polypeptides. Whether phosphorylation occurs on serine, threonine, or tyrosine residues is also not known.

Recent experimental evidence indicates that calcium- and calmodulin-modulated protein phosphorylation is the general mechanism mediating the neural response in mammalian tissues (*8*). Dephosphorylation of the phosphorylated proteins by specific phosphoprotein phosphatases offers a mechanism by which the biochemical pathways involving phosphorylation can be deactivated. Several investigators (*15*) have obtained evidence in plants for the regulation by phosphorylation and dephosphorylation of pyruvate dehydrogenase, hydroxymethylglutaryl–coenzyme A reductase, and quinate:NAD^+ 3-oxidoreductase (*15*); however, the role of calcium and calmodulin was not demonstrated. Lin *et al.* (*16*) reported phosphoprotein phosphatase from plants but did not indicate its link with calcium.

These findings on calcium-promoted protein phosphorylation should advance our understanding of the molecular mechanisms by which calcium regulates physiological processes such as phototropism, gravitropism, cytoplasmic streaming, chromosome movement, and other plant movements in response to various stimuli. Additional research is required to elucidate the nature of the protein kinases and substrates for protein kinases. Evidence for the existence of phosphoprotein phosphatase mediating selective dephosphorylation also needs to be obtained.

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13. Phosphorylation was studied in the soluble protein fraction from frozen coleoptiles in which calcium-independent protein phosphorylation was lower. Phosphorylation of the 100,000-dalton polypeptide was very low in extracts from fresh coleoptile but was pronounced in the extract from frozen coleoptiles.
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Nicotine Is Chemotactic for Neutrophils and Enhances Neutrophil Responsiveness to Chemotactic Peptides

Abstract. *Neutrophils contribute to chronic bronchitis and pulmonary emphysema associated with cigarette smoking. Nicotine was found to be chemotactic for human neutrophils but not monocytes, with a peak activity at ~ 31 micromolar. In lower concentrations (comparable to those in smokers' plasma), nicotine enhanced the response of neutrophils to two chemotactic peptides. In contrast to most other chemoattractants for neutrophils, however, nicotine did not affect degranulation or superoxide production. Nicotine thus may promote inflammation and consequent lung injury in smokers.*

Cigarette smoking is associated with the development of chronic bronchitis and pulmonary emphysema (*1*). Lung injury in smokers has been attributed to the effects of prolonged low-grade inflammation centered about the respiratory bronchioles and alveolar ducts (*2*) and especially to the release of polymorphonuclear neutrophil (PMN) elastase, an enzyme that produces emphysema when instilled intratracheally in animals (*3*). The recruitment of PMN's and mononuclear phagocytes to the airways of hamsters that have inhaled cigarette smoke (*4*) and the increased numbers of neutrophils in bronchoalveolar washings from human smokers (*5*) may be explained in part by the release of chemotactic factors from alveolar macrophages (*6*). However, a direct chemotactic effect of the components of cigarette smoke on inflammatory cells is an additional possibility. We now report that nicotine, which is readily absorbed from the respiratory tract and reaches plasma concentrations of 5 to 50 ng/ml (approximately 30 to 300 n*M*) in cigarette smokers (*7*), has effects on PMN's that may be relevant to lung inflammation in vivo.

We found that nicotine is chemotactic for human PMN's but not monocytes. Unlike many factors chemotactic for PMN's, it did not stimulate the release of superoxide anion (SOA) or cause degranulation. At low concentrations, nicotine enhanced directed migration of PMN's in response to optimum concentrations of complement-derived chemotactic activity (C5fr) and of *n*-formylmethionyl-leucyl phenylalanine (fMLP). As with its direct effect on PMN's, nicotine did not enhance fMLP-induced SOA release or degranulation.

Nicotine produced a concentration-dependent migratory response of PMN's obtained from heparinized venous blood of nonsmokers; the response was maximal at 30.8 μM (Fig. 1A). The average net number of cells migrating in response to this optimum nicotine concentration was indistinguishable from the response to $10^{-8} M$ fMLP ($P > 0.3$, Student's *t*-test). When a concentration gradient for nicotine was not present (Fig. 1B), PMN migration was not different from that observed in chambers in which culture medium only was used in the lower compartment. These data indicate that nicotine is chemotactic for PMN's, producing a stimulus to directed migration comparable to that of the well-studied potent chemotactic factor fMLP. Under similar test conditions, preparations of human monocytes showed no chemotactic response to nicotine.

