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Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines

(nicotinic acetylcholine receptors/α-bungarotoxin receptors/opioid peptides/tumor suppression)

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ABSTRACT **Using specific ligands, we find that lung cancer cell lines of diverse histologic types express multiple, high-affinity ($K_d = 10^{-9}$–10^{-10} M) membrane receptors for μ, δ, and κ opioid agonists and for nicotine and α-bungarotoxin. These receptors are biologically active because cAMP levels decreased in lung cancer cells after opioid and nicotine application. Nicotine at concentrations (≈100 nM) found in the blood of smokers had no effect on *in vitro* lung cancer cell growth, whereas μ, δ, and κ opioid agonists at low concentrations (1–100 nM) inhibited lung cancer growth *in vitro*. We also found that lung cancer cells expressed various combinations of immunoreactive opioid peptides (β-endorphin, enkephalin, or dynorphin), suggesting the participation of opioids in a negative autocrine loop or tumor-suppressing system. Due to the almost universal exposure of patients with lung cancer to nicotine, we tested whether nicotine affected the response of lung cancer cell growth to opioids and found that nicotine at concentrations of 100–200 nM partially or totally reversed opioid-induced growth inhibition in 9/14 lung cancer cell lines. These *in vitro* results for lung cancer cells suggest that opioids could function as part of a "tumor suppressor" system and that nicotine can function to circumvent this system in the pathogenesis of lung cancer.**

Lung cancer is classified into small-cell lung cancer (SCLC) representing 25% of all cases and non-small-cell lung cancers (non-SCLC) representing the remaining 75% (1). SCLCs exhibit many neuroendocrine features and produce a variety of neuropeptides, some of which act as autocrine growth regulators (1, 2). In this regard, Roth and Barchas (3) found [^{3}H]etorphine binding and opioid-like immunoreactivity in two human SCLC cell lines (NCI-H146 and NCI-H187) and suggested a possible autocrine role for opioids in SCLC. In contrast, although non-SCLC cell lines produce autocrine growth factors, they usually do not express neuroendocrine characteristics and have not been studied for endogenous opioid systems (4, 5).

Although there is a strong association between cigarette smoking, nicotine addiction, and the development of lung cancer, other roles of nicotine or its carcinogenic derivatives in the pathogenesis of lung cancer are unknown (6, 7). Nicotine binds stereospecifically to acetylcholine receptors in the peripheral and central nervous system leading to behavioral, cardiovascular, neuromuscular, endocrine, and metabolic effects (6, 8, 9). However, the possibility that nicotine could have a direct effect on lung cancer cells has not been generally studied. In fact, prior studies have indicated the presence of muscarinic but not nicotinic acetylcholine receptors (nAChR) in SCLC (10), whereas non-SCLCs have not been examined for either receptor type.

We have considered the possibility that the growth of lung cancer cells may be directly influenced by opioids and nicotine. The reasons for studying opioid–nicotine interactions include the addiction to nicotine from smoking and the use of narcotics for pain relief in lung cancer patients. In addition, several reports suggest an antagonism between opioid- and nicotine-stimulated pathways on behavioral and physiological processes in humans and whole animals (11–13). The data presented here show that lung cancer cell lines of diverse histologies express multiple opioid receptors as well as receptors for nicotine and α-bungarotoxin. Addition of opioids to the culture medium inhibits growth of these carcinoma cells, whereas the addition of nicotine together with the opioids reverses this process in a number of cell lines. These findings suggest a model in which endogenously produced opioids inhibit lung cancer growth, and exogenous nicotine reverses this inhibitory effect on growth.

MATERIALS AND METHODS

Materials. [^{3}H]Etorphine (30 Ci/mmol; 1 Ci = 37 GBq), [^{3}H]ethylketocyclazocine (EKC) (45 Ci/mmol), and U-50,488H (Upjohn) were donated by the National Institute on Drug Abuse (Rockville, MD); levorphanol was a gift from Hoffman–La Roche. ^{3}H-labeled [D-Ala^2,D-Leu^5]enkephalin (DADLE) (36 Ci/mmol), ^{3}H-labeled [D-Ala^2,*N*MePhe^4,Gly^5-ol]enkephalin (DAGO) (60 Ci/mmol), [3-iodotyrosyl-^{125}I, Leu^5]enkephalin (2000 Ci/mmol), and [^{3}H]α-bungarotoxin (83 Ci/mmol) were purchased from Amersham. (−)-[^{3}H]nicotine (63 Ci/mmol) and (±)-[^{3}H]nicotine (51 Ci/mmol) were purchased from DuPont/NEN. Commercial sources for the other chemicals were as follows: DAGO, DADLE, [D-Pen^2,D-Pen^5]enkephalin (DPDPE), (Peninsula Laboratories); morphine sulfate (Mallinckrodt); (−)-nicotine ditartrate, hexamethonium bromide, decamethonium bromide, mecamylamine hydrochloride, α-bungarotoxin, atropine, naloxone hydrochloride (Sigma).

