig. 2). ATP concentrations as low as 10 μM produced significant ($P \leq 0.01$) increases in InsP_3 and InsP_2 levels, which reached an intermediate plateau at about 1 mM ATP. Concentrations of ATP above 1 mM produced additional increases in InsP_3 and InsP_2 levels (Fig. 5). However,

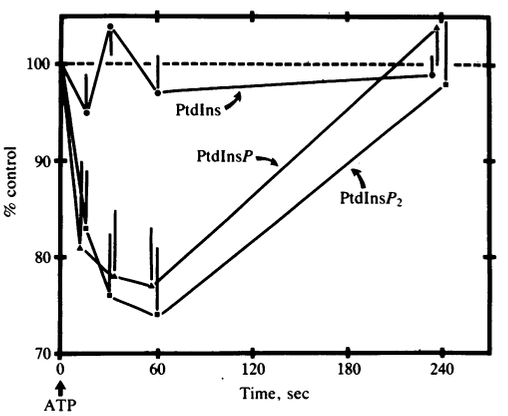

FIG. 4. Time course of ATP-induced inositol phospholipid hydrolysis. The radioactivities of cellular PtdInsP_2 (■), PtdIns$P$ (▲), and PtdIns (●) prelabeled to isotopic equilibrium with [^{3}H]inositol were determined for both unstimulated and ATP-stimulated (1 mM ATP) cells at each time point (n = 3). The results were calculated as described in the legend to Fig. 3. Shown is one of several experiments, each of which yielded similar results. Examples of control cpm (15 sec) were PtdInsP_2 (935 ± 60), PtdIns$P$ (1148 ± 53), PtdIns (37,983 ± 1502), and total (217,952 ± 1294); n = 3.

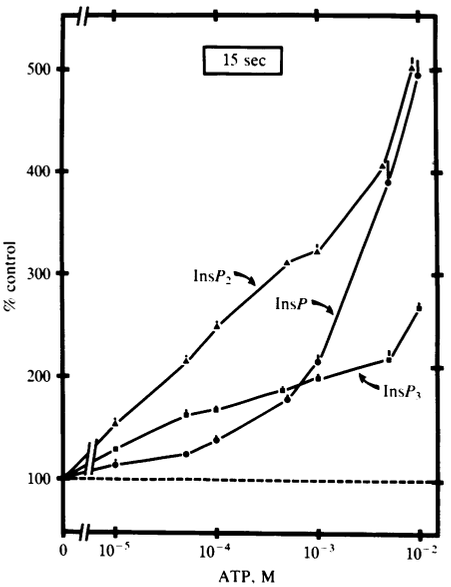

FIG. 5. Concentration dependence of ATP-induced inositol phosphate accumulation at 15 sec. InsP_3 (■), InsP_2 (▲), and Ins$P$ (●) levels were determined 15 sec after ATP addition as described in the legend to Fig. 3 (n = 3).

at 15 sec Ins$P$ levels increased only slightly above control levels at ATP concentrations below 100 μM but showed dramatic increases at ATP concentrations above 1 mM (Fig. 5).

A possible basis for the biphasic effect of ATP on inositol phosphate production could be permeabilization of the cells by ATP. Indeed, tetrabasic ATP (ATP^{4-}, not complexed with either Ca^{2+} or Mg^{2+}) has been shown to permeabilize the plasma membrane of some cell types (18). Although both Ca^{2+} and Mg^{2+} were present in our media, we tested to see if high concentrations of ATP could permeabilize endothelial cells. Using several criteria to assess plasma membrane integrity, including ethidium bromide uptake (19), trypan blue uptake, and lactate dehydrogenase release, we determined that ATP concentrations as high as 10 mM did not permeabilize the cells (data not shown). Moreover, since nucleotides other than ATP, which have not been shown to permeabilize cells, also increased inositol phosphate accumulation (see below), it is unlikely that ATP produced its effects by cell permeabilization.

