Carcinogenesis vol.22 no.3 pp.473–479, 2001

β-Adrenergic growth regulation of human cancer cell lines derived from pancreatic ductal carcinomas

D.L.Weddle, P.Tithoff, M.Williams and H.M.Schuller[1]

Carcinogenesis and Developmental Therapeutics Program, College of Veterinary Medicine, University of Tennessee, 2407 River Drive, Knoxville, TN 37996, USA

[1]To whom correspondence should be addressed

Exocrine ductal carcinoma of the pancreas has been associated with smoking, and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) causes this cancer type in laboratory rodents. Current knowledge on the growth regulation of this malignancy is extremely limited. Recent studies have shown overexpression of cyclooxygenase 2 (COX 2) and 5-lipoxygenase (5-lipox) in exocrine pancreatic carcinomas, suggesting a potential role of the arachidonic acid (AA) cascade in the regulation of this cancer type. In support of this interpretation, our data show high basal levels of AA release in two human cell lines derived from exocrine ductal pancreatic carcinomas. Both cell lines expressed m-RNA for β_2-adrenergic receptors and β_1-adrenergic receptors. Radio-receptor assays showed that β_2-adrenergic receptors predominated over β_1-adrenergic receptors. β_2-Adrenergic antagonist ICI118,551 significantly reduced basal AA release and DNA synthesis when the cells were maintained in complete medium. DNA synthesis of the cell line (Panc-1) with an activating point mutation in codon 12 of the ki-*ras* gene was significantly stimulated by NNK when cells were maintained in complete medium and this response was inhibited by the β-blocker ICI118,551, the COX-inhibitor aspirin, or the 5-lipox-inhibitor MK-886. The cell line without *ras* mutations (BXPC-3) did not show a significant response to NNK in complete medium. When the assays were conducted in serum-free medium, both cell lines demonstrated increased DNA synthesis in response to NNK, an effect inhibited by the β_2-blocker, aspirin, or MK-886. Panc-1 cells were more sensitive to the stimulating effects of NNK and less responsive to the inhibitors than BXPC-3 cells. Our findings are in accord with a recent report which has identified NNK as a β-adrenergic agonist and suggest β-adrenergic, AA-dependent regulatory pathways in pancreatic cancer as a novel target for cancer intervention strategies.

Introduction

Carcinoma of the exocrine pancreas is among the leading causes of cancer death in the US although it ranks only eleventh overall in cancer incidence (1,2). The high mortality of pancreatic cancer is attributed to the lack of early symptoms resulting in an advanced stage of disease at the time of diagnosis. The most common type of pancreatic cancer is ductal adenocarcinoma which comprises 75% of all exocrine pancreatic cancers (3).

Smoking is an established risk factor for pancreatic cancer (2), and the tobacco-specific nitrosamine NNK causes exocrine ductal pancreatic cancer in laboratory rodents (4). It is well established that NNK is metabolically converted into reactive metabolites (5) which react with the DNA molecule to form DNA methyl and pyridyloxobutyl adducts (5). Among these, the DNA adduct O^6-methylguanine has been associated with the expression of an activating point mutation in codon 12 of the Ki-*ras* gene (6). Activating point mutations in codon 12 of the Ki-*ras* gene (75%) and p53 mutations (50%) are common in exocrine ductal pancreatic carcinomas (7–9). Ductal pancreatic adenocarcinomas also frequently overexpress the epidermal growth factor receptor (EGF) (9) and EGF stimulates the growth of these cancer cells *in vitro* (10). More recently, it has been shown that ductal pancreatic carcinomas frequently overexpress COX 2 (11) and 5-lipox (12). These enzymes mediate the metabolic conversion of arachidonic acid (AA) into prostaglandins, thromboxanes and leukotrienes (13). Some of these products have growth stimulating properties in a variety of cell types (14,15). Accordingly, overexpression of COX 2 and/or 5-lipox may contribute to the aggressive growth of this cancer type.

Recent studies have shown that the growth of pulmonary adenocarcinoma is regulated by β-adrenergic receptors via the release of AA *in vitro* (16,17) and *in vivo* (18). This observation is supported by an earlier report that the growth of normal human bronchial epithelia *in vitro* is stimulated by β-adrenergic agonists (19). Moreover, the tobacco-specific nitrosamine NNK has been identified as a high affinity agonist for β-adrenergic receptors (17). Accordingly, NNK as well as classic β-agonists stimulated the growth of pulmonary adenocarcinoma *in vitro* and *in vivo* via the release of AA, whereas β-blockers or inhibitors of COX 2 or 5-lipox had an inhibitory effect (17,18). In light of the documented overexpression of AA-metabolizing enzymes in ductal pancreatic adenocarcinomas (11,12) and the established ability of NNK to induce this cancer type in laboratory rodents (5), we hypothesized that, in analogy to the findings in pulmonary adenocarcinoma, this type of pancreatic cancer may also express a β-adrenergic, AA-dependent growth regulating pathway.

Materials and methods

Cell culture

Human ductal pancreatic adenocarcinoma cell lines Panc-1 and BXPC-3 were purchased from the American Type Culture Collection (Manassas, VA) and maintained in an atmosphere of 5% CO_2 at 37°C in the culture medium recommended by the vendor. Panc-1 harbors an activating point mutation in codon 12 of the ki-*ras* gene (20) whereas BXPC-3 does not have *ras* mutations. Complete medium for Panc-1 cells comprised DMEM medium containing 4.5 g/l glucose, 20% fetal bovine serum (FBS), 2 mM glutamine and 50 000 U/500 ml penicillin, 50 000 µg/500 ml streptomycin. Complete medium for BCPC-3 cells comprised RPMI 1640 medium supplemented with 10% FBS, 2 mM glutamine and 50 000 U/500 ml penicillin, 50 000 µg/500 ml/streptomycin. Low serum medium used in some of the [^{3}H]thymidine

Abbreviations: AA, arachidonic acid; COX 2, cyclooxygenase 2; EGF, epidermal growth factor receptor; FBS, fetal bovine serum; 5-lipox, 5-lipoxygenase; NNK, nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

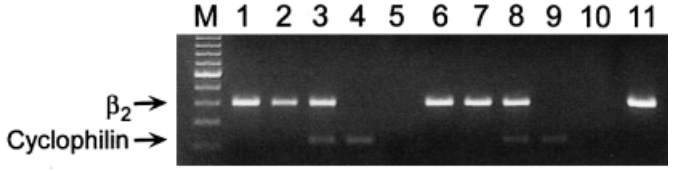

Fig. 1. Expression of mRNA for β_1-adrenergic receptors in the human pancreatic cancer cell lines BXPC-3 and Panc-1. The β_1 primer amplified a 159 bp fragment. Lanes 1 and 2, BXPC-3 with β_1 primers; lane 3, BXPC-3 with β_1 and cyclophylin primers; lane 4, BXPC-3 with cyclophylin primer; lane 5, BXPC-3 negative control without M-MLV reverse transcriptase; lanes 6 and 7, Panc-1 with β_1 primers; lane 8, Panc-1 with β_1 and cyclophylin primers; lane 9, Panc-1 with cyclophylin primer; lane 10, Panc-1 negative control without M-MLV reverse transcriptase; lane 11, transfected CHO cell line Rex 50 with β_1 primers (positive control).