Cell Lines and Growth Assays. Previously characterized SCLC and non-SCLC cell lines were grown in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum as described (14, 15). That cells were free of mycoplasma contamination was indicated by a molecular hybridization assay used according to the vendor's instructions (Gen-Probe, San Diego, CA). A semiautomated colorimetric assay (16), based on the ability of live cells to reduce a tetrazolium-based compound, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) to give a purple-colored product, was used to measure growth of the cells spectrometrically in the presence of various agonists and antago-

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Abbreviations: DAGO, [D-Ala^2,*N*MePhe^4,Gly^5-ol]enkephalin; DADLE, [D-Ala^2,D-Leu^5]enkephalin; Pen, penicillamine; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; EKC, ethylketocyclazocine; SCLC, small-cell lung cancer; non-SCLC, non-small-cell lung cancer; nAChR, nicotinic acetylcholine receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

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nists. Under the conditions used, the viable cell number is directly proportional to MTT reduction. Appropriate numbers of cells (1000–5000 per well) were cultured in 96-well tissue culture plates with various concentrations of the drugs, and 5 days later assayed with MTT. Absorbance was measured at 540 nm by using an automated microplate reader (Bio-Tek Instruments, Burlington, VT) with a program for determining mean and SD (usually < 8%) for each data point derived from eight replicate cultures. Cell growth was also measured by counting viable cells in a hemacytometer using trypan blue exclusion after brief trypsinization to obtain a single-cell suspension. Statistical analysis was performed with a microcomputer-based program (Statview 512+, Brain-Power, Calabasas, CA).

Receptor-Binding Assays. Cells were collected during the logarithmic phase of growth by centrifugation. The membrane preparations and receptor assays were performed as described (3). Membrane protein concentration was determined by using the Bio-Rad protein assay kit. Ligand binding to the intact membranes was measured as follows: in a final volume of 1 ml, aliquots of membranes (≈200 μg of protein) in 50 mM Tris·HCl buffer, pH 7.4, were incubated in triplicate for 60 min at 24°C with various concentrations of the ^{3}H-labeled ligand with or without excess of the specific nonradioactive ligand. Bound radioactivity was collected on Whatman GF/B glass fiber filters, which were then washed three times with 5 ml of ice-cold incubation buffer. Filters were placed in scintillation fluid, and radioactivity was counted. Specific binding was calculated as the difference between total binding and binding in the presence of excess nonradioactive ligand. For the nicotine receptor assays, both (−)-[^{3}H]nicotine and (±)-[^{3}H]nicotine were used with excess nonradioactive (−)-nicotine, and similar results were seen with both radioactive ligands. Scatchard analysis of the data was evaluated using a modification of the LIGAND computer program (17).

RIAs and cAMP Measurements. Cell extracts for RIA were prepared as described (3). The RIA kits for human β-endorphin, porcine dynorphin A (Peninsula Laboratories) and [L-Leu^5]enkephalin (Amersham) were used according to the vendors' instructions. For cAMP assays, cells were cultured for 4 days in 24-well plates in 1 ml of medium, and the medium was changed the day before drug treatment. The cells were incubated with the various drugs (100 nM) for 20 min at 37°C, extracts were prepared, and intracellular cAMP levels were measured by radiometric assay (Amersham kits) according to the vendor's instructions.

