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Inhibition of histamine *N*-methyltransferase activity in guinea-pig pulmonary alveolar macrophages by nicotine

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Abstract—Both *S*-(−)- and *R*-(+)-nicotine enantiomers are inhibitors of histamine N^{τ}-methylation activity in guinea-pig pulmonary alveolar macrophage cultures, exhibiting IC50 values of 7 and 8 μM, respectively. *S*-(−)-Nicotine is not biotransformed under the conditions of the experiment, however, *R*-(+)-nicotine undergoes significant *N*-methylation to produce *N*-methylnicotinium ion. *S*-(−)-Nicotine appears to inhibit the *N*-methylation of its optical antipode by the alveolar nicotine *N*-methyltransferase. The results indicate that a contributing factor in the toxicology of cigarette smoke inhalation may be due to the inhibition of pulmonary metabolism of histamine by nicotine.

Nicotine, the principal pharmacologically active component of tobacco smoke, has been implicated in the various adverse effects of smoking and health (Larson et al 1961; Larson & Silvette 1968). In this respect, many of the pharmacological properties of nicotine have been attributed to its effects on nicotinic cholinergic synapses. However, the pharmacology of nicotine is complex and cannot be entirely explained by nicotine mechanisms (Abood et al 1979). Recent studies (Ishihara et al 1981) have shown that smokers have higher circulating levels of histamine than non-smokers, and it has been known for some years that excessive smoking may be a contraindication in peptic ulceration (Packard 1960; Staszewski 1961; Finkbiner 1963; Ochsner 1964; Bargen 1980). More recently, a clinical study has shown that smokers undergoing therapy with cimetidine, a histamine H_2-receptor blocker, had a higher incidence of recurring gastric ulceration than non-smokers (Sontag et al 1984). These data would appear to indicate that smoking may result in an altered ability to metabolize histamine. In this respect, it has been shown recently (Godin & Crooks 1986) that histamine metabolism in guinea-pig lung homogenates can be inhibited by *S*-(−)-nicotine. The effect is due to *S*-(−)-nicotine competitively inhibiting histamine-N^{τ}-methyltransferase (HMT), the major enzyme involved in histamine metabolism. Since there is now convincing evidence linking elevated histamine levels with tumorigenesis (Bartholeyns & Fozard 1985; Scolnik et al 1985), the inhibition of histamine metabolism may be a significant factor to consider in the toxicology of tobacco products.

The aim of this present study was to determine whether histamine metabolism to N^{τ}-methylhistamine in guinea-pig cultured pulmonary alveolar macrophages (PAMs) is inhibited by *S*-(−)-nicotine. These cells are among the first lung cells to interact with the inhaled particulates of cigarette smoke. They have been shown to possess a number of methyltransferase activities (Zuckerman et al 1982; Pacheco et al 1985), including HMT activity (Gairola et al 1987), but appear to be deficient in oxidative enzyme activity (Bend et al 1973).

Materials and methods

[G-^{3}H]Histamine (specific activity 7·8 Ci mmol^{-1}) and (±)-[2′-^{14}C]nicotine (specific activity 57 mCi mmol^{-1}) were obtained from New England Nuclear (Boston, MA, USA); *S*-(−)-[methyl-^{3}H]nicotine (specific activity 68·6 Ci mmol^{-1}) and *R*-(+)-[methyl-^{3}H]nicotine (specific activity 76·5 Ci mmol^{-1}) were purchased from the Radiochemical Centre (Amersham, UK). *S*-(−)-Nicotine and histamine were purchased from the Aldrich Chemical Co. (Milwaukee, WI, USA), N^{τ}-methylhistamine was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other authentic metabolite standards were synthesized as previously described (Cundy et al 1984, 1985c).

Cell culture conditions. Three male Hartley guinea-pigs (500–600 g) were anaesthetized by an intraperitoneal injection of sodium pentobarbitone and exsanguinated by severing the abdominal aorta. The trachea was cannulated and the lungs lavaged with phosphate-buffered saline (PBS) at 37 °C. The broncheoalveolar lavage fluid from each animal was pooled and centrifuged at 400 *g* for 10 min and the cell pellet given a brief hypotonic shock to lyse the erythrocytes. The cells were washed once and cultured in complete medium containing RPMI-1640, 10% heat inactivated fetal calf serum, 20 mM glutamine and 1% antibiotic/antimycotic solution (all purchased from GIBCO, NY, USA). Each petri-dish containing $1{\cdot}2 \times 10^6$ cells in 1 mL of medium was maintained at 37 °C in humidified 5% CO_2/air for 1 h and the cultures were vigorously rinsed with warm Hanks balanced salt solution (HBSS) to remove the nonadherent cells. The adherent cell monolayers were then used in the incubations with radiolabelled histamine and nicotine. A microscopic examination of the Wright-Giemsa stained monolayer showed that over 98% of the adherent cells were macrophages. In the histamine concentration studies, the macrophage monolayers were carefully overlayered with the substrate mixture, which consisted of HBSS containing 1 mM glucose and [^{3}H]histamine, final concentration 0·06–50 μM. In the nicotine inhibition studies, substate mixtures contained [^{3}H]histamine (final concentration 2 μM) and varying concentrations of either *S*-(−)-nicotine or *R*-(+)-nicotine (final concentrations of either isomer 2–200 μM).