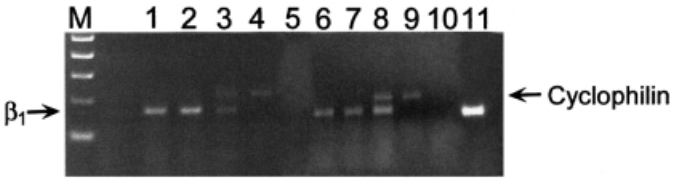

Fig. 2. Expression of mRNA for β_2-adrenergic receptors in the human pancreatic cancer cell lines BXPC-3 and Panc-1. The β_2 primer amplified a 401 bp fragment. Lanes 1 and 2, BXPC-3 with β_2 primers; lane 3, BXPC-3 with β_2 and cyclophylin primers; lane 4, BXPC-3 with cyclophylin primer; lane 5, BXPC-3 negative control without M-MLV reverse transcriptase; lanes 6 and 7, Panc-1 with β_2 primers; lane 8, Panc-1 with β_2 and cyclophylin primers; lane 9, Panc-1 with cylophylin primer; lane 10, Panc-1 negative control without M-MLV reverse transcriptase, lane 11, transfected CHO cell line NBR29 with β_2 primers (positive control).

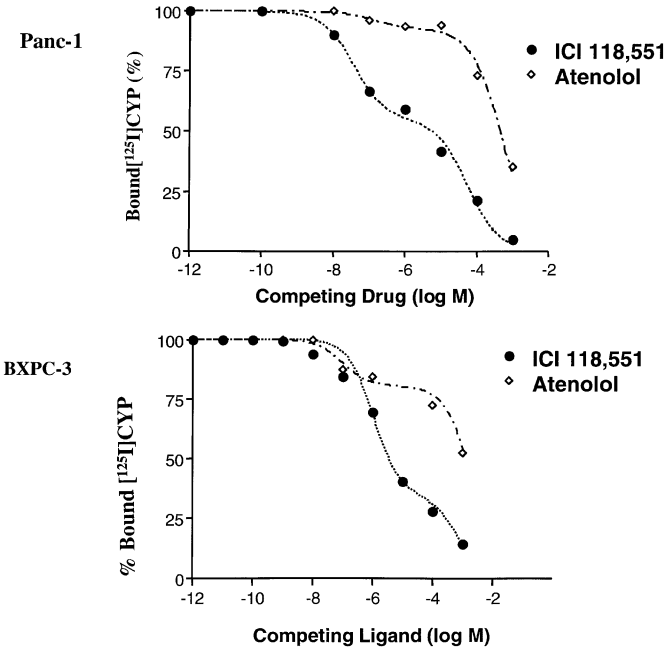

Fig. 3. Results of radio-receptor assays with crude membrane preparations from Panc-1 and BXPC-3 cells. Ascending concentrations of the β_2-adrenergic antagonist ICI118,551 or the β_1-adrenergic antagonist atenolol competed with the broad-spectrum β-adrenergic ligand [^{125}I]CYP (100 pM) for β-adrenergic binding sites under steady state conditions. Non-linear regression analysis for two-site binding isotherms yielded biphasic curves suggesting the presence of two populations of receptors (one with high and one with low affinity for the antagonist under study) in each cell line with β_2-receptors predominating over β_1-receptors (Panc-1: 70% β_2, 30% β_1; BXPC-3: 60% β_2, 40% β_1).

incorporation assays as specified in the text and figure legends was DMEM supplemented with 0.01% FBS, 2 mM glutamine and 50 000 U/500 ml penicillin, 50 000 µg/500 ml streptomycin.

RT–PCR

RNA was isolated using guanidine isothiocyanate/cesium chloride ultracentrifugation (21). Concentration of the RNA was determined by absorbance at 260 nm.

For the reverse transcription (RT) reaction, 2 µg RQ1 treated RNA, 1 µg oligo $dT_{12–18}$ primers (Gibco) and nuclease free water was heated to 82°C for 3 min, then placed on ice. To this was added 0.5 mM each dGTP, dATP, dCTP and dTTP, 10 mM DTT, 40 U RNasin ribonuclease inhibitor (Promega), 200 U Moloney-mouse leukemia virus reverse transcriptase (M-MLV, Gibco) and 10× buffer (100 mM Tris–HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$) in a final volume of 20 µl. The reaction mixture was incubated at 37°C for 1 h, followed by heat inactivation for 10 min at 92°C. A negative control reaction was performed without M-MLV.

The PCR reaction was performed with 5 µl of the RT reaction, which was mixed with 0.2 mM dNTPs, 5 µl 10× PCR buffer (100 mM Tris–HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1.25 U SuperTaq polymerase (Ambion, Austin, TX), 5% DMSO, a primer pair for cyclophilin used as an internal control (75, 125 nM; Ambion), and primers for the human β_1-or β_2-adrenergic receptors (250, 500 nM) and nuclease free water in a final volume of 50 µl. The β_1-adrenergic receptor primers were forward, 5′-CAAGTGCTGCGACTTCGTCACC-3′ and reverse, 5′-GCCGAGGAAACGGCGCTC-3′ which amplify a 159 bp fragment (22). The PCR conditions for the beta$_1$ primers were 1 cycle of 2 min at 94°C, 35 cycles of 94°C, 45 s; 55°C, 45 s; 74°C, 45 s, with a final extension for 5 min at 74°C. The β_2 adrenergic receptor primers were forward 5′-acgcagcaaagggacgag-3′ and reverse 5′-cacaccatcagaatgatcac-3′ which amplify a 401 bp fragment (23). The PCR conditions for the β_2 primers were: 1 cycle of 2 min at 94°C, 37 cycles of 94°C, 60 s; 56°C, 60 s; 72°C, 60 s, with a final extension for 5 min at 72°C. Reactions were run on a MJ Research PTC-200 thermal cycler.