RESULTS

Opioid and Nicotine Receptors Are Expressed on Diverse Types of Lung Cancer Cell Lines. We screened cell membrane preparations from 7 SCLC and 10 non-SCLC cell lines for total opioid-binding activity by using [^{3}H]etorphine, a narcotic agonist that has similar affinities for μ, δ, and κ receptor types (18) and found that both SCLC and non-SCLC cell lines contained significant levels of specific opioid-binding activity (Table 1). Using [^{3}H]nicotine or [^{3}H]α-bungarotoxin, we found that the lung cancer cell lines also express specific receptors for these ligands (Table 1). For both the opioid and nicotine receptor assays, specific binding constituted an average of ≈50–75% of total binding activity at the 2 nM concentration of ^{3}H-labeled ligands used and was linearly dependent on the protein concentration of the membrane preparation (between 10 and 200 μg of protein). The amount of specific binding for each ligand varied many fold among the lung cancer cell lines but was similar or higher than those reported in studies of mammalian brain (18–20). Statistical

Table 1. Binding of etorphine, (−)-nicotine, and α-bungarotoxin to membranes of lung cancer cell lines and inhibition of their growth by morphine

NCI lung cancer cell line	Specific binding of ^{3}H-labeled ligand,* fmol bound per mg of protein			Growth inhibition by morphine,† %
	Etorphine	Nicotine	α-Bungarotoxin	
SCLC				
H187‡	450	94	174	32
H69	202	237	559	92
H146	172	1024	4	48
H660 (extrapulmonary)	154	33	99	ND
H82	66	490	366	37
N417	39	1053	194	66
H345	18	77	70	ND
Non-SCLC				
H322 (bronchioalveolar)	293	169	134	44
H460 (large cell)	194	0	10	55
H157 (squamous)	157	407	0	65
H23 (adenocarcinoma)	119	402	76	14
H290 (mesothelioma)	78	74	0	71
H1373 (adenocarcinoma)	36	55	104	ND
H661 (large cell)	34	48	105	64
H125 (adenosquamous)	22	45	79	38
H358 (bronchioloaveolar)	20	36	59	72
H1299 (large cell)	7	19	18	64

NCI, National Cancer Institute; ND, not done.

*Two nanomolar concentrations of the radioactive ligands were tested for specific binding with and without 100 nM each of nonradioactive levorphanol for the [^{3}H]etorphine-binding assay, and (−)-nicotine and α-bungarotoxin for the [^{3}H]nicotine and [^{3}H]α-bungarotoxin assays.

†The % inhibition of growth over control (average of eight replicate cultures) in medium supplemented with 100 nM morphine in the 5-day MTT assay.

‡In cell line H187, opioid-binding activity was markedly inhibited (>90%) by heat (60°C for 15 min), trypsin (10 μg/ml for 15 min at 30°C), and proteinase K (10 μg/ml for 15 min at 30°C), whereas pretreatment with the sulfhydryl reagent, *N*-ethylmaleimide (0.5 mM) for 15 min at 30°C, followed by quenching with dithiothreitol (2.5 mM), reduced specific binding by 86%.

analysis showed no significant correlation between the levels of etorphine, nicotine, or α-bungarotoxin binding in the different cell lines.

Determination of Specific Opioid Receptor Types on Lung Cancer Cell Lines. Opioids exert their effects by interacting with the μ, δ, and κ opioid receptor types (for review, see ref. 21). Using highly specific ligands such as DAGO (μ agonist), DPDPE (δ agonist), and U-50,488H (κ agonist), we found that the lung cancer cell lines exhibited multiple opioid receptor types (Table 2). The major difference found between SCLC and non-SCLC cell lines is the absence of high-affinity κ agonist binding in the non-SCLC lines. Using the SCLC line H187 and the non-SCLC line H157, we found the binding to be saturable for the different opioid ligands used (Fig. 1). Scatchard analysis of the binding data showed that the SCLC line H187 exhibited high-affinity sites for [^{3}H]DAGO, with a best fit for a single high-affinity binding component (Fig. 1, B_{max} = 175 fmol/mg of protein; K_d = 5.8 nM), whereas the Scatchard plot for [^{3}H]EKC (using nonradioactive U-50,488H for displacement) could be resolved into two linear components with K_d values of 1.2 nM and 7 nM (B_{max} = 120 fmol/mg of protein and 900 fmol/mg of protein, respectively) (Fig. 1). No high-affinity binding to H187 was detected for [^{3}H]DADLE (using nonradioactive DPDPE for displacement). The non-SCLC line H157 expressed high-affinity binding sites for [^{3}H]DAGO (B_{max} = 800 fmol/mg of protein; K_d = 6 nM) and [^{3}H]DADLE (B_{max} = 1700 fmol/mg of protein; K_d = 2.2 nM), whereas no high-affinity binding to [^{3}H]EKC was detected (Fig. 1).

