

P₂-Purinergeric Receptor Agonists Inhibit the Growth of Androgen-independent Prostate Carcinoma Cells

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Abstract

To develop a new approach to the treatment of advanced, hormone-refractory prostate cancer, the signal transductions regulating the growth of human androgen-independent prostate carcinoma cell lines were studied. Agonist-stimulated Ca²⁺ mobilization, a critical regulatory event in other secretory cell types, was studied as a means of identifying previously undescribed plasma membrane receptors that may transduce a growth inhibitory signal. In all of the cell lines tested, P₂-purinergeric receptor agonists, including ATP and certain hydrolysis-resistant adenine nucleotides, induced a rapid, transient increase in cytoplasmic free Ca²⁺ that was detectable at 50 to 100 nM ATP, was maximal at 100 μM ATP, and was inhibited ~ 50% by chelation of extracellular Ca²⁺. Within 8 s after addition, ATP stimulated accumulation of the polyphosphatidylinositol products inositol (1, 4, 5) trisphosphate, inositol (1, 3, 4) trisphosphate, and inositol tetrakisphosphate. In addition to stimulating phosphatidylinositol turnover and Ca²⁺ mobilization, ATP and hydrolysis-resistant ATP analogues induced > 90% inhibition of the growth of all lines tested. These data demonstrate that human androgen-independent prostate carcinoma cells express functional P₂-purinergeric receptors linked to phospholipase C, and that agonists of this receptor are markedly growth inhibitory, suggesting a novel therapeutic approach to this common adult neoplasm. (*J. Clin. Invest.* 1992; 89:191-196.) **Key words:** signal transduction • calcium • phosphatidylinositol turnover • hormone-refractory • adenine nucleotides

Introduction

Prostate cancer is second only to lung cancer as a cause of cancer deaths in men in the United States (1). The standard treatment for metastatic disease is hormonal therapy using antiandrogens in combination with medical or surgical castration, or castration alone (2-6). While there is a > 80% initial response rate to hormonal therapy, essentially all hormonally

treated patients relapse with androgen-independent tumor, which is refractory to further hormonal manipulation and to traditional cytotoxic chemotherapy (7, 8).

Little is known about the growth regulation of androgen-independent prostate cancer. As part of a search for new approaches to controlling the growth of this tumor, we examined human androgen-independent prostate carcinoma cell lines for expression of cell surface receptors that transduce a growth-inhibitory signal. These studies began by testing agonists known to induce phospholipase C activation and phosphatidylinositol (PtdIns)¹ turnover. PtdIns turnover is the central pathway controlling intracellular free Ca²⁺ ([Ca²⁺]_i) levels (9). Although PtdIns turnover has been shown to be critical to the hormonal response of other secretory cell types (10), hormone-stimulated Ca²⁺ transients or PtdIns turnover have not been reported in androgen-independent prostate carcinoma cells. A further reason for studying agonist-stimulated Ca²⁺ mobilization was provided by recent data showing that an increase in [Ca²⁺]_i plays an important role in the death of normal prostatic epithelial cells following androgen withdrawal (11-13). These data suggested that activation of a receptor linked to phospholipase C might be capable of inducing cell death in a Ca²⁺-dependent manner, even in the absence of androgen dependence.

Despite the prevalence of prostate cancer, prostate carcinoma cell lines have been difficult to establish. There are only three well-characterized human prostate carcinoma cell lines; DU 145 and PC-3, both androgen independent, and LNCaP, an androgen-sensitive cell line. In the present study we used DU 145 and PC-3, plus a more metastatic variant of PC-3, PC-3-M, to examine the signal transduction responses of androgen-independent prostate cancer. The data presented here show that androgen-independent prostate carcinoma cells express functional cell surface receptors for adenine nucleotides known as P₂-purinergeric receptors, and that stimulation of this receptor leads to activation of PtdIns turnover and a large increase in [Ca²⁺]_i, derived from Ca²⁺ release from internal stores and the opening of plasma membrane Ca²⁺ channels. Furthermore, these data show that P₂-purinergeric receptor agonists induce concentration-dependent inhibition of the growth of androgen-independent prostate carcinoma cells.