One half of the PCR reaction (25 µl) was run on a 1.5% agarose (Gibco) gel for 2.15 h at 75 V. A 100 bp DNA ladder (Gibco) was run on the same gel. The gel was imaged by ethidium bromide staining using a UVP (Upland, CA) GDS 7500 or an Ultra Lum (Paramount, CA) TUI-5000 gel documentation system.

The PCR fragments were sequenced using the forward primers used to amplify the fragment by RT–PCR with the ABI Terminator Cycle Sequencing reaction kit on an ABI 373 DNA sequencer (Perkin-Elmer, Foster City, CA). Sequences were entered into DNASIS software (S.Hitachi, San Francisco, CA). The sequences using the forward primers were compared with the sequence of human β_1 (GenBank accession no. J03019, bases 747–887) or β_2-adrenergic receptors (GenBank accession no. M15169, bases 1677–2060).

Radio-receptor assays

Radio-receptor assays were conducted as previously described with crude membrane fractions (17). Briefly, ascending concentrations of non-radioactive ligands (atenolol, ICI115,118) competed with the β-adrenergic radioligand [^{125}I]iodocyanopindolol ([^{125}I]CYP; 100 pM; 2200 Ci/mmol; NEN, Boston, MA), under steady state conditions (incubation for 45 min at room temperature) established by saturation binding assays with the radio-ligand at various temperatures and with various incubation times. Non-specific binding was determined by incubations in the presence of 1 mM alprenolol (RBI, Natick, MA). The reactions were terminated by the addition of 2 ml ice-cold 10 mM Tris buffer, and collection of bound radioactivity on Whatman GF/C filters by vacuum filtration (Brandel cell harvester). Radioactivity bound to the filters was determined with a γ counter (Packard). The binding data were analyzed by non-linear regression for two-site isotherms using the computer program Prism/Graph-pad for the Macintosh.

Determination of AA release from pre-labeled cells

The release of AA by AC cells was determined as previously described (24). Briefly, Panc-1 or BXPC-3 cells were seeded into 6-well plates (10^5 cells/well) in complete medium. When they had reached 50% confluency, the cells were incubated with [^{3}H]AA (0.25 µCi/ml, sp. act. 200–240 Ci/mmol; American Radiolabeled Chemicals, St Louis, MO) for 24 h. Following two washes with medium containing 0.1% BSA, cells were incubated in medium with 0.1% BSA for 45 min followed by incubation with NNK or β-blockers as specified in the figure legends. BSA was added to the medium to trap released fatty acids, thus inhibiting subsequent metabolism and reacylation. Accordingly, the radioactivity in the supernatant reflected cumulative deacylation of [^{3}H]AA from phospholipid pools. The incubation times and drug concentrations used were based on published data with pulmonary adenocarcinoma cell lines (17). Following incubation, the medium was removed and placed into scintillation vials with scintillation cocktail (20 ml; Microscint). The remaining monolayers were dissociated by trypsin/EDTA (1 ml of 10×) and placed into scintillation vials with 20 ml scintillation cocktail. Radioactivity was determined by liquid scintillation spectrophotometry (Top Count, Packard,