Characteristics of [^{3}H]Nicotine Binding to Membranes of Lung Cancer Cell Lines. The binding observed with (−)-nicotine was saturable; half-maximal binding activity was 1 and 5 nM for H187 and H157, respectively (Fig. 2). Scatchard analysis of the [^{3}H]nicotine binding showed high-affinity sites for the SCLC cell line H187 (B_{max} = 119 fmol/mg of protein, K_d = 1.4 nM) and the non-SCLC cell line H157 (B_{max} = 5042 fmol/mg of protein, K_d = 14 nM) (Fig. 2). [^{3}H]Nicotine binding was displaced by various nicotine antagonists including hexamethonium (a ganglionic antagonist, marking C_6 nAChRs seen in the peripheral nervous system) and decamethonium (a C_{10} antagonist active at the neuromuscular junction) (Table 2). Mecamylamine (marking receptors in both the central and peripheral nervous system) partially displaced [^{3}H]nicotine binding to SCLC cell lines but only marginally inhibited its binding to non-SCLC membranes. In contrast, atropine, a muscarinic acetylcholine antagonist, did not compete well for nicotine binding in these cell lines (data not shown).

Table 2. Opioid and nicotine receptor types expressed in lung cancer cell lines

NCI lung cancer cell line	Receptor type	
	Opioid*	Nicotine†
SCLC		
H69	μ, δ, κ	C_6, C_{10}, Mec
H146	μ, κ	C_6, C_{10}, Mec
N417	μ, δ, κ	C_6, C_{10}
H187	μ, κ	C_6, C_{10}, Mec
H345	ND	C_6, C_{10}
Non-SCLC		
H23	μ, δ	C_6, C_{10}
H157	μ, δ	C_6, C_{10}
H358	δ	C_6, C_{10}
H322	ND	C_6, C_{10}, Mec
H125	ND	C_6, C_{10}

NCI, National Cancer Institute; ND, not done.
*One hundred nanomolar nonradioactive ligands were used in the binding assays with 1 nM of the ^{3}H-labeled ligand. DAGO was used with [^{3}H]DAGO for the μ receptor, DPDPE with [^{3}H]DADLE for the δ receptor, and U50,488H with [^{3}H]EKC for the κ receptor.
†For nicotine receptor analysis 100 nM of nonradioactive (−)-nicotine or nicotine antagonists were tested for their ability to displace [^{3}H]nicotine (2 nM) binding. Hexamethonium used for the C_6 receptors and decamethonium used for the C_{10} receptors competed as efficiently as (−)-nicotine for [^{3}H]nicotine binding. Mecamylamine (Mec) at 100 nM, identifying both central and peripheral nACHRs, only partially displaced [^{3}H]nicotine binding.

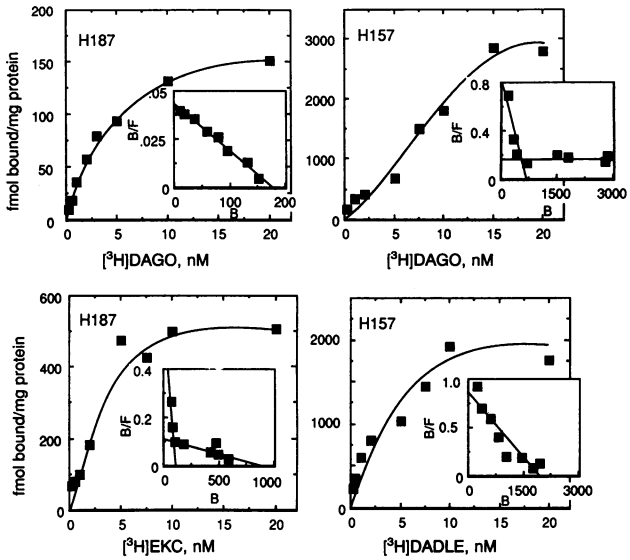

FIG. 1. Saturation binding and Scatchard analysis of specific opioid binding to membranes of lung cancer cell lines H187 (SCLC) and H157 (non-SCLC). Various concentrations of the ^{3}H-labeled ligands were incubated with cell membranes with and without excess nonradioactive compounds as described. The ^{3}H-labeled ligands used are shown; the specific nonradioactive ligands were used as follows: DAGO with [^{3}H]DAGO (μ receptor), DPDPE with [^{3}H]DADLE (δ receptor), and U50,488 H with [^{3}H]EKC (κ receptor). Each experiment was repeated twice with similar results. B, fmol bound/mg of protein; B/F, bound/free.