Methods

Cells. The human prostate carcinoma cell lines PC-3 and DU 145 were obtained from the American Type Culture Collection (Rockville, MD). PC-3-M (14) was a gift of Dr. James Kozlowski, Northwestern Univer-

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1. **Abbreviations used in this paper:** PtdIns, phosphatidylinositol; [Ca²⁺]_i, intracellular free Ca²⁺; AMP-PNP, β,γ-imido ATP; ATPγS, adenosine 5'-O-(3-thiotriphosphate); ADPβS, adenosine 5'-O-(2-thiodiphosphate); Ins(1,4,5)P₃, inositol 1,4,5-trisphosphate; Ins(1,3,4)P₃, inositol 1,3,4-trisphosphate.

sity Medical School (Chicago, IL). PC-3 cells were grown in Ham's F-12K medium plus 7% FBS; DU 145 cells were grown in Eagle's modified essential medium plus 10% FBS; PC-3-M cells were grown in RPMI 1640 with 10% FBS. All media were supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 300 µg/ml glutamine, and 25 mM Hepes. Tissue culture media and supplements were obtained from Biofluids, Inc., Rockville, MD. Signal transduction experiments were performed 2 d after propagation, while cells were in logarithmic growth.

Assay of intracellular free Ca^{2+} . $[Ca^{2+}]_i$ was determined by the fura2 spectrofluorometric technique (15). Cells were harvested, washed, and resuspended in Ca^{2+} medium (1 mM $CaCl_2$, 5 mM KCl, 1 mM $MgCl_2$, 140 mM NaCl, 20 mM Hepes, 5 mM dextrose, pH 7.4), incubated with 2 µM fura2/acetoxymethyl ester (AM) (Molecular Probes Inc., Eugene, OR) at room temperature for 30 min, diluted fivefold with Ca^{2+} medium, and incubated at room temperature for an additional 60 min. After loading, cells were washed and resuspended in Ca^{2+} medium at $1.5\text{--}2.0 \times 10^6$ cells/ml and kept on ice until use. Cell viability was determined by trypan blue exclusion and was typically > 95%. Calcium measurements were made at 37°C in a quartz cuvette. Fura2 fluorescence was continuously monitored in a dual wavelength fluorometer (Deltascan; Photon Technology International, South Brunswick, NJ) using excitation wavelengths of 340 and 380 nm, and an emission wavelength of 510 nm.

Assay of inositol phosphates. Inositol phosphates were analyzed as described previously (16). Briefly, cells were incubated for 48 h with 6 µCi/ml myo-[3H]inositol (45–80 Ci/mmol, Dupont/New England Nuclear, Boston, MA) in inositol-free RPMI 1640 medium (NIH Media Unit) without serum. The cells were harvested, incubated in Ca^{2+} medium with 10 mM LiCl for 15 min at 37°C, washed, and resuspended in Ca^{2+} medium plus 10 mM LiCl. Cell suspensions were preincubated at 37°C for 15 min and treated with reagents as indicated. Reactions were terminated by addition of TCA to a final concentration of 10%, and stored on ice for 30 min. After centrifugation at 10,000 g for 10 min, the supernatants were removed, extracted twice with water-saturated ether, and analyzed by anion-exchange HPLC using a Partisil 10 SAX column (25 cm \times 0.45 cm; Whatman Inc., Clifton, NJ) and an ammonium phosphate gradient (0.01 M–1.4 M, pH 3.8, 1 ml/min flow rate). Radioactivity was monitored with an on-line Raytest radiodetector (RSM Analytische Instrumente GmbH, Straubenhardt, Germany) equipped with a 2-ml flow cell, and quantitated using software provided by the manufacturer. The scintillant (Monoflow 4; National Diagnostics Inc., Summerville, NJ) was used at a scintillant:eluate ratio of 4:1 (vol:vol). Inositol phosphates were identified by the elution times of [3H]inositol phosphate standards (Dupont/New England Nuclear).

In vitro growth. Cells were seeded at an initial density of 2.5×10^3 cells/cm² (PC-3), or 1.5×10^3 cells/cm² (DU 145 and PC-3-M) in 24-well plates (Costar Corp., Cambridge, MA). After incubation for 24 h the cells were treated as indicated (day 0). The cells were retreated on days 2, 4, and 6, with one change of medium on day 4. The cells were harvested on the day indicated, and viable cell number determined by hemacytometer counts of trypan blue-excluding cells.