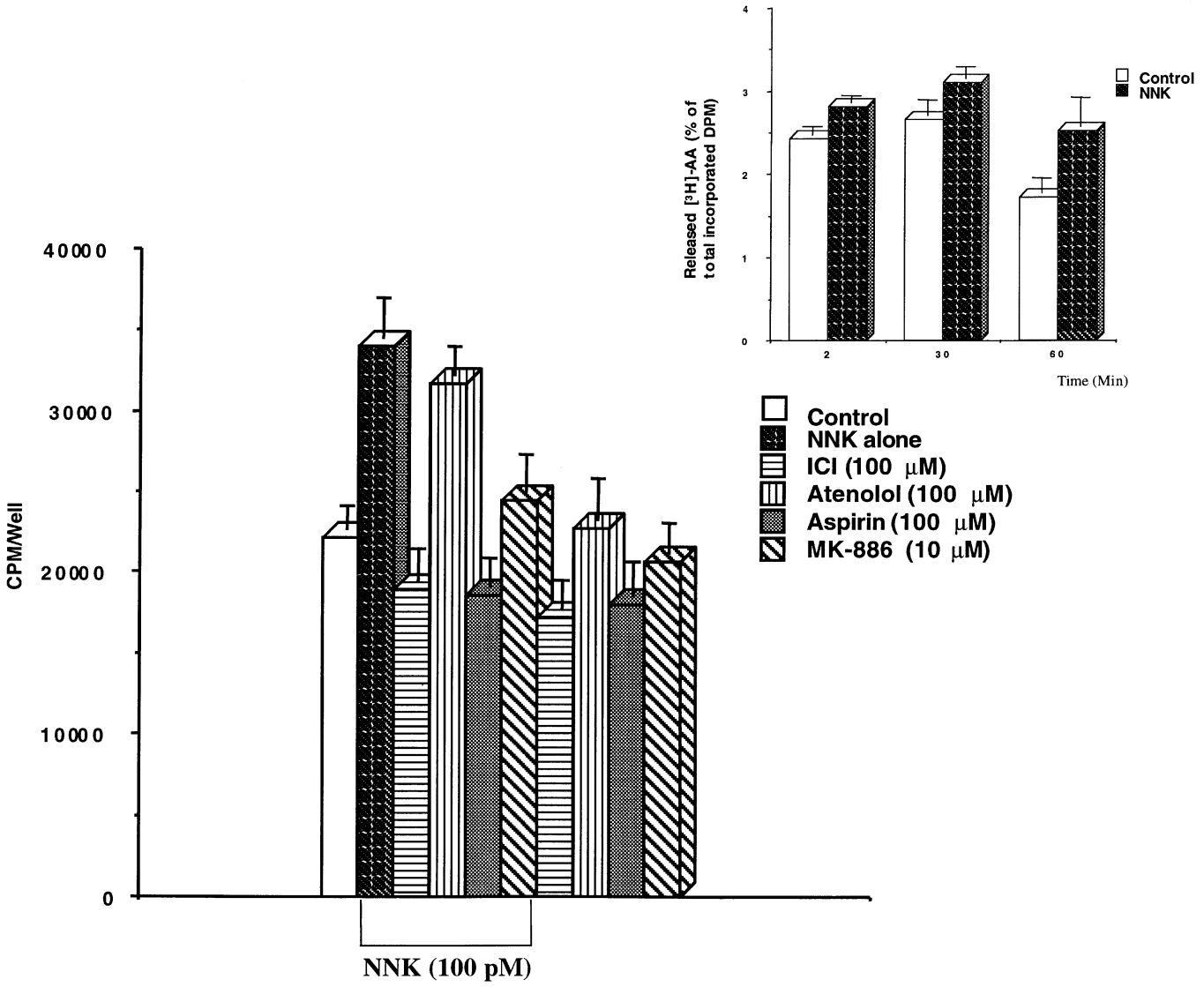

Fig. 4. Results of [^{3}H]thymidine incorporation assays in Panc-1 cells maintained in complete medium. Non-parametric ANOVA showed that variation among group medians was significantly ($P < 0.0001$) greater than expected by chance. NNK (100 pM) caused a 1.5-fold increase in DNA synthesis ($P < 0.02$). The β_2-blocker ICI118,551 (100 μM) significantly reduced DNA synthesis of unstimulated cells ($P < 0.01$) or of NNK-stimulated cells ($P < 0.001$), whereas the β_1-blocker atenolol did not significantly reduce DNA synthesis in unstimulated or NNK-exposed cells. The COX inhibitor aspirin (100 μM) or the 5-lipox inhibitor MK-886 (10 μM) decreased DNA synthesis in unstimulated cells slightly. But this effect was not statistically significant. In contrast, both of these inhibitors significantly reduced DNA synthesis in response to stimulation by NNK (aspirin, $P < 0.008$; MK-886, $P < 0.03$). Inset, AA release over time in unstimulated and NNK-treated (10 nM) Panc-1 cells. NNK significantly ($P < 0.05$) increased the release of AA at all time intervals.

Meride, CT). Released AA was expressed as percent of total incorporated cellular AA. Statistical analysis of data was by ANOVA and group means compared using the Student *t*-test.

[^{3}H]Thymidine incorporation assays

Cells were seeded into 96-well plates (15×10^3 cells/well, five wells/treatment group) in complete medium and allowed to settle for 24 h. [^{3}H]Thymidine (76 Ci/mmol, 0.5 Ci/well, Amersham) and NNK (at concentrations from 10 pM–1 μM) were then added. For assays in low serum medium, cells were seeded in complete medium and allowed to settle for 4 h. Following two washes with PBS, the medium was then replaced with DMEM containing 0.01% FBS followed by the addition of NNK. The β_1-adrenergic antagonist atenolol (100 μM), the β_2-antagonist ICI118,551 (100 μM), the COX inhibitor aspirin (100 μM) or the 5-lipox inhibitor MK-886 (10 μM) were added immediately prior to NNK. Following an incubation period of 24 h in an atmosphere of 5% CO_2 at 37°C, the cells were washed twice with PBS, followed by 0.1 N NaOH. Scintillation fluid (Microscint) was then added to the wells and radioactivity measured with a microplate scintillation and luminescence counter (Top Count, Packard, Meride, CT). Statistical evaluation of data was by non-parametric ANOVA and Mann–Whitney test. Viability of cells as assessed by trypan blue dye exclusion was >97% with all inhibitor concentrations used in this study. The reductions in DNA synthesis observed are therefore indicative of inhibited cell proliferation and were not caused by cytotoxic effects.

Results and discussion

RT–PCR revealed expression of mRNA for β_1- and β_2-adrenergic receptors in both cell lines (Figures 1 and 2). The β_1 primers amplified a 159 bp fragment while the β_2 primers amplified a 401 bp fragment. PCR fragments amplified by the human β_1 primers in the pancreatic cancer cells were 100% identical to the published sequence (GenBank accession no. J03019, bases 747–887). PCR fragments amplified by the human β_2 primers in pancreatic cancer cells were 100% identical to the published sequence (GenBank accession no. M15169, bases 1677–2060).

Radio-receptor assays in which ascending concentrations of atenolol or ICI118,551 competed for β-adrenergic binding sites

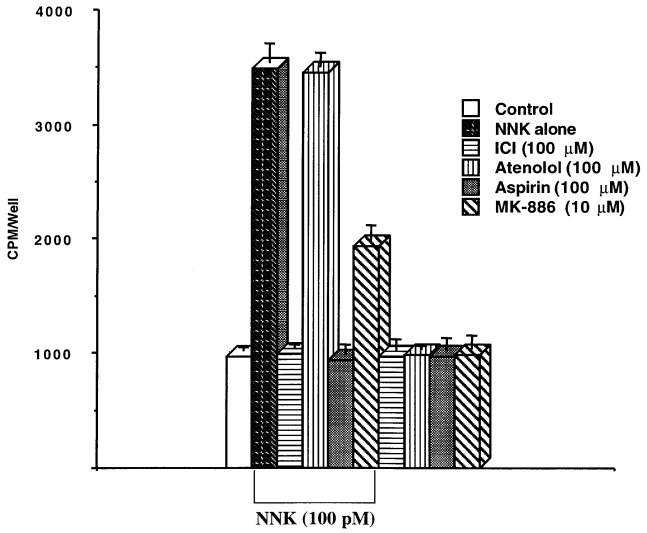

Fig. 5. Results of [^{3}H]thymidine incorporation assays in Panc-1 cells maintained in serum-free medium. Non-parametric ANOVA showed that variation among group medians was significantly ($P < 0.0001$) greater than expected by chance. NNK (100 pM) caused a 3.6-fold increase in DNA synthesis ($P < 0.007$) which was completely inhibited down to the levels of control cells by ICI118,551 (100 μM), aspirin (100 μM, $P < 0.007$) and partially inhibited by the 5-lipox inhibitor MK-886 (10 μM). The group medians for each of these inhibitors was significantly different from the group means of cells treated with NNK alone ($P < 0.007$). Atenolol did not inhibit DNA synthesis of unstimulated or NNK-treated cells under these conditions.

with [^{125}I]CYP (100 pM) generated typical two-site binding curves in both cell lines (Figure 3) indicating the presence of functional β_1- and β_2-adrenergic receptors. Non-linear regression analysis for two-site isotherms of the binding data revealed that the majority of the binding sites were of the β_2-adrenergic type (70% Panc-1 cells; 60% BXPC-3 cells).

