ganglia and striated muscle. At these peripheral synapses, nicotine so clearly mimics acetylcholine, the endogenous neurotransmitter, that cholinergic receptors in ganglia and striated muscle are designated nicotinic cholinergic receptors (*8*). In the brain, too, nicotine appears to act as a cholinergic agonist (*9*). However, the pharmacological characteristics of brain nicotinic cholinergic receptor sites labeled by [^{3}H]acetylcholine (*1*) or [^{3}H]nicotine (*10*) suggest that they differ from ganglia and striated muscle receptors.

We suggest two possible explanations for the increase in nicotinic cholinergic receptor binding sites following repeated administration of nicotine:

1) Nicotine may interfere with the metabolism or release of acetylcholine, resulting in decreased availability of the transmitter at cholinergic synapses in the brain and subsequent up-regulation of the receptors. However, this would imply that muscarinic receptors labeled by [^{3}H]quinuclidinyl benzilate are less sensitive than nicotinic receptors labeled by [^{3}H]acetylcholine to decreases in acetylcholine availability, since no significant change in [^{3}H]quinuclidinyl benzilate binding is found after repeated nicotine administration.

2) At the dose used in this study, nicotine or a metabolite of nicotine may antagonize receptors in the brain. Although no adverse effects of nicotine were obvious in these experiments, high concentrations of nicotine decrease activity in autonomic ganglia and at the neuromuscular junction by depolarization blockade (*11*). And cotinine, a major metabolite of nicotine, competes for [^{3}H]acetylcholine binding sites in the brain (*1*). Although the affinity of cotinine for [^{3}H]acetylcholine binding sites is low (175 μM), it is comparable to that of several centrally acting drugs that are classified as nicotinic cholinergic antagonists (*1*). In addition, one or more metabolites of nicotine (possibly hydroxynicotine) confers protection against the lethal effects of nicotine in mice (*12*).

If a metabolite of nicotine were acting as a nicotinic cholinergic antagonist in the brain, it could explain the up-regulation of nicotinic cholinergic binding sites. In addition, it could provide a model for and insight into neurochemical processes underlying nicotine addiction and withdrawal.

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Formaldehyde Damage to DNA and Inhibition of DNA Repair in Human Bronchial Cells

Abstract. *Cultured bronchial epithelial and fibroblastic cells from humans were used to study DNA damage and toxicity caused by formaldehyde. Formaldehyde caused the formation of cross-links between DNA and proteins, caused single-strand breaks in DNA, and inhibited the resealing of single-strand breaks produced by ionizing radiation. Formaldehyde also inhibited the unscheduled DNA synthesis that occurs after exposure of cells to ultraviolet irradiation or to benzo*[a]*pyrene diolexpoxide but at doses substantially higher than those required to inhibit the resealing of x-ray–induced single-strand breaks. Therefore, formaldehyde could exert its mutagenic and carcinogenic effects by both damaging DNA and inhibiting DNA repair.*

Formaldehyde (HCHO) is a highly reactive chemical (*1*) that is genotoxic in several species (*2*) including the rat, in which it is a respiratory carcinogen (*3*). The chemical is a common environmental pollutant occurring in tobacco smoke, consumer products, many occupational settings, and gasoline and diesel exhaust (*4*). It is also formed endogenously in the cell during demethylation reactions and during the metabolism of *N*-nitrosodimethylamine (*5*) and certain drugs (*6*). Exposure to HCHO has a variety of pathobiological consequences [for reviews, see (*2, 4*)].

The potential carcinogenic hazard of HCHO in the human respiratory tract is currently being debated (*7*). Since its effects on the respiratory tract are essentially unknown, we evaluated its ability to kill and to damage the DNA of human bronchial cells grown as clones in a serum-free medium as described (*8*). We compared the effects of HCHO on epithelial cells and fibroblasts that originated from the same bronchial specimen, and determined whether HCHO could interfere with the repair of DNA that had been damaged by other genotoxic agents. The repair mechanisms studied were (i) the rejoining of x-ray–induced DNA single-strand breaks and (ii) the unscheduled DNA synthesis induced by ultraviolet radiation or (+)-(7β,8α)-dihydroxy - (9α,10α) - epoxy - 7,8,9,10 -tetrahydrobenzo[*a*]pyrene (BPDE). We also investigated the direct effects of HCHO on DNA including the formation of DNA protein cross-links and DNA single-strand breaks.