Reagents. ATP, ADP, AMP, adenosine, α,β -methylene ATP, β,γ -methylene ATP, and β,γ -imido ATP (AMP-PNP) were from Sigma Chemical Co., St. Louis, MO. Adenosine 5'-O-(3-thiotriphosphate) (ATP γ S) and adenosine-5'-O-(2-thiodiphosphate) (ADP β S) were from Boehringer-Mannheim Biochemicals Inc., Indianapolis, IN, and 2-methylthio ATP was supplied by Research Biochemicals Inc., Natick, MA.

Results

ATP-stimulated Ca^{2+} response. The addition of 100 µM ATP to fura2-loaded PC-3 cells induced a rapid, transient increase in $[Ca^{2+}]_i$ (Fig. 1 A). The Ca^{2+} transient was detectable immediately after ATP addition, and reached a maximum within 1 min. Within ~ 2 min the cells established a new basal level of

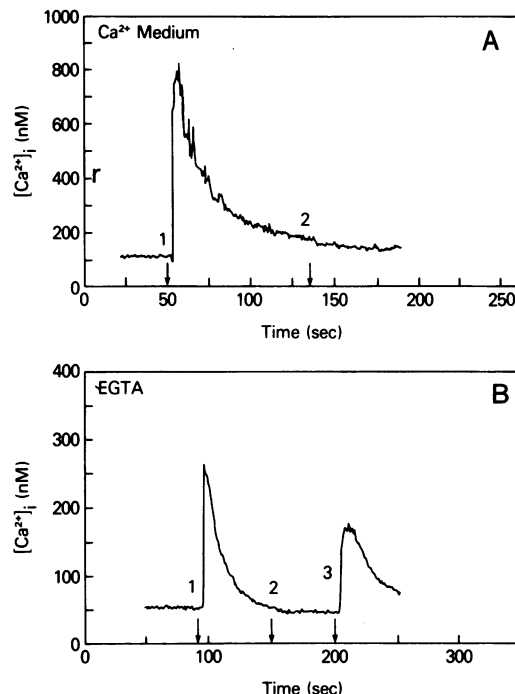


Figure 1. ATP-stimulated Ca^{2+} transient in PC-3 prostate carcinoma cells. Cells were loaded with fura2, the baseline Ca^{2+} level was established, and ATP or ionomycin were added at the times indicated. 1 and 2, ATP (100 µM); 3, ionomycin (5 µg/ml). (A) Tracing from assay performed in medium containing 1 mM Ca^{2+} . (B) Tracing from assay performed in medium containing 2.5 mM EGTA.

$[Ca^{2+}]_i$. After one addition of 100 µM ATP the Ca^{2+} response to a subsequent ATP addition was completely inhibited (Fig. 1 A). Washing the cells, resuspending in agonist-free medium, and incubating at 37°C resulted in full recovery of ATP responsiveness within 10 min (data not shown). This dramatic, agonist-induced desensitization did not result from depletion of internal Ca^{2+} stores, as shown by the ability of ionomycin to increase $[Ca^{2+}]_i$ after stimulation with ATP in EGTA-containing medium (Fig. 1 B). In the absence of significant free extracellular Ca^{2+} the ATP-induced Ca^{2+} response was inhibited ~ 50%, indicative of a Ca^{2+} response derived both from release from internal stores and the opening of plasma membrane Ca^{2+} channels (Fig. 1 B). These data are supported by studies with plasma membrane Ca^{2+} channel blockers showing that preincubation with the Ca^{2+} channel blockers diltiazem (200 µM) or verapamil (20 µg/ml) inhibited the ATP-stimulated Ca^{2+} transient 49 and 30%, respectively. The ATP-stimulated Ca^{2+} response in DU 145 and PC-3-M was of similar intensity, duration, and relative contribution of internal and external stores (data not shown).

Concentration dependence of the ATP-stimulated Ca^{2+} transient. The concentration dependence of the ATP response in the three androgen-independent cell lines is shown in Fig. 2. In PC-3 cells, Ca^{2+} mobilization was detectable at 100 nM ATP and was maximal at 100 µM. In DU 145 and PC-3-M cells the ATP-stimulated Ca^{2+} response was detectable at 50 nM and was maximal at 100 µM. The EC_{50} for Ca^{2+} mobilization was 1 µM in PC-3-M, 5 µM in PC-3, and 6 µM in DU 145. These EC_{50} values are similar to those reported for the P_2 -purinergic receptor-associated Ca^{2+} transient in thyrocytes, (17), adrenal chromaffin endothelial cells (18), and astrocytes (19).