Regulation of Intracellular cAMP Levels by Opioids and Nicotine. Opioids act, in part, through lowering intracellular cAMP levels (22, 23). Decreases in intracellular cAMP levels compared to control levels found after agonist (100 nM) application to SCLC cell line H187 were 60% for DAGO, 30% for morphine (μ), 10% for DADLE (δ), 30% for U-50,488H (κ), and 38% for nicotine. Likewise, decreases in intracellular cAMP levels were found in the non-SCLC cell line H157 with morphine (34%) and DADLE (55%) but not with the κ agonist U-50,488H. Forskolin (10 μM) stimulated cAMP levels (5- to 10-fold) in H187 and H157 cells, and these increases were reduced by morphine (62%). Pretreatment of the cells with pertussin toxin at 100 ng/ml for 3 hr before the addition of opioids resulted in an increase of ≈20–30% in cAMP levels

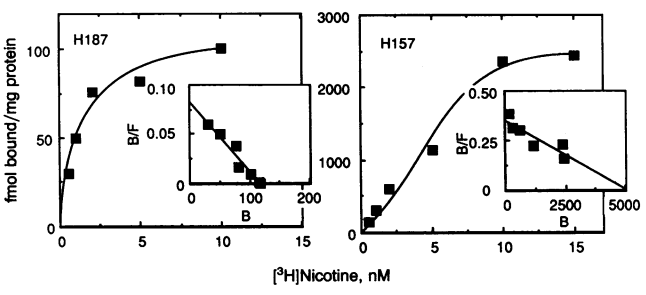

FIG. 2. Saturation binding and Scatchard analysis of specific [^{3}H]nicotine binding to membranes of lung cancer cell lines H187 and H157. One micromolar (−)-nicotine was used as the nonradioactive ligand. Each experiment was repeated three times.

over control levels. In addition, the guanine nucleotide GTP (100 μM) reduced [^{3}H]etorphine binding by 75% relative to control binding. Together, these data suggest involvement of an inhibitory guanine nucleotide-binding protein in the action of opioids in these cell lines.

Opioid-Peptide Immunoreactivities Are Expressed in Human Lung Cancer Cells. Cell extracts of five SCLC (N417, H82, H69, H187, and H60) and five non-SCLC (H460, H596, H125, H157, and H23) lines were assayed for β-endorphin-, [Leu^5]enkephalin-, and dynorphin A-like immunoreactivities. Each line contained intracellular immunoreactivity for one or more of these opioids. β-Endorphin was expressed between 1.3 and 25 pg/mg of total protein in six lines, [Leu^5]enkephalin ranged from 2.1 to 66 pg/mg in seven lines, whereas dynorphin A was detected at levels of 8–50 pg/mg of protein in six lines. High levels of β-endorphin immunoreactivity were also found in the culture fluid of both the SCLC line H187 (1500 pg per 10^6 cells per 6 hr) and the non-SCLC line H157 (250 pg per 10^6 cells per 6 hr).

Effects of Exogenously Added Opioids on the Growth of Lung Cancer Cells *in Vitro*. Agonists for the three opioid receptor types caused a concentration-dependent inhibition of cell growth of N417 (SCLC) and H157 (non-SCLC) in the MTT assay (Fig. 3). Inhibition of growth was seen at opioid concentrations as low as 1–10 nM; maximum inhibition of growth occurred at 50–200 nM. Similar growth-inhibitory results were seen with morphine in 14 other lung cancer cell lines tested (Tables 1 and 3), and this inhibitory effect of opioids was confirmed by direct cell counting (data not shown). There was no statistical correlation between growth inhibition by morphine and the amount of specific [^{3}H]etorphine, [^{3}H]nicotine, or [^{3}H]α-bungarotoxin binding found at the 2 nM concentration of radioactive ligands used (Table 1). Concomitant administration of the opioid antagonist naloxone (100 nM) reversed the inhibitory effect of morphine (Fig. 3 *A* and *D*, Fig. 4), whereas at higher concentrations ($IC_{50} \approx$ 500 nM) naloxone itself inhibited growth (data not shown). Similar dose-dependent effects of naloxone have been seen with other tumor systems (24, 25).