Cells exposed to HCHO developed both DNA protein cross-links and single-strand breaks. Using methods previously described (*9*), we found that the number of DNA protein cross-links induced by 100 μM HCHO in epithelial cells and fibroblasts was similar (0.65 and 0.83 unit, respectively), and that the frequency of these cross-links was proportional to the concentration of HCHO. The rate at which these cross-links were removed was also similar in the two cell types; the half-removal time was approximately 2 hours (data not shown). This is consistent with results obtained in mouse L1210 cells (*10*). Formaldehyde-induced DNA single-strand breaks were also detected after removal of the cross-links with proteinase K. The frequency of DNA single-strand breaks induced by 500 μM HCHO was 4.2 per 10^{10} daltons in epithelial and 3.5 per 10^{10} daltons in fibroblastic cells

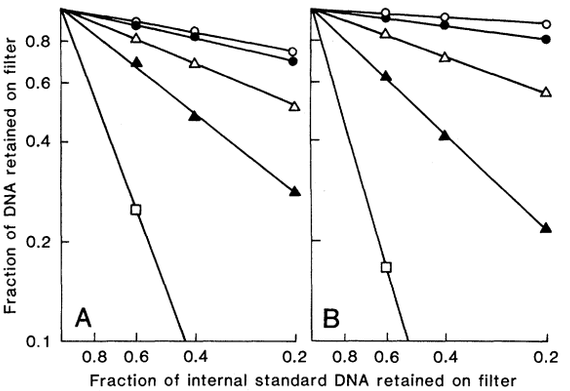

Fig. 1. Effect of HCHO on the repair of x-ray–induced DNA single-strand breaks in human bronchial epithelial cells and fibroblasts. [2-^{14}C]Thymidine (0.02 μCi; 52 mCi/mmole) was added to growing cells for 3 days and then removed 1 to 2 days before the experiment. Formaldehyde (Fisher certified acs; 37 percent by weight) was diluted to 5 m*M* in distilled water immediately before being added to cells (5×10^4 to 10×10^4) in serum-free LHC-1 medium (*8*). After treatment for 1 hour, the cells were rinsed twice with cold phosphate-buffered saline (PBS). The cells were removed at 4°C with a rubber policeman and put into a solution of Dulbecco's Ca^{2+} and Mg^{2+}-free PBS containing 15 m*M* EDTA. The alkaline dilution technique was used to assess DNA damage. To measure DNA single-strand breaks the cells were collected onto polycarbonate filters (2 μm pore size; Nucleopore), lysed with 2 percent sodium dodecyl sulfate (SDS) and 0.1*M* glycine, and 0.02*M* EDTA (*p*H 9.6); 2 ml of the same solution containing Proteinase K (0.5 mg/ml) was then put through each filter at 0.4 ml/min. EDTA (0.02*M*; acid form), 0.1 percent SDS, and tetrapropyl ammonium hydroxide were then added in the amount required to to give a *p*H of 12.2. Eluted fractions were collected and assayed for radioactivity. The combination of the polycarbonate filters, Proteinase K digestion, and SDS in the eluting solution completely removed the DNA protein cross-linking effect of HCHO at concentrations up to 1 m*M*. An internal standard ([^{3}H]thymidine-labeled L1210 cells that received 300 rad at 4°C) was included in each assay. An estimate of the frequency of single-strand breaks was quantitated from the relative elution (RE) of DNA which was derived from $\log R_0 - \log R_F$, where R_0 and R_F represented the fraction of DNA retained on the filter in untreated and HCHO-treated cells, respectively. The frequency of DNA single-strand breaks was calculated by comparing the RE when 27 percent of the tritiated internal standard DNA was retained on the filter (12 hours of elution) to that produced in L1210 cells after 300 rad of x-rays, assuming a single-strand break efficiency of 2.7×10^{-12} per rad per dalton. The abscissa represents a corrected time scale obtained from [^{3}H]thymidine-labeled L1210 cells that had received 300 rad. (A) Fibroblasts. (B) Epithelial cells. Symbols: ○, control cells; ●, cells exposed to 100 μ*M* HCHO for 1 hour; □, cells exposed to 800 rad without subsequent incubation; △, cells exposed to 800 rad and incubated for 1 hour in fresh medium at 37°C; ▲, cells exposed to 800 rad and incubated for 1 hour in medium containing 100 μ*M* HCHO.

and was dose-dependent. The removal rate of the single-strand breaks was also similar in the two cell types (data not shown).

The relation between exposure to HCHO and the resealing of DNA single-strand breaks induced by x-rays is presented in Fig. 1. We showed previously (*9*) that epithelial cells and fibroblasts efficiently reseal x-ray–induced single-strand breaks at comparable rates. Exposure of cells to 800 rad resulted in a high frequency of single-strand breaks and a rapid elution of DNA. Cells incubated for 1 hour after exposure to x-rays showed a substantial decrease in the number of breaks. Cells exposed to 100 μ*M* HCHO showed insignificant numbers of breaks, but the resealing rate of the x-ray–induced breaks was substantially decreased in both cell types. Repair was also inhibited if the cells were treated with HCHO for 1 hour, then exposed to x-rays, and then incubated in HCHO-free medium. Therefore, the effect of HCHO persisted for at least 1 hour.