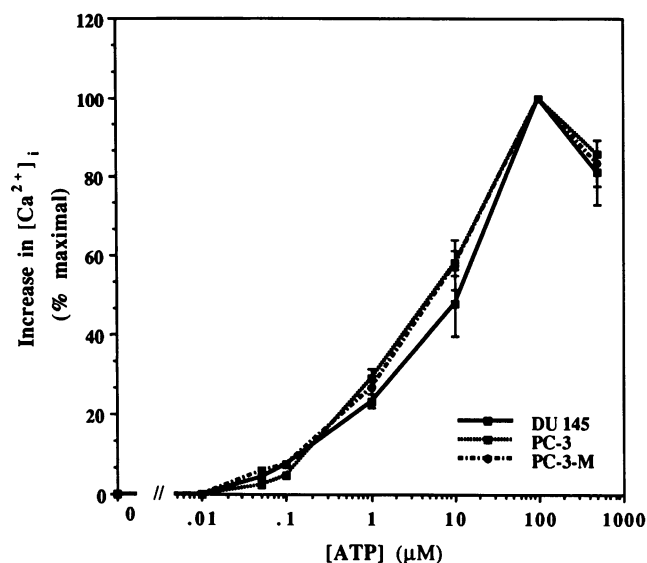


Figure 2. Concentration dependence of the ATP-stimulated Ca^{2+} transient in three human androgen-independent prostate carcinoma cell lines. Cells were loaded with fura2 and ATP-stimulated fluorescence was recorded during incubation of cell aliquots at 37°C. The data presented are the means \pm SD ($n = 3$).

Pharmacologic characterization of the purinergic receptor expressed by androgen-independent prostate carcinoma cells. Purinergic receptors, which have not been purified or cloned, are classified pharmacologically into two major subclasses, P_1 and P_2 , according to the relative activity of a series of agonists. P_1 receptors are more responsive to adenosine than to ADP and ATP, while P_2 receptors are more responsive to ATP and ADP than to AMP and adenosine (20, 21). PC-3 cells expressed purinergic receptors of the P_2 subclass (Fig. 3). P_2 -purinergic receptors have been further divided into two main subtypes, P_{2x} and

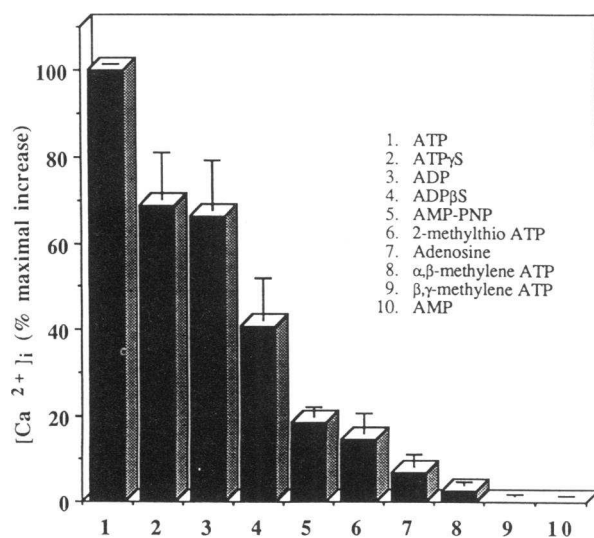


Figure 3. Pharmacologic characterization of the purinergic receptor expressed by PC-3 cells. The data represent the percent increase in $[\text{Ca}^{2+}]_i$ after addition of each purine (100 μM final concentration), expressed as percent maximal response, which is the response to 100 μM ATP. Data shown are the results of three separate experiments. Values are the means \pm SD.