Preliminary studies with NNK concentrations from 10 pM to 1 μM demonstrated the greatest increase in DNA synthesis with the 100 pM concentration. This concentration of NNK was therefore used in most assays unless otherwise stated. Both cell lines expressed a β-adrenergic, AA-dependent growth stimulating pathway as evidenced by their response to NNK alone, established β-blockers or inhibitors of COX or 5-lipox in [^{3}H]thymidine incorporation assays (Figures 4–7). Panc-1, which demonstrates an activating point mutation in codon 12 of the Ki-*ras* gene, was sensitive to the growth-stimulating effects of NNK resulting in a low but significant ($P < 0.02$) increase (1.5-fold) in DNA synthesis when maintained in complete medium (Figure 4). This effect was further enhanced (3.6-fold, $P < 0.0001$) when the assay was conducted in low serum (0.01%) medium (Figure 5). In contrast, cell line BXPC-3 which lacks *ras* mutations demonstrated no significant growth stimulation in response to NNK when the cells were maintained in complete medium (Figure 6), whereas a low (1.5-fold) but significant ($P < 0.008$) response to NNK was observed in cells maintained in medium containing only 0.01% FBS (Figure 7). A possible explanation for the different sensitivities of the two cell lines to NNK may be that the activating point mutation of the Ki-*ras* gene in Panc-1 cells rendered these cells more susceptible to the growth stimulating effects of NNK. The site-specific antagonist for β_2-adrenergic receptors ICI118,551 significantly ($P < 0.001$) inhibited DNA synthesis of unstimulated cells maintained in complete medium in both cell lines (Figures 4 and 6), whereas the site-selective antagonist for β_1-adrenergic receptors atenolol had no significant effect. The growth inhibiting effect of ICI118,551 was significantly ($P < 0.001$) greater in BXPC-3 cells (reduction of 82.4%) than in Panc-1 cells (reduction of 25%), suggesting a lower sensitivity of the cell line with activating *ras* mutations (Panc-1) to this β_2-antagonist. The COX inhibitor aspirin and the 5-lipox inhibitor MK-886 both decreased DNA synthesis in unstimulated BXPC-3 cells maintained in complete medium (aspirin: reduction of 61.3%, $P < 0.008$; MK-886: reduction of 49.3%, $P < 0.008$). In contrast, reductions of 18 and 7%, respectively in aspirin- or MK-886-treated Panc-1 cells maintained in complete medium were not statistically significant. ICI118,551 (100 μM) significantly ($P < 0.001$) reduced the growth inhibiting effect of NNK (100 pM) (Figures 4 and 6). In support of data which have shown that NNK competes with significantly higher affinity for β_2-adrenergic binding sites than ICI118,551 (17), concentrations of 10 μM, 1 μM or 100 pM of this β-blocker did not inhibit NNK-stimulated DNA synthesis in either cell line (data not shown). [^{3}H]Thymidine incorporation assays conducted in low serum (0.01% FBS) medium showed that the β_2-blocker ICI118,551, as well as the COX-inhibitor aspirin or the 5-lipox-inhibitor MK886, completely inhibited the growth stimulating effects of NNK in Panc-1 cells (Figure 5). NNK-induced growth stimulation was even further reduced below the levels of control cells by all three of these inhibitors in BXPC-3 cells (Figure 7). These findings further support the interpretation of our data with cells maintained under optimum growth conditions in complete medium, which had suggested a lower sensitivity of the *ras*-mutated cell line (Panc-1) to inhibitors of this β-adrenergic regulatory pathway.

Determination of AA release showed high basal levels of AA release in both cell lines maintained in complete medium (Figure 4, inset; Figure 6, inset). NNK caused a time-dependent increase ($P < 0.05$) over control levels in Panc-1 cells (Figure 4, inset) whereas NNK failed to significantly increase AA release in BXPC-3 cells (exemplified in Figure 6, inset). The β_2-blocker ICI118,551 did not significantly reduce NNK-stimulated AA release in Panc-1 cells (data not shown) while significantly ($P < 0.001$) reducing AA release in unstimulated or NNK-exposed BXPC-3 cells (Figure 5, inset). These data provide further support for the interpretation that the cell line with an activating point mutation in the Ki-*ras* gene (Panc-1) was more sensitive to the stimulation of the β-adrenergic regulatory pathway by NNK, while being relatively resistant to the inhibiting effects of the β-blocker. As ras activation is an event downstream of AA release, these findings also suggest that the β_2-adrenergic receptor itself may be mutated in these cells.

Collectively, our data demonstrate that two human exocrine ductal pancreatic adeno-carcinoma cell lines expressed a β_2-adrenergic receptor-controlled signal transduction pathway which stimulated DNA synthesis via the release of AA. Recent reports have shown that agonists of β_2 adrenergic receptors stimulate the activation of a *ras* and Src tyrosine kinase-dependent mitogen-activated protein (MAP) kinase pathway in fibroblasts (25). Activating point mutations in the Ki-*ras* gene are common in ductal pancreatic carcinomas (7,8), and growth stimulation by the EGF receptor via activation of tyrosine kinase, ras and the MAP kinase cascade has been demonstrated in cell lines derived from these cancers (10). In light of the fact that reactive NNK metabolites cause activating point mutations in the *ras* gene (9,10), it is conceivable that