When bronchial cells were exposed to x-rays and HCHO simultaneously a synergistic effect was observed (*11*). Significant potentiation of the toxicity of x-rays occurred with 100 μ*M* HCHO and 400 rad and with 300 μ*M* HCHO and 200 and 400 rad. Only slight toxicity was observed in cells exposed to 100 and 300 μ*M* HCHO alone (76 and 56 percent colony-forming efficiency of untreated cells, respectively).

We also investigated the mechanism of inhibition of unscheduled DNA synthesis by HCHO in confluent fibroblasts. The effects of ultraviolet irradiation and BPDE were studied in experiments with confluent fibroblasts. In the presence of hydroxyurea, an inhibitor of semiconservative DNA synthesis (*12*), HCHO alone caused no significant increase in unscheduled DNA synthesis (Fig. 2). As expected, exposure to ultraviolet radiation and to BPDE caused significant amounts of such synthesis. The most interesting finding was that 100 μ*M* HCHO did not inhibit the unscheduled DNA synthesis induced by ultraviolet radiation or BPDE. Substantially higher concentrations of HCHO, 600 and 1000 μ*M*, were required to significantly inhibit such synthesis. In contrast, 100 μ*M* HCHO inhibited the repair of x-ray–induced single-strand breaks. This finding indicates that the ligation step of excision repair may be preferentially sensitive to HCHO.

A number of mechanisms may be involved in the inhibition of DNA repair by HCHO. The high reactivity of the chemical probably causes methylolation of chromatin or other proteins including enzymes critical to DNA repair processes. The potentiating effect of HCHO on the cytotoxicity of x-rays may depend on interaction with enzymes critical for re-

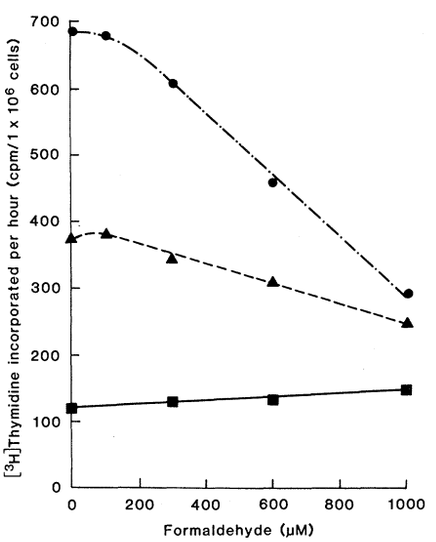

Fig. 2. The effect of HCHO on unscheduled DNA synthesis induced by ultraviolet radiation and BPDE in confluent bronchial fibroblasts. Cells (2×10^5) were inoculated into 60-mm^2 dishes in LHC-1 and medium 199 (1:1 by volume) and grown in the presence of [2-^{14}C]thymidine for 48 hours to uniformly label the cellular DNA. The dishes were left for another 48 hours until the cells had just reached confluency. The cells were then incubated with 10 m*M* hydroxyurea for 30 minutes to inhibit semiconservative DNA synthesis (*11*). With the hydroxyurea still present, [^{3}H]thymidine (4 μCi/ml, 80 Ci/mmole) was added and the cells were exposed to ultraviolet radiation (10 Jm^{-2}) or BPDE (5 μg/ml) added from a stock solution of 5 mg/ml in tetrahydrofuran and then incubated for 1 hour at 37°C. The medium was subsequently removed and the cells were washed five times with cold PBS, removed from the plate in PBS containing 15 m*M* EDTA with a rubber policeman, precipitated with 7.5 percent trichloroacetic acid (TCA), and collected on 0.3 μm filters (Millipore PHWP). The filters were washed twice with cold 5 percent TCA and twice with ice-cold 95 percent ethanol. The precipitate on filters was heated at 70°C for 1 hour in 0.1*N* HCl, and the amount of unscheduled DNA synthesis was estimated from incorporation of [^{3}H]thymidine. The yield of DNA and the number of cells were quantitated from [^{14}C]thymidine incorporation as measured by spectroscopy. Symbols: ■, cells exposed to HCHO alone; ▲, cells exposed to BPDE (5 μg/ml); ●, cells exposed to ultraviolet radiation (10 Jm^{-2}).

pair of x-ray–induced DNA damage. The comparatively low concentrations (100 μM) that decrease such repair are comparable to the effective levels of certain potent carbamylating and alkylating agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea (*13*) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (*14*). Millimolar concentrations of dimethylsulfate are required to inhibit excision repair of ultraviolet radiation–induced DNA damage (*15*).