P_{2y} . The order of agonist potency for P_{2x} receptors is β,γ -methylene ATP = α,β -methylene ATP > ATP = 2-methylthio ATP; the order of agonist potency for P_{2y} receptors is 2-methylthio ATP \gg ATP \gg α,β -methylene ATP = β,γ -methylene ATP (22). The data shown here are more consistent with expression of a P_{2y} -purinergic receptor, although the relatively weak activity of 2-methylthio ATP suggests that androgen-independent prostate carcinoma cells may express a subclass of P_2 -purinergic receptor different from the P_{2x} or P_{2y} subtypes. This figure also shows that the hydrolysis-resistant ATP analogues α,β -methylene ATP, ATP γ S, AMP-PNP, and ADP β S were agonists of the prostatic P_2 -purinergic receptor. Because of their diminished susceptibility to ectonucleotidases (21) these analogues should be markedly more active in vivo than unmodified nucleotides. The DU 145 and PC-3-M cell lines also expressed purinergic receptors of the P_2 subclass, with the same relative agonist potency, and these cell lines were responsive to the same nucleotidase-resistant analogues as PC-3 (data not shown).

ATP-stimulated inositol phosphate production. The data in Fig. 1 indicated that $\sim 50\%$ of the ATP-stimulated Ca^{2+} transient derived from release of Ca^{2+} from internal stores. Hormone-stimulated Ca^{2+} release is initiated by the binding of inositol 1,4,5-trisphosphate [$\text{Ins}(1,4,5)\text{P}_3$] to specific $\text{Ins}(1,4,5)\text{P}_3$ receptors on intracellular Ca^{2+} storage sites (9, 23). $\text{Ins}(1,4,5)\text{P}_3$ is produced by phospholipase C-catalyzed hydrolysis of the membrane lipid phosphatidylinositol 4,5-bisphosphate (24, 25). To study further the signals induced by ATP in the prostate carcinoma cell lines, inositol phosphate production after ATP addition was measured. PC-3-M cells were metabolically labeled for 48 h with myo- $[\text{H}^3]$ inositol, treated with 100 μM ATP for the time indicated, protein and lipid were precipitated with TCA, and the aqueous phase was analyzed for inositol phosphate isomers by anion exchange HPLC with radiometric detection. HPLC tracings from a study of the time course of the inositol phosphate response to ATP are shown in Fig. 4, and the data in Table I quantitate the changes in individual inositol phosphate isomers. $\text{Ins}(1,4,5)\text{P}_3$ levels, which were very low in untreated control PC-3-M cells, increased rapidly after ATP addition. $\text{Ins}(1,4,5)\text{P}_3$ levels were 3.3-fold higher than in control cells at 8 s after ATP addition, 3.7-fold higher than control values at 30 s, and declined to close to unstimulated values at 30 min following ATP addition. $\text{Ins}(1,4,5)\text{P}_3$ is a substrate for both an $\text{Ins}(1,4,5)\text{P}_3$ 5-phosphatase that produces inositol 1,4-bisphosphate, and a 3-kinase that produces inositol 1,3,4,5-tetrakisphosphate (26), which is thought to act in concert with

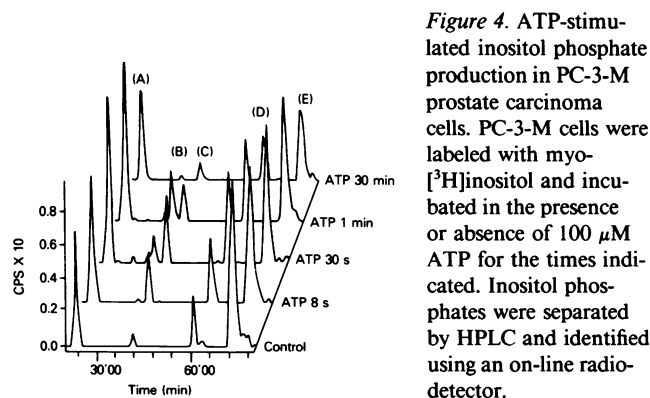


Figure 4. ATP-stimulated inositol phosphate production in PC-3-M prostate carcinoma cells. PC-3-M cells were labeled with myo- $[\text{H}^3]$ inositol and incubated in the presence or absence of 100 μM ATP for the times indicated. Inositol phosphates were separated by HPLC and identified using an on-line radio-detector.