To determine whether nicotine might also modify the response of PMN's to other chemotactic factors, we incubated

PMN's with various concentrations of nicotine, washed the cells, and then tested them for a chemotactic response to fMLP ($10^{-8}M$) or human C5fr (twice the median effective dose). Nicotine increased the number of cells migrating in response to both chemoattractants (Fig. 1C). This effect was significant at 0.31 μM nicotine (50 ng/ml) and nearly as great as the effect at 30.8 μM. The enhanced response of cells incubated with nicotine did not represent a changed dose-response relation to fMLP, but rather an increased response to various concentrations of fMLP (Fig. 1D). Incubation with nicotine had no effect on the chemotactic response of monocytes to fMLP.

Since many chemotactic factors stimulate SOA production and degranulation by PMN's, we looked for such effects of nicotine. Nicotine had no direct effect on

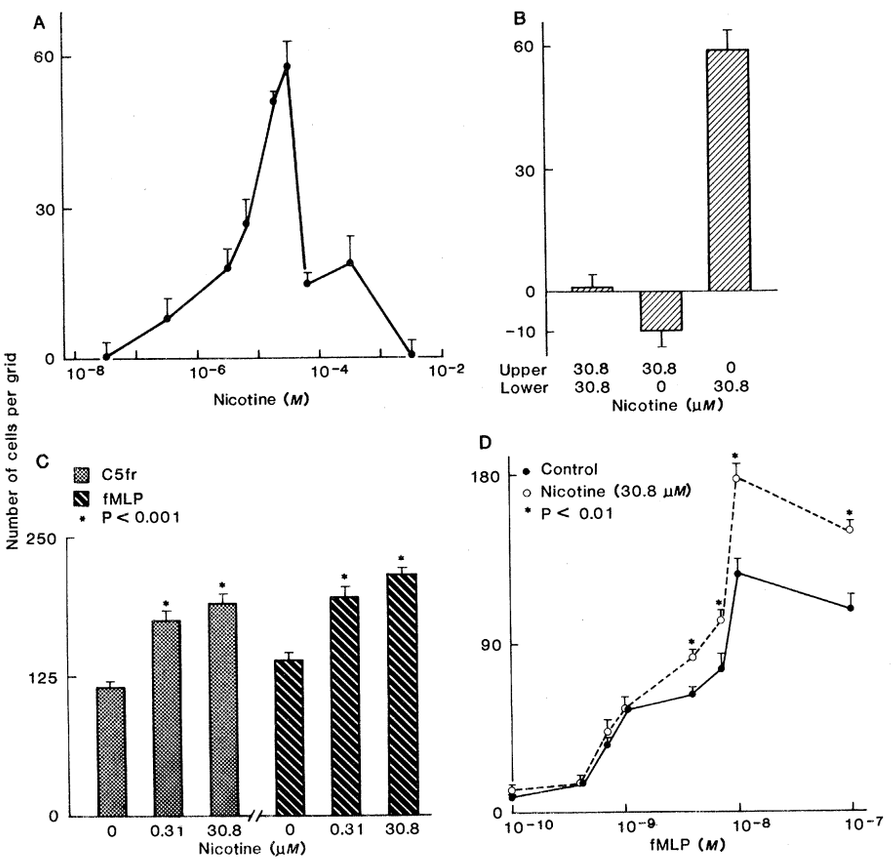

Fig. 1. (A to D) Nicotine chemotactic activity for PMN and nicotine-induced enhancement of the PMN response to chemotactic peptides. PMN's (*13*) were tested for chemotaxis in modified Boyden chambers (*14*). Chemotactic activity is expressed as the number of responding cells per high-power grid (×400), corrected for background movement as established by chambers in which only medium was in the lower compartment. Each value is the mean ± 1 standard error ($N = 15$). (A) PMN migration in response to nicotine. (B) Confirmation that nicotine causes directed migration of PMN's. The chemotactic gradient was abolished or reversed by placing nicotine in both the upper and lower compartments or the upper compartment only. (C) Nicotine enhancement of the PMN response to chemotactic peptides. PMN's were incubated with nicotine (*15*), then tested for chemotactic responsivity to optimum concentrations of C5fr (twice the median effective dose) or fMLP ($10^{-8}M$). Slight enhancement of the chemotactic response to both peptides was seen after exposure to 0.03 μM nicotine, but the differences were not significant. (D) Nicotine enhancement of the neutrophil response to fMLP.