Nicotine metabolism studies were carried out utilizing either (±)-[^{14}C-2′]nicotine, *R*-(+)-[^{3}H-N′CH$_3$]nicotine or *S*-(−)-[^{3}H-N′-CH$_3$]nicotine. Substrate solutions comprised HBSS containing 1 mM glucose and the appropriate radiolabelled nicotine (final concentration 20 μM). In some cases, histamine (final concentration 20 μM) was included in the substrate solution. All plates were incubated for 1 h in 5% CO_2/air at 37 °C and the cell-free reaction mixtures were transferred to tubes for analysis. The viability of the macrophage cells was shown to be greater than 90 percent after 1 h incubation in all experiments. The adherent cell monolayers were lysed in 0·2 mL of freshly prepared ice-cold 0·05% Triton solution and the cell lysates were analysed by HPLC radiochromatographic analysis (see below). Three experiments were performed for each measurement. The protein content of all lysates was measured according to the method of Lowry et al (1951).

Analytical chromatography. The analysis of nicotine metabolites was carried out using a previously described high-performance cation-exchange liquid radiochromatography procedure capable of quantitating nicotine and six of its potential metabolites (Cundy et al 1985a; Cundy & Crooks 1987). For the determina-

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tion of histamine N^{τ}-methylation, cell lysates were co-injected with authentic standards of histamine and its metabolites onto a Partisil-10 SCX 10μ particle cation exchange column (25 × 0·4 cm) (Whatman, Clifton, NJ, USA) to which was attached a 7 × 0·4 cm CSX-1 Whatman pellicular cation exchange guard column and analyzed using a previously reported procedure (Godin & Crooks 1986).

Results and discussion

The effect of histamine concentration on HMT activity in guinea-pig alveolar macrophages is shown in Table 1. Histamine N^{τ}-methylation was found to be linear up to 20 μM substrate concentration. However, an apparent inhibition of HMT activity was observed at 50 μM histamine. This substrate inhibition

Table 1. Effect of histamine concentration on HMT activity in guinea-pig alveolar macrophages.

Histamine (μM)	HMT activity* (pmoles N^{τ}-methylhistamine formed mg protein^{-1}h^{-1})
0·06	0·56 ± 0·12
1·0	10·2 ± 3·1
10·0	178 ± 31
20·0	359 ± 25
50·0	161 ± 30

* ± s.d., n = 3

has been observed previously with guinea-pig lung HMT in related studies (Godin & Crooks 1986). In the nicotine inhibitory studies, a substrate concentration of 2 μM histamine was utilized.

The effect of both *S*-(−)- and *R*(+)-isomers of nicotine on HMT turnover in alveolar macrophage is presented in Table 2.

Table 2. Inhibition of HMT activity in cultured guinea-pig alveolar macrophages by nicotine enantiomers.

Concn of nicotine (μM)	% HMT inhibition by nicotine*†	
	R-(+)-Enantiomer	*S*-(−)-Enantiomer
2·0	35·0 ± 26·6	25·0 ± 11·4
20	90·5 ± 5·2	95·3 ± 2·2
200	99·9 ± 2·7	95·3 ± 3·5

* ± s.d.
† [Histamine] = 2 μM

These data clearly show that both nicotine enantiomers are good inhibitors of histamine N^{τ}-methylation. IC50 values were calculated to be 7 and 8 μM, respectively, for *S*-(−)-nicotine and *R*-(+)-nicotine, indicating a lack of stereoselectivity in the inhibitory potency of the enantiomers.