Table 1. ATP-stimulated Inositol Phosphate Production in PC-3-M Prostate Carcinoma Cells

ATP Tx	I(1,4)P ₂	I(1,3,4)P ₃	I(1,4,5)P ₃	IP ₄
None	969±70	10±4	156±60	340±59
8 s	1273±9	32±7	519±85	585±17
30 s	1450±120	220±88	579±8	678±116
60 s	1524±5	385±52	465±102	765±28
30 min	988±116	15±0	169±8	371±9

PC-3-M cells were labeled for 48 h with 6 μ Ci/ml myo-[³H]inositol, washed, and incubated in the presence or absence of 100 μ M ATP for the times indicated. Inositol phosphates were separated by HPLC and quantitated using an on-line radiodetector.

Ins(1,4,5)P₃ to prolong agonist-stimulated Ca²⁺ signals (27). Inositol 1,3,4,5-tetrakisphosphate is dephosphorylated by inositol 5-phosphatase to inositol 1,3,4-trisphosphate [Ins(1,3,4)P₃] (28). Ins(1,3,4)P₃ levels were almost undetectable in untreated cells, increased 3-fold at 8, 22-fold at 30, 38-fold at 60 s, respectively, and returned to basal levels by 30 min. There was also an increase of inositol tetrakisphosphate that was maximal at 60 s. These data show a time course of inositol phosphate production consistent with the pattern of Ca²⁺ response after ATP stimulation, i.e., Ins(1,4,5)P₃ levels increased rapidly following ATP addition, reaching a maximal accumulation at the time of the peak of the Ca²⁺ transient. The maximal increase in IP₄ and Ins(1,3,4)P₃ occurred ~ 30 s after the peak in Ins(1,4,5)P₃ accumulation, as would be expected if Ins(1,4,5)P₃ produced in response to ATP is the precursor for the ATP-stimulated increase in IP₄ and Ins(1,3,4)P₃.

P₂-Purineric receptor agonists inhibit the growth of androgen-independent prostate carcinoma cells. Our primary goal in studying receptor-linked phosphatidylinositol turnover and Ca²⁺ mobilization in prostate carcinoma cells was to use signal transduction studies to explore the biology of prostate cancer and to identify a receptor transducing a growth inhibitory signal in these cells. Having shown that androgen-independent prostate carcinoma cell lines express P₂-purineric receptors coupled to phospholipase C, we examined the effect of agonists of this receptor on cell growth. As shown in Fig. 5, addition of ATP every 2 d resulted in > 90% inhibition of the growth of each of the androgen-independent prostate carcinoma cell lines. In contrast to classical cytotoxic agents, this effect developed gradually over an 8-d period. The efficacy of ATP varied considerably with the treatment schedule. For example, a single addition of ATP typically inhibited growth about 40% (data not shown).

Having established an optimal schedule we tested whether the marked effect on cell growth induced by ATP was also induced by hydrolysis-resistant P₂-purineric receptor agonists. As shown in Fig. 6, the hydrolysis-resistant agonists AMP-PNP and ATP γ S were as effective as ATP in inhibiting cell growth. If ATP and the hydrolysis-resistant ATP analogues were inhibiting growth through activation of phospholipase C and increased [Ca²⁺]_i one would expect the concentration dependence of the ATP-stimulated Ca²⁺ transient to follow the concentration dependence of ATP-induced growth inhibition. As can be seen in Fig. 7, Ca²⁺ mobilization and growth inhibition have the same ATP concentration-dependence. These data, together with the effectiveness of hydrolysis-resistant P₂-puriner-

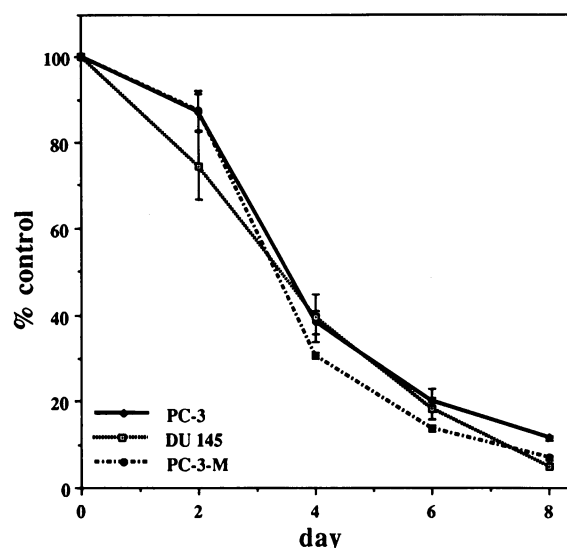


Figure 5. Effect of ATP on the growth of three androgen-independent prostate carcinoma cell lines. Cells were harvested (day minus 1), transferred to 24-well plates, and allowed to adhere for 24 h. Adenine nucleotides (100 μ M) were added on days 0, 2, 4, and 6. Viable cell number was determined at the times indicated by hemacytometer counts of trypan blue-excluding cells. The data are expressed as percent of the number of viable cells in untreated control cultures seeded at the same initial concentration. Values are the means \pm SD ($n = 3$).