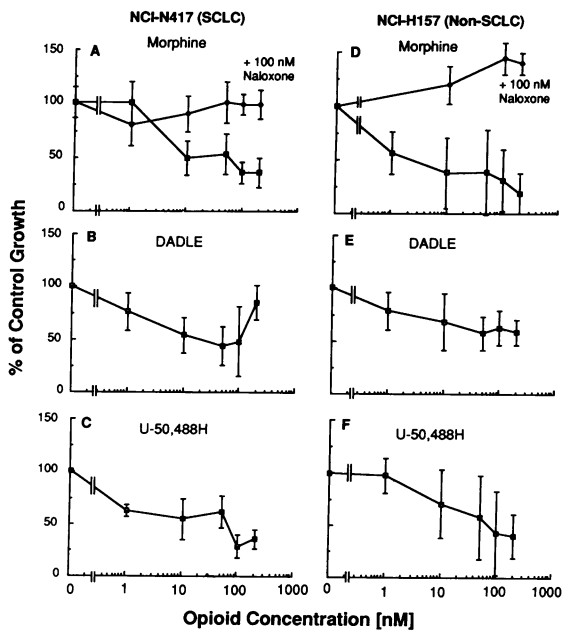

FIG. 3. Effect of various concentrations of opioid agonists on the growth of SCLC N417 (*A*, *B*, and *C*) and non-SCLC H157 (*D*, *E*, and *F*) cell lines as detected by the MTT assay. For growth effect of morphine, reversal with naloxone (100 nM) is also shown. Each experiment was repeated three times, and the data were pooled. Thus, each data point represents the mean of 24 culture wells.

Table 3. Nicotine and naloxone reversal of the inhibitory effect of morphine on the growth of lung cancer cell lines

Drug treatment*	Growth†, % of control
Control	100 ± 7
Morphine (100 nM)	45 ± 5
Morphine + (−)-nicotine	76 ± 13
Morphine + naloxone	93 ± 6
(−)-Nicotine (100 nM)	92 ± 2
Naloxone (100 nM)	89 ± 3
Naloxone + (−)-nicotine	76 ± 4

Data are expressed as mean ± SEM.
*Drugs used in combination were both at 100 nM.
†n = 14 different lung cancer lines. A significant degree of reversal by nicotine of morphine-induced growth inhibition was seen in SCLC cell lines H69, H187, N417, and non-SCLC lines H125, H157, H290, H358, H460, and H661 but not in SCLC lines H82, H146, or non-SCLC lines H23, H322, or H1299. The data from all 14 lines were analyzed statistically, and one-way analysis of variance yielded significant differences ($P < 0.05$) between morphine and the other treatments. Similarly, Kruskal–Wallis nonparametric one-way analysis of variance by ranks yielded an H statistic of 34, with the following mean ranks: control 45; morphine, 11; nicotine, 34; morphine plus nicotine, 24.

Opioid–Nicotine Interactions in Human Lung Cancer Cell Lines. In the MTT assay, nicotine alone, at concentrations from 0.1 nM to 1 μM, had no significant growth effect on either SCLC or non-SCLC cell lines growing in liquid culture (data not shown). However, when nicotine (100 nM) was added to the culture medium along with the opioids, the dose-dependent growth inhibition by morphine or DADLE was reversed. In fact, in some cases the combination of morphine and nicotine actually stimulated growth (Fig. 4). In tests of this effect of nicotine on many lung cancer cell lines, we found the inhibitory effect of morphine on lung cancer cell growth was partially or totally reversed in 9/14 lines by adding 100 nM nicotine to the culture medium containing the opioid (Table 3).