Table 1. Effect of nicotine on PMN superoxide anion release and degranulation in vitro. PMN's were incubated with various concentrations of nicotine for 10 minutes at 37°C in air with 5 percent CO_2; fMLP was then added to the indicated groups. Supernatant fluids were assayed after an additional 15 minutes. Values are means ± standard errors for three different experiments. N.D., no data.

Nicotine	fMLP ($10^{-8}M$) stimulus	Superoxide (nanomoles per 10^6 cells)*	Lysozyme (percent released)†	Myeloperoxidase (milliunits per 10^6 cells)‡	Elastase (micrograms of elastin solubilized per 10^6 cells)§	Viability (percent)‖
0 (control)	No	2.2 ± 0.5	11.8 ± 6.4	3.8 ± 0.1	12.4 ± 0.8	92 ± 3
0 (control)	Yes	21.9 ± 0.7	52.6 ± 2.6	114.2 ± 16.0	596.7 ± 19.7	N.D.
3.1×10^{-8}	No	2.4 ± 1.3	10.3 ± 1.1	4.4 ± 0.6	11.7 ± 1.7	90 ± 2
3.1×10^{-8}	Yes	19.7 ± 2.0	54.5 ± 1.8	112.8 ± 1.9	570.8 ± 64.2	N.D.
3.1×10^{-7}	No	1.8 ± 0.4	7.5 ± 1.7	4.7 ± 0.7	12.3 ± 4.2	79 ± 11
3.1×10^{-7}	Yes	20.9 ± 0.8	55.0 ± 2.2	110.9 ± 9.4	559.7 ± 20.8	N.D.
3.1×10^{-5}	No	2.3 ± 1.0	5.5 ± 3.2	3.6 ± 0.8	10.9 ± 2.7	91 ± 4
3.1×10^{-5}	Yes	21.3 ± 0.7	56.4 ± 1.8	111.8 ± 5.6	554.6 ± 15.8	N.D.
3.1×10^{-4}	No	1.0 ± 1.4	9.5 ± 1.0	4.6 ± 0.2	9.1 ± 2.6	86 ± 4
3.1×10^{-4}	Yes	21.4 ± 1.1	55.9 ± 1.4	110.9 ± 4.2	577.5 ± 49.7	N.D.
3.1×10^{-3}	No	0.0 ± 1.4¶	6.3 ± 2.4	3.4 ± 0.3	8.6 ± 1.3	55 ± 12
3.1×10^{-3}	Yes	11.0 ± 0.5¶	54.5 ± 1.4	92.5 ± 8.5¶	553.6 ± 18.3	N.D.
1.5×10^{-2}	No	0.1 ± 0.7¶	6.3 ± 0.9	4.6 ± 1.3	N.D.	29 ± 23
1.5×10^{-2}	Yes	0.0 ± 0.6¶	21.3 ± 6.2¶	21.1 ± 1.4¶	171.9 ± 10.8	N.D.

*There were 1.5×10^6 PMN's per assay in RPMI 1640 salts; each assay was corrected for cytochrome *c* reduction occurring in a companion assay with superoxide dismutase (33 μg/ml) (*16*). The change in absorbance at 550 nm for cytochrome *c* reduction was assumed to be 2.1×10^4 cm^2/mmole (*17*). †There were 1.5×10^6 PMN's per assay in RPMI 1640 salts containing 10 μM cytochalasin B. Activity, measured as the change in optical density per minute at 450 nm, was quantified in a Beckman DU-8 recording spectrophotometer at 25°C (*18*). Percent lysozyme released = (activity in PMN supernatant fluid)/(activity released by 0.1 percent Triton X-100) 100. ‡There were 1.5×10^6 PMN's per assay in RPMI 1640 salts containing 10 μM cytochalasin B. Activity of PMN supernatant fluid was measured as the change in optical density per minute at 25°C (1 unit of peroxidase decomposes 1 μmole of H_2O_2 per minute) (*19*). §There were 7.5×10^6 PMN's per assay in RPMI 1640 salts containing 10 μM cytochalasin B. The activity of PMN supernatant fluid was measured by solubilizing [^{14}C]elastin (230 dis/min per microgram of elastin (in 16 hours at 37°C)(*20*). ‖Five hundred cells were counted per viability assay. Percent viability = (number of cells excluding trypan blue)/5. ¶Significantly different from the corresponding control value at $P < 0.01$ (analysis of variance).