gic receptor agonists, strongly associate the growth inhibition after treatment with purineric receptor agonists with agonist-stimulated phosphatidylinositol turnover and Ca²⁺ mobilization.

Discussion

Hormonal therapy of prostate cancer was introduced in 1941 by Huggins and Hodges, who predicted that the growth of prostate cancer would be blocked by androgen withdrawal, based on the observation that the survival and growth of normal pros-

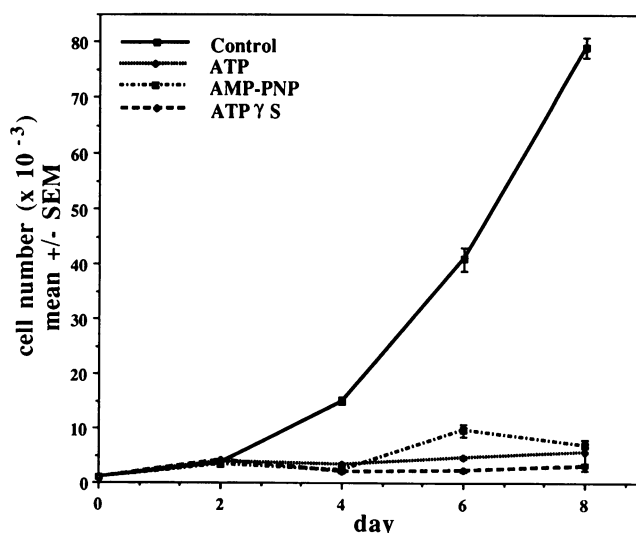


Figure 6. Inhibition of the growth of PC-3-M cells by ATP and hydrolysis-resistant ATP analogues. Cells were plated and treated as described in the Fig. 5 legend.

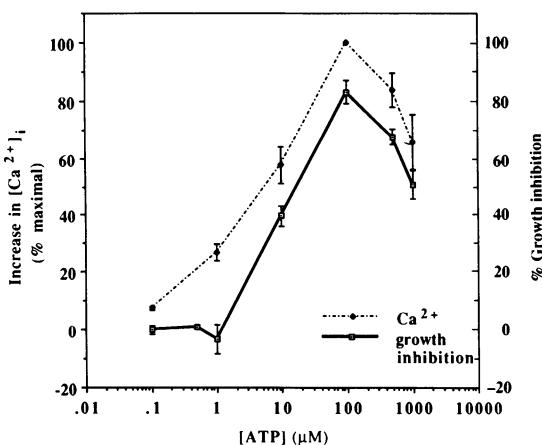


Figure 7. Correlation of the concentration dependence of the ATP-stimulated Ca^{2+} transient and ATP-induced growth inhibition of PC-3-M prostate carcinoma cells. The ATP-stimulated Ca^{2+} transient is expressed as percent maximal response. ATP-induced growth inhibition is expressed as percent growth inhibition of ATP-treated cells versus untreated control cells. All data are the means \pm SD ($n = 3$).

tatic epithelium is dependent on androgen (29, 30). Unfortunately, despite high initial response rates essentially all patients eventually relapse with androgen-independent disease, in which further antiandrogen therapy is ineffective. Recurrence with androgen-independent tumor may result from progressive malignant transformation of hormone-dependent tumor cells, or, as some data suggest, hormone-independent cells may derive from the outgrowth of androgen-independent cancer cell clones present in the tumor before hormonal therapy (31). Regardless of the underlying mechanism, the failure of androgen ablation as a curative therapy and the high frequency of recurrence with androgen-independent disease clearly indicate the importance of developing a new approach to the treatment of prostate cancer, targeted to the androgen-independent cancer cell.