Nucleotide Specificity of Inositol Phosphate Accumulation. Since P_2-purinergic receptors appeared to mediate the effects of ATP on prostacyclin formation, we performed similar experiments to determine the nature of the ATP receptor mediating inositol phosphate accumulation. Table 1 shows that, whereas both ATP and ADP produced similar large increases in inositol phosphate levels, neither AMP nor adenosine significantly affected basal inositol phosphate levels, thus substantiating the P_2-purinergic receptor nature of both ATP-stimulated prostacyclin formation and inositol phosphate accumulation.

As with prostacyclin formation, nucleotides other than ATP produced similar increases in inositol phosphate levels, with an order of efficacy at 1 mM of ATP > GTP, UTP ≥ TTP > CTP at 10 min in the presence of 10 mM LiCl (to inhibit inositol-1-phosphatase) (Table 2). Similar results were obtained when inositol phosphates were measured at 15 sec (data not shown). Table 2 also shows that the stable analog of ATP α,β-methylene-ATP also stimulated inositol phosphate formation, albeit to a lesser degree than ATP, suggesting that like prostacyclin formation, ATP hydrolysis was not required for its effect on inositol phosphate accumulation.

Table 1. Effect of adenosine and adenine nucleotides on inositol phosphate levels

	% of total cpm in each sample			
	Ins$P$	InsP_2	InsP_3	n
Control	1.69 ± 0.09	1.15 ± 0.09	0.99 ± 0.05	8
ATP	8.91 ± 0.09	3.70 ± 0.09	1.86 ± 0.08	4
ADP	8.21 ± 0.17	3.01 ± 0.03	1.62 ± 0.07	4
AMP	2.17 ± 0.14	1.12 ± 0.01	0.96 ± 0.03	4
Adenosine	1.60 ± 0.02	1.02 ± 0.05	0.96 ± 0.03	4

Inositol phosphate levels were measured 10 min after the addition of each compound (each 1 mM). LiCl (10 mM) was included in the medium. The average total cpm in the control samples was 62,412 ± 5956 (n = 8).

The effect of α,β-methylene-ATP was not additive with that of ATP nor did it antagonize the effect of ATP (Table 2).

Effect of Other Agents on Inositol Phosphate Accumulation. Two additional agents that were found to stimulate prostacyclin formation, bradykinin and thrombin, also stimulated inositol phosphate accumulation. In particular, Table 2 shows that bradykinin (1 μM) at 15 sec produced significant increases in InsP_3 and InsP_2 levels but had no effect on Ins$P$ levels. In contrast, thrombin (2 units/ml) only stimulated Ins$P$ accumulation both at 10 min (Table 2) and at earlier time points (not shown). Components of the chromaffin cell secretory granule that did not stimulate prostacyclin formation (catecholamines, opioid peptides) also did not stimulate inositol phosphate accumulation (data not shown). Thus, the effects on endothelial cells of both bradykinin and thrombin as well as ATP may be mediated by inositol phospholipid metabolism, although their mechanisms of action may differ.

DISCUSSION

The primary finding in our studies is that ATP acts on bovine adrenal medullary endothelial cells in culture by means of P_2-purinergic receptors to activate both phospholipase C and prostacyclin synthesis. The two effects may represent sequential rather than separate discrete actions of ATP based on the following observations. (*i*) The metabolism of PtdInsP_2 and PtdIns$P$ and the resulting formation of InsP_3 and InsP_2 (which was not dependent on the presence of extracellular Ca^{2+}) either preceded or paralleled the formation of prostacyclin. (*ii*) At early time points, the concentration dependence of ATP-induced InsP_3 and InsP_2 accumulation was similar to the concentration dependence of ATP-induced

Table 2. Effect of various nucleotides, thrombin, and bradykinin on inositol phosphate levels

	% of total cpm in each sample			
	Ins$P$	InsP_2	InsP_3	n
Control	1.43 ± 0.07	0.61 ± 0.03	0.66 ± 0.02	6
ATP	6.23 ± 0.35	2.35 ± 0.08	1.55 ± 0.04	3
GTP	5.03 ± 0.33	1.56 ± 0.03	1.16 ± 0.02	3
TTP	4.00 ± 0.01	1.15 ± 0.04	0.95 ± 0.05	3
CTP	3.06 ± 0.06	0.91 ± 0.05	0.85 ± 0.05	3
α,β-Methylene-ATP	3.58 ± 0.08	1.61 ± 0.08	1.16 ± 0.05	3
+ ATP	6.32 ± 0.17	2.67 ± 0.09	1.65 ± 0.10	3
Thrombin	2.46 ± 0.10	0.58 ± 0.04	0.52 ± 0.04	4
Bradykinin	1.37 ± 0.08	1.02 ± 0.03	1.08 ± 0.02	4