SOA production or on release of azurophil granule contents (lysozyme, elastase, and myeloperoxidase) or specific granule contents (lysozyme) (Table 1). Concentrations of nicotine up to 3 m*M* also failed to modify the effects of fMLP on SOA production or degranulation. Higher nicotine concentrations significantly reduced SOA and enzyme release but also reduced cell viability as estimated by trypan blue dye exclusion.

Our findings indicate that nicotine is chemotactic for PMN's. Unlike C5fr, fMLP, and a number of other factors chemotactic for PMN's, it does not enhance SOA generation or degranulation. In this respect, nicotine is similar to the peptide Gly-His-Gly (*8*) and to the chemotactic factor generated from plasma by SOA (*9*).

Our data also indicate that nicotine enhances PMN responsiveness to other chemotactic factors. Nicotine may thus alter receptor affinity for other chemoattractants or affect membrane fluidity, causing a faster start-up response to chemoattractants, as reported for *n*-propanol and *n*-butanol in combination with fMLP (*10*). The effects we observed may be mediated by noncholinergic nicotine receptors on PMN's (8.7×10^4 per cell) (*11*). This possibility deserves further study, but the described PMN nicotine receptor affinity for nicotine is 36 n*M*, while significant nicotine effects in our study were observed in the range 0.31 to 31 μ*M*. The differences between the chemotactic responses of PMN's and monocytes to nicotine suggest that monocytes do not have nicotine receptors.

Our results contrast with the inhibition by nicotine of the chemotactic response of PMN's to casein reported by Bridges *et al.* (*12*). Of the many differences between our experimental protocol and that of Bridges *et al.*, the most important appears to be that we used subcytotoxic rather than toxic concentrations of nicotine.

Since the optimum concentration for nicotine-induced PMN chemotaxis is approximately 100 times higher than that typically found in the plasma of cigarette smokers, nicotine might appear to be a negligible stimulus to the ingress of PMN's into the lungs of smokers. However, during smoking the intrapulmonary concentration of nicotine, especially in the bronchoalveolar lining fluid, may be much higher than the plasma concentration. Moreover, nicotine, even at concentrations found in the plasma of smokers, makes PMN's more responsive to other chemotactic factors in vitro. Nicotine may also enhance PMN recruitment in response to the tobacco smoke–induced release of the PMN chemotactic factor by alveolar macrophages (*6*). This possibility should be amenable to testing in vitro.

Nicotine is the most widely used addictive alkaloid in the world. Our results suggest that it may promote pulmonary inflammation and may thus have a prominent role among smoke components in the pathogenesis of lung injury in cigarette smokers.

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Heterogeneity of Normal Human Diploid Fibroblasts: Isolation and Characterization of One Phenotype

Abstract. *Cultures of human diploid fibroblasts contain cells that respond to exposure to the first component of complement (C1) by initiating DNA synthesis and growth. The plasma membranes of these cells have specific binding sites for the C1q subcomponent of C1. A fluorescence-activated cell sorter was used to isolate a subset of cells with a high affinity for C1q, and the growth and synthesis activities of these high-affinity cells were studied after numerous replications in vitro. These cells synthesize DNA and grow faster than the parent cultures and low-affinity cells, and they produce two to three times as much protein. About 40 percent of their total protein synthesis activity is directed to collagen production, unusually high proportions of collagen types III and V being produced. These properties and the high affinity of the cells for C1q are retained for at least six cell transfers. This phenotype has the properties expected of fibroblasts in healing wounds and inflamed tissues.*

Resident fibroblasts are responsible for the production and maintenance of the connective-tissue matrix and for repair after injury. The growth and synthesis activities of these cells are controlled by intrinsic genetic factors and by environmental ligands present in the tissue fluids such as epidermal growth factor, platelet-derived and fibroblast growth factors, complement proteins, prostaglandins, and factors released by lymphoid cells and macrophages (*1–3*).

These environmental ligands modulate cell activities by a concentration-dependent, reversible binding to specific sites on the surfaces of fibroblasts (*4*). However, a mechanism of this kind cannot account for the behavior of these cells in some aspects of growth and differentiation and in certain pathologic situations.