Our approach to this problem has been to study the signal transduction pathways regulating the growth of human androgen-independent prostate carcinoma cell lines. As reported here, ATP and several modified purine nucleotides stimulate PtdIns turnover and Ca^{2+} mobilization in androgen-independent prostatic cancer cells. The relative potency of these agonists is consistent with the expression of a purinergic receptor of the P_2 subtype. P_2 -purinergic receptors are expressed in a wide variety of tissues and cell lines, including urinary bladder and vas deferens, aortic and adrenal chromaffin endothelial cells, hepatocytes, alveolar type II pneumocytes, mast cells, and neutrophils, and a variety of tumor cell lines, including PC12 pheochromocytoma, neuroblastoma, and HL-60 acute myelocytic leukemia cells (see 21 for review), but they have not been reported on cells of prostatic origin. In concordance with the wide distribution of P_2 -purinergic receptors, extracellular ADP and ATP have been shown to have a variety of physiologic effects, including regulation of cardiovascular function, platelet aggregation, smooth muscle contraction, and peripheral and central neurotransmission. While the level of ATP in human plasma is maintained in the 100–500 nM range, much higher concentrations can be achieved locally. Two mechanisms for this are the release of ATP as a cotransmitter by adrenergic and cholinergic neurons, and the release of intracellular ATP after cell injury, a potentially ubiquitous source of

extracellular ATP, because most cells have cytoplasmic ATP stores of > 5 mM (21).

ATP has been reported to stimulate (32) or to inhibit (33, 34) cell growth, depending on the cell type and the constituents of the culture medium. While the effect of ATP on prostate cancer cell lines has not been reported, it is known that ATP can inhibit the growth of some tumor cells, and it has been suggested that tumor cells are more susceptible to the growth inhibitory effects of ATP than nonmalignant cell types (35–37). These studies postulated that ATP and ADP increase the plasma membrane permeability of human tumor cells, allowing the entry of ADP and ATP into cellular adenine nucleotide pools, causing inhibition of DNA synthesis as a consequence of the elevation of intracellular ATP levels. The data presented here are not consistent with ATP-induced plasma membrane permeabilization. Because the concentration of Ca^{2+} in the assay medium is $\sim 10,000$ -fold higher than the cytosolic-free Ca^{2+} concentration, there would be increased fura2 fluorescence with ATP concentrations > 100 μM if ATP were inducing rapid permeabilization, while we have observed a clear decline in fura2 fluorescence after the optimal 100- μM dose (Fig. 2). If ATP were inducing rapid permeabilization, a second addition of ATP would not be associated with complete homologous desensitization in the fura2 Ca^{2+} assay (Fig. 1). In addition, ATP-treated cells did not show an increase in trypan blue uptake compared to untreated cells for at least 24 h after ATP addition (data not shown). The data presented here demonstrate that hydrolysis-resistant ATP analogues were as effective as ATP in inhibiting growth (Fig. 6), which is consistent with P_2 -purinergic receptor-mediated growth inhibition. Furthermore, the concentration dependence of growth inhibition followed the concentration dependence for Ca^{2+} mobilization (Fig. 7). These data show that there was a 50% decrease in the Ca^{2+} response and in growth inhibition as the ATP concentration increased from 100 to 500 μM , which would not be predicted if the mechanism of growth inhibition were through an increase in plasma membrane permeability and increased intracellular ATP levels.

While P_2 -purinergic receptors are typically coupled to inositol phospholipid-specific phospholipase C, it has been shown that these receptors can be coupled to other signal transduction events including activation of phospholipase A_2 and the opening of ATP-activated cation channels (see 38 for review). Therefore, although the data we have presented are consistent with a P_2 -purinergic receptor-activated event, and we have shown that growth inhibition is associated with PtdIns turnover and increased $[\text{Ca}^{2+}]_i$, further work would be required to establish a direct link between PtdIns turnover, Ca^{2+} mobilization, and growth inhibition.

In summary, the data presented here demonstrate that androgen-independent prostate carcinoma cells express functional P_2 -purinergic receptors, and that agonists of this receptor induce PtdIns turnover, Ca^{2+} mobilization, and a marked inhibition of cell growth. These data suggest that signal transduction studies in general, and hydrolysis-resistant P_2 -purinergic receptor agonists specifically, may be useful in developing a new approach to the treatment of advanced prostate cancer.

Acknowledgments

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