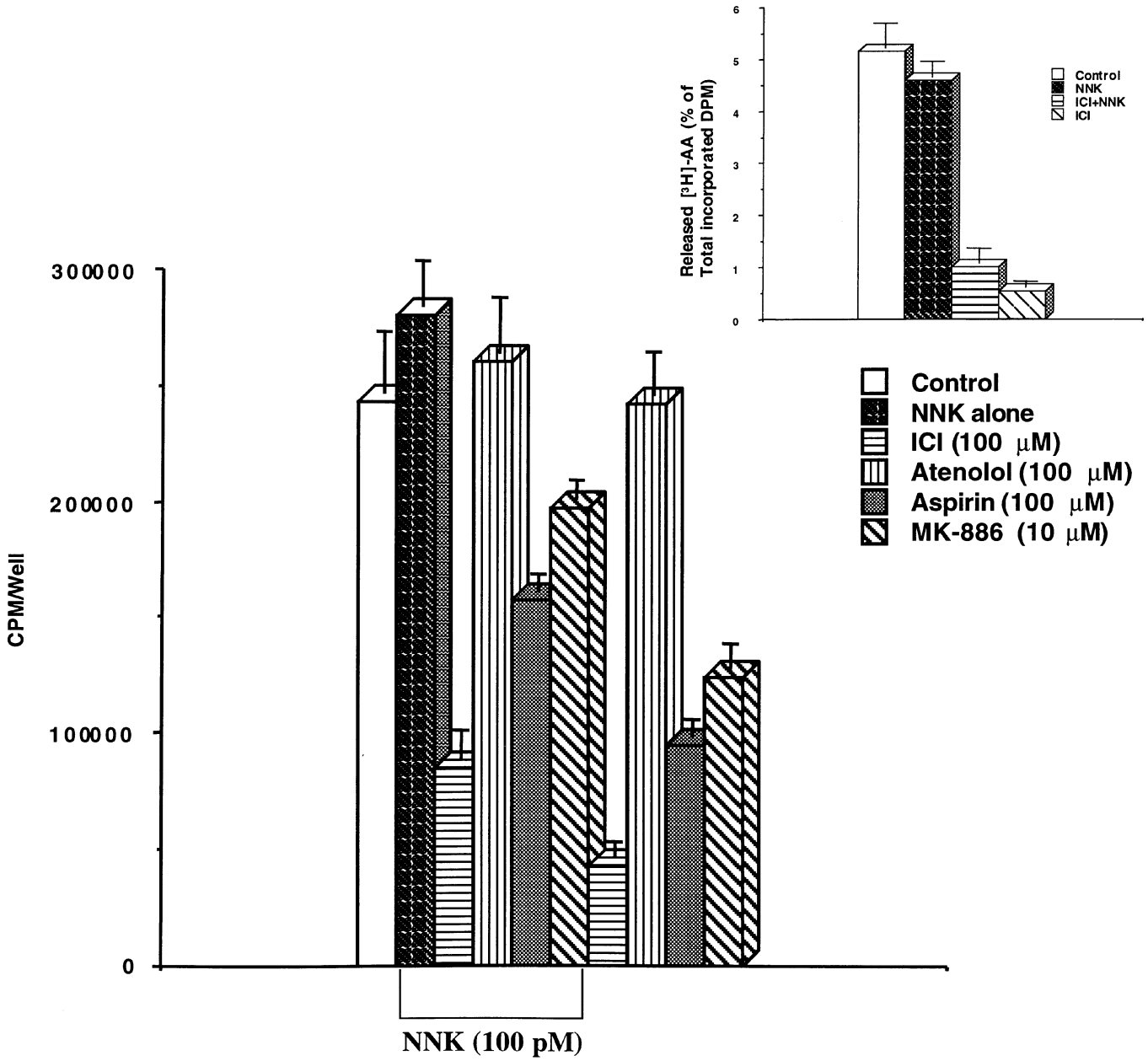

Fig. 6. Results of [^{3}H]thymidine incorporation assays in BXPC-3 cells maintained in complete medium. Non-parametric ANOVA showed that variation among group medians was significantly ($P < 0.0001$) greater than expected by chance. NNK (100 pM) caused a slight but statistically not significant increase in DNA synthesis. ICI118,551 (100 μM), apsirin (100 μM) and MK-886 (10 μM) each significantly ($P < 0.007$) reduced DNA synthesis in unstimulated and NNK-exposed cells. ICI118,551 yielded the strongest inhibition in both, unstimulated (82.4%) and NNK-stimulated (69.6%) cells. The β_1-blocker atenolol (100 μM) had no effect. Inset, AA release in BXPC-3 cells: NNK (100 pM) did not increase the very high basal levels of AA release. However, the β_2-blocker ICI118,551 (100 μM) significantly ($P < 0.001$) inhibited AA release in control and NNK-treated cells.

binding of NNK to β-adrenergic receptors may further stimulate this pathway via activation of Src tyrosine kinase and *ras*. On the other hand, AA itself or its metabolites can act as second messengers and stimulate or inhibit transcriptional events and the cell cycle in a cell type-specific manner (14,15). Moreover, the greater sensitivity of Panc-1 cells to β_2-adrenergic stimulation in conjunction with their reduced responsiveness to the β_2-antagonist suggest that a subset of ductal pancreatic carcinomas may express a mutated β_2-adrenergic receptor rendering it more susceptible to agonists and less responsive to antagonists. Further studies are clearly needed to dissect the complex signaling events involved.

Our data suggest that β-blockers as well as inhibitors of COX or 5-lipox may be useful chemopreventive agents for ductal pancreatic adenocarcinoma in smokers. Broad-spectrum β adrenergic antagonists (β-blockers), such as propranolol or alprenolol, are widely used for the therapy of hypertension (26) and atherosclerosis (27,28). Moreover, the broad-spectrum COX inhibitor aspirin as well as β-blockers are widely used for the therapy and prevention of heart attacks (26,27). As these cardiovascular diseases are among the many adverse health effects caused by smoking (29,30), epidemiologial data to determine the chemopreventive effects of these agents on ductal pancreatic adenocarcinoma development should be readily available.