The critical factors determining the concentration of HCHO that can be safely handled by the cell remain to be identified. Exogenous HCHO will readily react with respiratory mucus and the exterior surface of the target cells so that only a small fraction of the HCHO reaches the nucleus. Since HCHO is formed endogenously in the cell during demethylation reactions, cells must maintain pathways for its detoxification. Many xenobiotics are also metabolized by demethylation reactions. Formaldehyde is metabolized to formate, but this agent did not cause the formation of DNA single-strand breaks or affect the repair of such breaks induced by x-rays (data not shown).

Since HCHO damages DNA, inhibits DNA repair, and potentiates the cytotoxicity of x-rays in human bronchial epithelial cells, and since the HCHO may act in concert with physical and chemical agents to produce toxic, mutagenic, and carcinogenic effects (*11*), we suggest that the mutagenic and carcinogenic effects of this chemical alone or in combination with other agents should be further investigated.

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Dopamine Modulation of the Effects of γ-Aminobutyric Acid on Substantia Nigra Pars Reticulata Neurons

Abstract. *Studies were conducted to assess whether basal ganglia output neurons originating in the substantia nigra pars reticulata might be affected by dopamine released from dendrites of neighboring substantia nigra pars compacta neurons. Dopamine applied by iontophoresis increased the baseline firing rates of approximately half of the substantia nigra pars reticulata cells tested. The more significant finding, unrelated to the increase in firing, was the ability of dopamine to attenuate the inhibitory responses of these cells to iontophoretically applied γ-aminobutyric acid. These findings suggest a role for dopamine as a neuromodulator and further suggest that it can act at sites beyond the striatum to modify transmission from the basal ganglia to motor nuclei.*

The nigrostriatal dopamine system is involved in several disorders of movement that originate in the basal ganglia—most notably Parkinson's disease. How dopamine influences movement ultimately depends on how it acts within the basal ganglia to modify transmission of information to premotor nuclei outside the basal ganglia. The substantia nigra (SN) pars reticulata, located ventral to the nigral dopamine neurons, functions as one of two basal ganglia output nuclei (*1*), projecting largely to the motor thalamus and superior colliculus (*2*). The SN receives an innervation, in part utilizing γ-aminobutyric acid (GABA), from the striatum and globus pallidus (*3*). Thus, dopamine neurons might affect SN pars reticulata output pathways in two ways: (i) indirectly, by release of dopamine from terminals within the striatum, or (ii) directly, by local release of the transmitter (*4*) from dendrites that extend into the pars reticulata (*5*). Since pars reticulata neurons are strategically involved in the transmission of messages from the basal ganglia to motor effector sites, we conducted studies to determine how these neurons might be affected by dendritically released dopamine. We now report that iontophoretically applied dopamine directly affected the firing rates of approximately half of the pars reticulata neurons. Of potentially greater importance, however, is our observation that dopamine acted as a neuromodulator by markedly and reproducibly diminishing the responses of reticulata cells to the inhibitory transmitter GABA.

Male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with chloral hydrate (400 mg per kilogram of body weight, intraperitoneally) and mounted in a stereotaxic apparatus. Techniques for extracellular, single-unit recording and microiontophoresis were used as described (*6*, *7*). Cells of the SN pars reticulata were identified by criteria described in detail elsewhere (*6*, *8*). These cells could be distinguished from dopamine neurons of the SN pars compacta by their location and by differences in the shape of their action potentials, discharge frequencies, and firing patterns. Efforts were made to antidromically activate the cells used in these studies from the ventromedial nucleus (VM) of the thalamus. Pulses, 200 μsec in duration and 0.2 to 0.5 mA in intensity, were delivered from a bipolar stimulating electrode stereotaxically positioned in the left VM nucleus (ipsilateral to the recording site). Three criteria were used to establish whether cells could be excited antidromically: (i) stable latency of the antidromic response, (ii) ability of cells to follow stimulus frequencies greater than 300 Hz, and (iii) collision of antidromic and spontaneous spikes.

The ability of applied dopamine to modify responses of reticulata cells to other transmitters applied iontophoretically was evaluated as follows. Repeated 30-second iontophoretic pulses of GABA, glycine, acetylcholine, or glutamic acid were applied at a fixed current, separated by 30-second periods of baseline activity. After completion of at least three applications of a transmitter, dopamine (10 nA) was simultaneously applied by iontophoresis during three or more additional pulses of the original