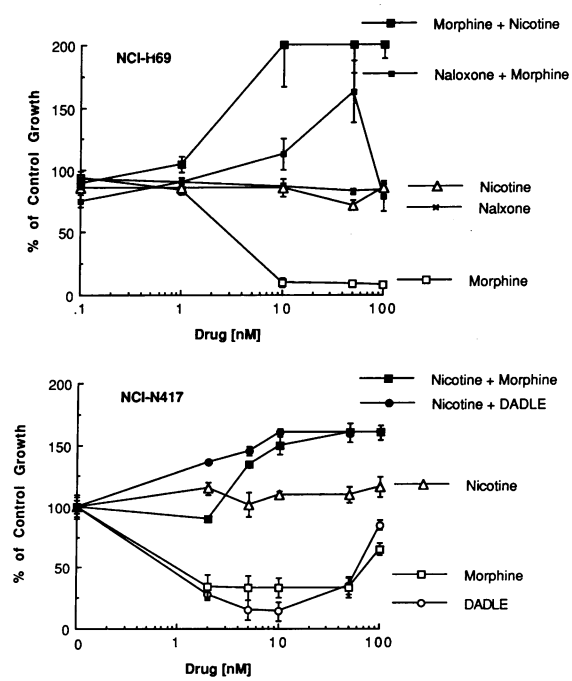

FIG. 4. Nicotine reversal of the inhibitory effect of morphine and DADLE on growth in SCLC cell lines H69 and N417 in the MTT assay. The drug concentrations (nM) for the agents used alone is indicated. Nicotine or naloxone added with the opioids were at concentrations of 100 nM.

DISCUSSION

We have found that SCLC and non-SCLC cell lines express specific membrane receptors for the μ, δ, and κ opioid agonists. They also express receptors for (−)-nicotine and α-bungarotoxin with pharmacologic properties similar to nAChRs seen in the brain, neuromuscular junction, and the peripheral nervous system (26, 27). In addition, these cell lines produce immunoreactive β-endorphin, enkephalins, and dynorphin-related opioid peptides. However, of most interest is the observation that opioid agonists inhibit lung cancer growth *in vitro* and that nicotine can reverse this opioid-induced inhibition of growth in a number of cell lines.

Although they did not study lung cancers, Zagon and coworkers (28) found expression of endogenous opioids and specific opioid receptors in several benign and malignant human and animal tumors; opioid peptides also have been described in human neuroendocrine tumors (3, 29). Although the mechanism(s) whereby opioids inhibit growth of lung cancer cells and nicotine can modulate this effect of opioids are unknown, their elucidation should be of general interest to tumor cell biology. Growth-inhibitory effects of opioids have already been reported from *in vivo* and *in vitro* studies of diverse tumors types (24, 25, 30–32). In addition, endogenous systems coupling nicotine and opioid effects in bovine adrenal chromaffin cells have been reported (33, 34).

The fact that lung cancer cells express opioid receptors, produce endogenous opioid peptides, and have their growth inhibited by exogenously added opioids represents a paradox. We propose as an explanation for this paradox that expression of opioid peptides and their cognate receptors represent another system of "tumor suppression", the function of which can be inactivated in cancer cells. Possible modes of inactivation include lack of production of biologically active opioid peptides or their receptors or the presence of a factor inhibiting their function. Our results indicate that nicotine can function to antagonize opioid action in lung cancer cells. Although nicotine alone, at concentrations from 1 nM to 1 μM, did not affect the growth of cells significantly, nicotine added with opioids to the medium partially or totally reversed the inhibitory effect of opioids on growth in some of the cell lines. The candidacy of nicotine as an inhibitor of opioid function is strengthened by the presence of nicotine and opioid receptors on the same lung cancer cells and steady-state blood nicotine concentrations of 15–40 ng/ml (≈90–240 nM) in cigarette smokers (8).

In conclusion, we hypothesize that the normal function of the endogenous opioid pathway would be to inhibit cell proliferation. However, with the exposure to nicotine this "suppressive" effect is overcome in the bronchial epithelial cells destined to become lung cancer cells. Nicotine can activate protein kinase C (35) and, thus, potentially could serve as a tumor promoter for lung tissue. Tolerance is a central feature of nicotine addiction (8, 9) and nAChRs have become desensitized stoichiometrically by phosphorylation on tyrosine residues (36). This raises the question of the role of activated oncogenes with tyrosine kinase activity in the desensitization of nicotinic receptors in lung cancer cells. Finally, whether the carcinogenic metabolites (7) of nicotine can also bind to the lung cancer nicotine receptors will be interesting to resolve. In any event, the demonstration of high-affinity, stereospecific nicotine receptors on lung cancer cells suggests further characterization of these receptors and their effects on cellular metabolism. In addition, the inhibitory effects on cell growth by opioids that are widely used clinically need to be further studied to see whether such effects are of therapeutic value.

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