The striking similarities between our current findings in cell

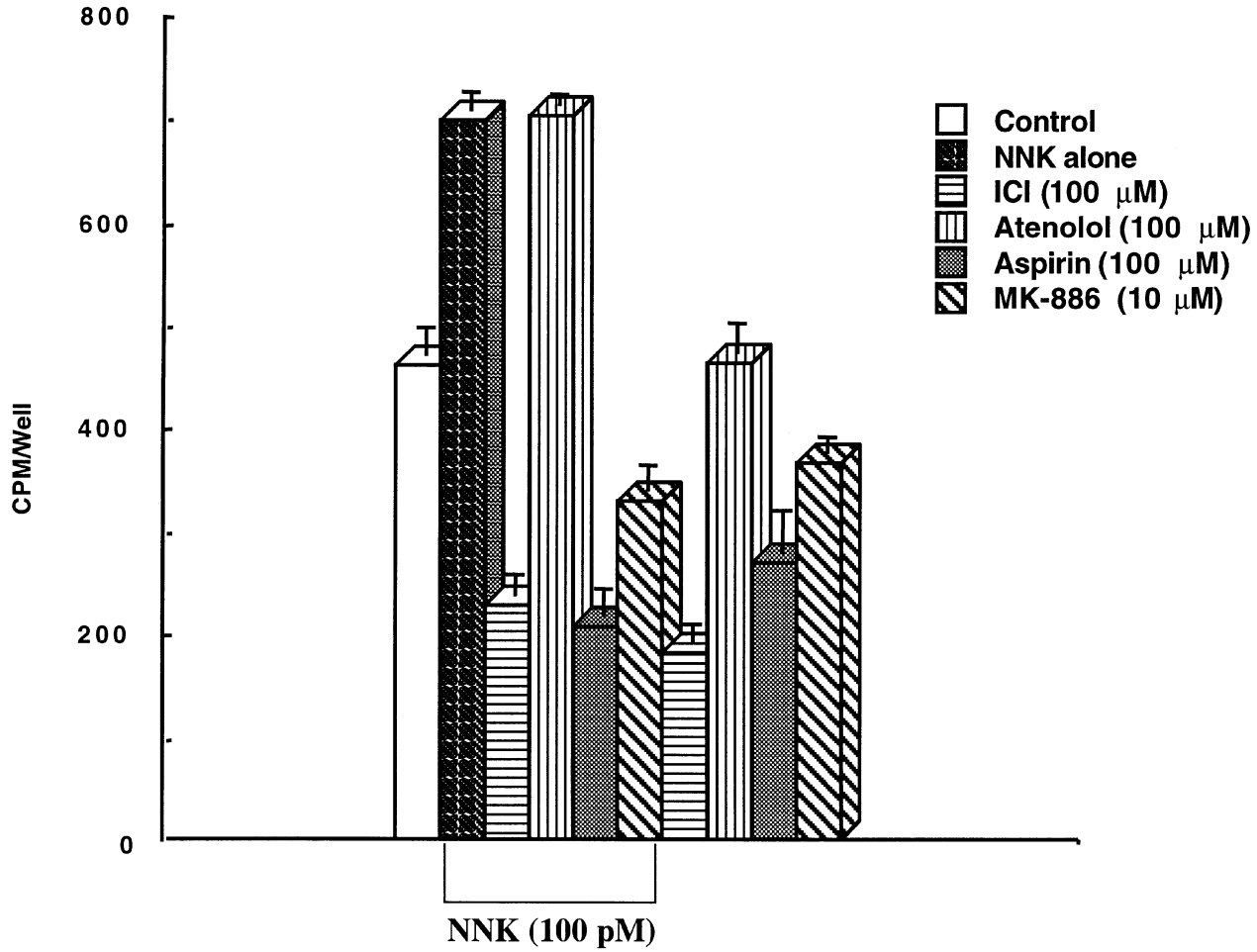

Fig. 7. Results of [^{3}H]thymidine incorporation assays in BXPC-3 cells maintained in serum-free medium. Variation among group means was significantly greater ($P < 0.0001$) than expected by chance by non-parametric ANOVA. NNK (100 pM) caused a 1.5-fold increase in DNA synthesis ($P < 0.04$). ICI118,551 (100 μM), aspirin (100 μM) or MK-886 (10 μM) each reduced DNA synthesis in unstimulated or NNK-treated cells below the levels of controls ($P < 0.008$).

lines derived from pancreatic adenocarcinomas and recent reports on the β-adrenergic, AA-dependent regulation of pulmonary adenocarcinomas (16–18) raises the possibility that a significant subset of adenocarcinomas in a variety of organs may be under β-adrenergic control. Adenocarcinomas which overexpress COX 2 and/or 5-lipox such as have been reported in the breast, colon, and prostate (12) seem prime candidates. In addition to providing hitherto unexplored chemopreventive and clinical management opportunities with β-blockers for these cancers, this novel aspect of cell type specific cancer regulation may also help to identify factors which selectively promote the development of this cancer family. β-Adrenergic agonists are the active ingredients of a host of prescription and over the counter drugs for the treatment of upper and lower respiratory tract diseases, such as the common cold, sinusitis, asthma, bronchitis/bronchiolitis and obstructive pulmonary disease (32) Moreover, many of the recently emerging weight loss treatments raise the overall metabolism by stimulating the sympathetic branch of the autonomic nervous system directly with non-selective adrenergic stimulants (e.g. ephedrine) or indirectly by stimulating the thyroid gland, which in turn results in an increase of physiologic adrenegic agonists. All of these seemingly harmless drugs have the potential to selectively promote the development of adenocarcinomas which are under β-adrenergic control. Further studies are urgently needed to address these concerns.

Acknowledgement

This work was supported by Public Health Service Grant RO1 CA42829 with the National Cancer Institute.

References

1. Di Magno,E.P., Reber,H.A. and Tempero,M.A. (1999) AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. *Gastroenterology*, **117**, 1464–1484.
2. Gold,E.B. and Goldin,S.B. (1998) Epidemiology of and risk factors for pancreatic cancer. *Surg. Oncol. Clin. N. Am.*, **7**, 67–91.
3. Kloppel,G. (1993) Pathology of nonneuroendocrine pancreatic tumors. In Go,V.L.W., Dimagno,E.P., Gardner,J.D., Lebenthal,E., Reber,H.A. and Scheele,G.A. (eds) *The Pancreas: Biology, Pathobiology, and Disease*. Raven Press, New York, pp. 871–879.
4. Rivenson,A., Hoffmann,D., Prokopczyk,B. and Hecht,S.S. (1988) Induction of lung and exocrine pancreas tumors in F344 rats by tobacco-specific and area-derived nitrosamines. *Cancer Res.*, **48**, 6912–6917.
5. Hecht,S.S. (1996) Recent studies on the mechanisms of bioactivation and detoxification of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific lung carcinogen. *CRC Crit. Rev. Toxicol.*, **26**, 163–181.
6. Belinsky,A., Devereux,T.R., Maronpot,R.R., Stoner,G.D. and Anderson,M.W. (1989) The relationship betweeen the formation of promutagenic adducts and the activation of the K-ras protooncogene in lung tumors from A/J mice teated with nitrosamines. *Cancer Res.*, **40**, 5305–5311.
7. Mangrey,S. and King,T.C. (1998) Molecular pathobiology of pancreatic adenocarcinoma. *Front. Biosci.*, **15**, 1148–1160.
8. Perugini,R., McDade,T.P., Vittinberga,F.J. and Callery,M.P. (1998) The molecular and cellular biology of pancreatic cancer. *Crit. Rev. Eukaryot. Gene Expr.*, **8**, 377–393.