Studies were also carried out using radiolabelled nicotine enantiomers, to determine the metabolic stability of the nicotine isomers in the pulmonary alveolar macrophage cultures. Cell free supernatants and cell lysates were analysed separately for oxidative and *N*-methylated metabolites. Analysis of cell-free supernatants from experiments with *S*-(−)-, *R*-(+)-, and (±)-radiolabelled nicotines indicated in all cases an absence of extracellular metabolism. Interestingly, analysis of cell lysates from the above experiments afforded quite different results. No oxidative or *N*-methylated metabolites of *S*-(−)-nicotine could be detected in cell lysates, however, in experiments with *R*-(+)-nicotine, a significant amount of the *N*-methylated metabolite, *N*-methylnicotinium ion (NMN), was detected in the cell lysate. At a concentration of 20 μM *R*-(+)-nicotine, 22·2 (± 0·8) percent conversion to NMN was observed in the lysate; the turnover of the 'nicotine *N*-methyltransferase' activity was 227 ± 25 pmoles (mg protein)$^{-1}$h^{-1}. No other metabolic products of *R*-(+)-nicotine were detected.

Interestingly, when identical experiments were carried out utilizing radiolabelled (±)-nicotine, conversion to NMN and turnover of the 'nicotine *N*-methyltransferase' reaction dropped dramatically to 2·1 (± 0·7) percent and 42 pmoles (mg protein)$^{-1}$h^{-1}, respectively. This phenomenon has been observed in previous studies with guinea-pig homogenates (Cundy et al 1985a, b) and was shown to be due to the remarkable substrate-inhibitor properties of nicotine enantiomers towards guinea-pig lung 'nicotine *N*-methyltransferase', wherein *S*-(−)-nicotine acts as a competitive inhibitor of the *N*-methylation of its optical antipode.

Addition of histamine (20 μM) to macrophage incubations inhibited the *N*-methylation of *R*-(+)-nicotine in lysates. This observation is analogous to results from similar studies utilizing guinea-pig lung homogenates (Cundy 1984).The inhibition may be due to competition of the above two methyltransferases for the same cofactor, SAM, or the two methyltransferases may be identical, in which case, the mechanism of *R*-(+)-nicotine inhibition may be due to it acting as an alternate substrate for HMT. In this latter respect it is interesting to note that the two methyltransferases have remarkably similar characteristics (Crooks & Cundy 1988).

The above data indicate that nicotine is capable of inhibiting the major route of histamine metabolism, i.e. N^{τ}-methylation, in cultured alveolar macrophages, which may be a contributing factor in the observed elevation of circulating histamine levels in smokers compared to non-smokers. The toxicological significance of elevated histamine levels is of particular relevance. Histamine is involved in a variety of biochemical events that are related to both health and disease. The biogenic amine is implicated in inflammation, allergic reactions, and gastric acid secretion. Recent studies have shown that histamine levels are elevated in tissues from tumor-bearing animals (Burtin et al 1981, Watanabe et al 1981, Bartholeyns & Bouchlier 1984), and several reports indicate a role for histamine in tumorigenesis (Bartholeyns & Fozard 1985; Scolnik et al 1985). Recent DNA binding studies (Scolnik et al 1984) suggest that histamine interacts in a way similar to that proposed by Abraham (1981) for the polyamines, probably resulting in the modulation of genomic expression of DNA. Such an interaction may be related to changes similar to those proposed by Reddy et al (1982), for the activation of oncogenes.

The ability of macrophages to biotransform both histamine and *R*-(+)-nicotine into *N*-methylated products is worthy of comment. While the *N*-methylation of histamine is a deactivating endogenous metabolic reaction, the formation of *R*-(+)-*N*-methylnicotinium ion is of potential toxicological significance. Nicotine *N*-methylation is catalysed by an *S*-adenosylmethionine-dependent methyltransferase that is widely distributed in tissues, with highest activity in the lung (Cundy et al 1985b). Several in-vivo studies have detected *N*-methylated derivatives of nicotine, cotinine, nornicotine and nicotine *N'*-oxide as urinary metabolites of nicotine in dog, guinea-pig and man (McKennis et al 1963, Cundy et al 1985c; Sato & Crooks 1985; Pool et al 1986; Neurath & Pein 1987). The major methylated urinary metabolite, NMN, has been shown to be pharmacologically active and causes release of noradrenaline from the axonal membrane (Euler & Persson 1970; Hedqvist 1970) as well as inhibition of re-uptake mechanisms. The observed stereospecificity in the *N*-methylation of nicotine by alveolar macrophages is interesting. Although the *S*-(−)-isomer of nicotine is considered to be the nicotine species present in cigarette smoke, studies in our laboratory (Pool 1987; Crooks, Godin, Pool, unpublished observations) and in others (Klus & Kuhn 1977) have estab-

lished the presence of amounts of *R*-(+)-nicotine in smoke condensate ranging from 1·6 to 11·9 percent, which probably results from pyrolytic racemization of the *S*-(−)-isomer. In this respect, recent in-vitro studies have shown that human liver homogenate contains a SAM-dependent methyltransferase that is capable of catalysing the *N*-methylation of both nicotine enantiomers (Crooks & Godin 1988) in a stereoselective manner.

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