Inositol phosphate levels were measured at 10 min except that for bradykinin they were measured at 15 sec. Other than for experiments using bradykinin, LiCl (10 mM) was included in the medium. Concentrations were as follows: nucleotides, 1 mM; thrombin, 2 units/ml; bradykinin, 1 μM. The average total cpm in the control samples was 150,497 ± 19,343 (n = 6). Control percentages were similar at 15 sec and at 10 min.

prostacyclin formation. (*iii*) In most tissues examined to date, InsP_3 stimulates the release of Ca^{2+} from internal stores, causing an increase in intracellular free Ca^{2+} (7, 8). An elevation in intracellular free Ca^{2+} concentrations has been shown to activate the enzyme phospholipase A_2, which, in turn, liberates arachidonic acid from membrane phospholipids (20). Arachidonic acid is then rapidly converted to prostacyclin (21). (*iv*) The metabolism of inositol phospholipids results in the formation of not only inositol phosphates but also of diacylglycerol (7, 8, 22). Diacylglycerol may be an additional source of arachidonic acid through its enzymatic degradation by diacylglycerol lipase (23).

The dramatic effects of ATP on cultured adrenal medullary endothelial cells suggest that endothelial cells in the intact adrenal gland may respond to ATP released from neighboring chromaffin cells. It has been estimated that chromaffin cell secretory granules contain about 150 mM ATP and 20 mM ADP and GTP (24). The release of ATP from chromaffin cells following their stimulation with acetylcholine is rapid and amounts to 10–20% of total cellular ATP (4, 5). Since the distance between chromaffin cells and the fenestrated vascular endothelium is as little as 100–200 nm with only a thin basement membrane separating these two cell types (25), it is likely that ATP released from chromaffin cells bathes the adjacent endothelial cells at high concentrations.

Our evidence that ATP interacts with P_2-purinergic receptors on endothelial cells to induce inositol phospholipid metabolism and prostacyclin formation is consistent with previous reports showing that P_2-purinergic receptors mediate both endothelium-dependent relaxation and prostacyclin formation in endothelial cells derived from other tissues (14, 26). As with P_2-purinergic receptors found on certain other cell types (13, 27, 28), the receptors on adrenal medullary endothelial cells were sensitive to nucleotides other than ATP, such as UTP, TTP, GTP, and CTP. Moreover, the observation that the stable ATP analog α,β-methylene ATP was less effective than ATP in stimulating prostacyclin formation and inositol phosphate accumulation is consistent with a P_{2y}-purinergic receptor subclassification as described by Gordon (14).

There are at least two mechanisms by which ATP could indirectly regulate local blood flow in the adrenal medulla by acting on endothelial cells. (*i*) ATP-induced prostacyclin release from endothelial cells may not only relax smooth muscle and cause vasodilation in the medulla, it may also prevent catecholamine-induced platelet aggregation (29). (*ii*) ATP may stimulate the formation of endothelium-derived relaxing factor (EDRF), which might also vasodilate the medulla (27). The adrenal medullary blood vessels that would probably respond to prostacyclin and EDRF include the medullary arteries that supply oxygen-rich blood directly to the medulla (25, 30). However, an action on peripheral segments of the central vein that drain the adrenal cortex and supply portal blood rich in glucocorticoids to the medulla cannot be excluded (30).

The function, therefore, of ATP released from chromaffin cells may be to influence the critical role that endothelial cells play in controlling blood flow. The concept that ATP released from chromaffin cells may regulate local blood flow can be extended to any secretory cell that contains ATP within its secretory granules. The colocalization of ATP with other hormones and neurotransmitters is common (14, 31) and may represent a general mechanism whereby secretory cells ensure the delivery of hormones to their target organs through the action of ATP on the local vasculature.

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