9. Sakorafas,G.H., Tsiotou,A.G. and Tsitos,G.G. (2000) Molecular biology of pancreatic cancer; oncogenes, tumor suppressor genes, growth factors, and their receptors from a clinical perspective. *Cancer Treat. Rev.*, **26**, 29–52.
10. Douziech,N., Calvo,E., Laine,J. and Morisset,J. (1999) Activation of MAP kinases in growth responsive pancreatic cancer cells. *Cell Signal*., **11**, 591–602.
11. Yip-Schneider,M.T., Barnard,D.S., Billings,S.D., Cheng,L., Herilman,D.G., Lin,A., Marshall,S.J., Crowell,P.L., Marshall,M.S. and Sweeneyn,C.J. (2000) Cyclooxygenase-2 expression in human pancreatic adenocarcinomas. *Carcinogenesis*, **21**, 139–146.
12. Hong,S.H., Avis,I., Vos,M.D., Martinez,A., Treston,A.M. and Mulshine,J.L. (1999) Relationship of arachidonic acid metabolizing enzyme expression in epithelial cancer cell lines to the growth effect of selective biochemical inhibitors. *Cancer Res*., **1**, 2223–2228.
13. Goetzl,E.J., An,S. and Smith,W.L. (1995) Specificity of expression of eicosanoid mediators in normal physiology and human disease. *FASEB J.*, **9**, 1051–1058.
14. Rao,G., Bass,A., Glasgow,W., Eling,T., Runge,M. and Alexander,T. (1994) Activation of mitogen-activated protein kinases by arachidonic acid and its metabolites in vascular smooth muscle cells. *J. Biol. Chem.*, **269**, 32586–32590.
15. Blobe,G.C., Khan,W.A. and Hannum,Y.A. (1995) Protein kinase C: cellular target of the second messenger arachidonic acid. *Prostaglandins Leukot. Essent. Fatty Acids*, **52**, 129–135.
16. Park,P.G., Merryman,J.I., Orloff,M. and Schuller,H.M. (1995) β-Adrenergic signal transduction in peripheral lung adenocarcinoma: Implications for individuals with preexisting chronic lung disease. *Cancer Res*., **55**, 3504–3508.
17. Schuller,H.M., Tithof,P.K., Williams,M. and Plummer III,H.K. (1999) The tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is a β-adrenergic agonist and stimulates DNA synthesis in lung adenocarcinoma via β-adrenergic receptor-mediated release of arachidonic acid. *Cancer Res*., **59**, 4510–4515.
18. Schuller,H.M., Porter,B. and Riechert,A. (2000) β-Adrenergic modulation of NNK-induced lung carcinogenesis in hamsters. *J. Cancer Res. Clin. Oncol.*, **126**, 624–630.
19. Willey,J.C., Laveck,M.A., McLendon,I.A. and Lechner,J.F. (1985) Relationship of ornithine decarboxylase activity and cAMP metabolism to proliferation of normal bronchial epithelial cells. *J. Cell. Physiol.*, **124**, 207–212.
20. Weisz,B., Giehl,K., Gana-Weisz,M., Egozi,Y., Ben-Baruch,G., Marciano,D., Gierschik,P. and Kloog,Y. (1999) A new functional ras antagonist inhibits human pancreatic tumor growth in nude mice. *Oncogene*, **22**, 2579–2588.
21. MacDonald,R.J., Swift,G.H., Przybyla,A.E. and Chirgwin,J.M. (1987) Isolation of RNA using quanidinium salts. *Methods Enzymol.*, **152**, 219–227.
22. Ihl-Vahl,R., Marquetant,R., Bremerich,J. and Strasser,R.H. (1995) Regulation of β-adrenergic receptors in acute myocardial ischemia: subtype-selective increase of mRNA specific for β_1-adrenergic receptors. *J. Mol. Cell. Cardiol.*, **27**, 437–452.
23. Fujii,N., Shibata,T., Homma,S., Ikegami,H., Murakami,K. and Miyazaki,H. (1997) Exercise-induced changes in β-adrenergic-receptor mRNA level measured by competitive RT–PCR. *J. Appl. Physiol.*, **82**, 1926–1931.
24. Tithof,P.K., Peters-Golden,M. and Ganey,P.E. (1998) Distinct phospholipases A_2 regulate the release of arachidonic acid for eicosanoid production and superoxide anion generation in neutrophils. *J. Immunol.*, **160**, 954–960.
25. Luttrell,L.M., Ferguson,S.S.G., Daaka,Y. *et al*. (1999) β-Arrestin-dependent formation of β_2 adrenergic receptor–src protein kinase complexes. *Science*, **283**, 655–661.
26. Hoffman,B.B. and Lefkowitz,R.J. (1990) Adrenergic receptor antagonists. In Goodman Gilman,A., Rall,T.W., Nies,A.S. and Taylor,P. (eds) *The Pharmacological Basis of Therapeutics*. Pergamon Press, New York, pp. 221–243.
27. Bondjers,G. (1994) Anti-atherosclerotic effects of β-blockers. *Eur. Heart J.*, **15** (suppl. C), 8–15.
28. Burris,J.F. (1993) β-Blockers, dyslipidemia, and coronary heart disease. *Arch. Intern. Med.*, **153**, 2085–2092.
29. Gutstein,D.E. and Valentin,F. (1998) Pathophysiological basis for adjunctive therapies in the treatment and secondary prevention of acute myocardial infarction. *Clin. Cardiol.*, **21**, 161–168.
30. US Office on Smoking and Health (1989). The Health Consequences of Smoking: Cardiovascular Diseases: A Report of the Surgeon General. US Printing Office, Washington, DC, pp. 179–203.
31. Howard,G., Wagenknecht,L.E., Burke,G.L., Diez-Roux,A., Evans,G.W., McGovern,P., Nieto,J. and Tell,G.S. (1998) Cigarette smoking and the progression of atherosclerosis. *Jama*, **279**, 119–124.
32. Hoffman,B.B. and Lefkowittz,R.J. (1990) Catecholamines and sympathomometic drugs. In Goodman Gilman,A., Rall,T., Nies,A.S. and Taylor,P. (eds) *The Biochemical Basis of Therapeutics*. Pergamon Press, New York, pp. 618–637.

Received August 8, 2000; revised November 21, 2000; accepted November 27, 2000