Comparison of Monoamine Oxidase A in Peripheral Organs in Nonsmokers and Smokers

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Smokers have reduced levels of brain monoamine oxidase A (MAO A) leading to speculation that MAO A inhibition by tobacco smoke may underlie some of the neurophysiologic effects of smoking. Because smoking exposes peripheral organs as well as the brain to MAO A–inhibitory compounds, we determined whether smokers would also have reduced MAO A in peripheral organs. **Methods:** We measured MAO A in peripheral organs in a group of 9 smokers and compared it with a group of nonsmokers studied previously. MAO A was measured using PET and serial scans with the MAO A–specific radiotracers ^{11}C-clorgyline and deuterium-substituted ^{11}C-clorgyline (^{11}C-clorgyline-D2) using the deuterium isotope effect to assess binding specificity. The time course of radiotracer in the arterial plasma was also measured and data from the tissue time–activity curves and the arterial input function were analyzed using a 3-compartment model to estimate k_3, which represents the rate-limiting step for the irreversible binding of labeled clorgyline to MAO A. **Results:** Tracer uptake at plateau was reduced with deuterium substitution for the heart, lungs, and kidneys, indicating specificity for MAO. There was no difference in organ uptake at plateau between nonsmokers and smokers though, for the smokers, the efflux of tracer from peak uptake to plateau was slower for the lungs. The area under the time–activity curve for the arterial plasma was also significantly reduced for smokers versus nonsmokers and the reduction occurred in the first few minutes after radiotracer injection. Smokers had an ~50% reduction in k_3 when compared with nonsmokers; however, k_3 did not differ for nonsmokers and smokers for the heart and the kidneys. **Conclusion:** Because MAO A breaks down serotonin, norepinephrine, dopamine, and tyramine, and because the lung is a major metabolic organ in degrading some of these substances, reduced lung MAO A may contribute to some of the physiologic effects of smoking. This study also revealed that the concentration of the radiotracers in the arterial plasma is significantly lower for the smoker versus the nonsmoker and that this appears to be caused in part by retention of the radiotracer in lungs. If this is generally true for other substances that are administered intravenously, then this needs to be considered as a variable that may contribute to different short-term behavioral responses to intravenously administered drugs for nonsmokers versus smokers.

Key Words: PET; cigarette smoke; monoamine oxidase; lung; arterial plasma

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Monoamine oxidase (MAO; EC 1.4.3.4) is a key regulatory and protective enzyme because its substrates include many physiologically active amines such as neurotransmitters, drugs, and dietary amines (*1*). MAO is particularly well suited for regulatory and protective actions because it occurs in virtually every organ in the body. It also occurs in 2 different subtypes, MAO A and MAO B, which are different gene products and have different substrate and inhibitor specificities (*2*). MAO A preferentially oxidizes norepinephrine and serotonin and is selectively inhibited by clorgyline (*3*), whereas MAO B preferentially breaks down benzylamine and phenylethylamine and is selectively inhibited by L-deprenyl (*4*). Both forms oxidize dopamine and tyramine (*5*).

The regulatory and protective roles of MAO are illustrated by the effectiveness of MAO inhibitor drugs as antidepressants and by reports of serious and sometimes fatal elevations in blood pressure when individuals who are treated with nonsubtype-selective irreversible MAO inhibitor drugs ingest foods containing the vasoactive dietary amine tyramine (*6*). In fact, the common warning to avoid coadministration of certain prescription and over-the-counter drugs with MAO inhibitor drugs points out the importance of robust MAO activity in peripheral organs.

We have previously shown that smokers have reduced levels of brain MAO A and B (*7,8*). More recently we have also shown that smokers have reduced MAO B in some peripheral organs, including heart, lungs, kidneys, and spleen (*9*). Because reduced MAO B in peripheral organs could potentially alter sympathetic tone and contribute to

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some of the physiologic effects of smoking, we questioned whether smokers would also have reduced MAO A in peripheral organs. We measured MAO A in peripheral organs in nonsmokers and smokers using PET and serial scans with the MAO A–specific radiotracers ^{11}C-clorgyline and deuterium-substituted ^{11}C-clorgyline (^{11}C-clorgyline-D2) (*10,11*). ^{11}C has a 20.4-min half-life and decays by positron emission. Binding specificity for MAO A was assessed in different organs based on the deuterium isotope effect. The deuterium isotope effect refers to a reduction in the rate of a reaction that occurs when a deuterium atom is substituted for hydrogen atom in a chemical bond that is cleaved in the rate-limiting step of a reaction (*12*). MAO is well known to exhibit a robust deuterium isotope effect and, thus, a comparison of the rate of binding of ^{11}C-clorgyline and ^{11}C-clorgyline-D2 provides an assessment of the specificity of the imaging method for detecting MAO A activity. We have previously shown that MAO A can be quantified in brain, heart, lungs, kidneys, and spleen using this dual-tracer approach (*10*).

MATERIALS AND METHODS

Subjects

These studies were approved by the Institutional Review Board at Brookhaven National Laboratory and written informed consent was obtained from each subject after the procedures had been explained. Nine healthy subjects (8 male, 1 female; mean age, 39 ± 7 y) were recruited by newspaper advertisements and word-of-mouth (Table 1 for subject information). Exclusion criteria were as described previously (*10*) except for smoking status. Smokers were instructed to have their last cigarette before entering the imaging laboratory. A blood sample for plasma cotinine analysis (by gas chromatography; Quest Diagnostics) was taken before the first PET scan. A group of 9 nonsmokers studied previously (*10*) was used for comparison.

PET Scans

PET scans were run on a whole-body, high-resolution positron emission tomograph (Siemens/CTI ECAT HR+, with 4.6 × 4.6 × 4.2-mm NEMA [National Electrical Manufacturers Association] resolution at the center of the field of view) in 3-dimensional dynamic acquisition mode. Subjects were positioned with their torso in the field of view of the tomograph. A transmission scan was obtained with a ^{68}Ge rotating rod source before each emission scan to correct for attenuation. Catheters were placed in an antecubital vein for radiotracer injection and in the radial artery for blood sampling. ^{11}C-Clorgyline and ^{11}C-clorgyline-D2 were prepared as described previously (*13,14*) (Fig. 1 for radiotracer structures). Each subject received both tracers with a time interval of 2–3 h between injections. The average doses of ^{11}C-clorgyline and ^{11}C-clorgyline-D2 were 246 ± 56 MBq (6.6 ± 1.5 mCi) and 226 ± 72 MBq (6.1 ± 1.95 mCi), respectively. The specific activity was 9 × 10^3 MBq/μmol (250 mCi/μmol) at the time of injection. The timing sequence for the PET scans and arterial sampling were described previously (*10*).

TABLE 1
Subject Information

Parameter	Nonsmokers (ethnicity)	Smokers (ethnicity)
No. of subjects	9 (8 AA/1 H)	9 (8 AA/1 C)
Age (y)	39 ± 6	39 ± 7
Sex	9 M	8 M/1 F
Cigarettes/day	—	15 ± 10
Smoking years	—	22 ± 11
Cotinine (ng/mL)	—	320 ± 111

AA = African American; H = Hispanic; C = Caucasian.

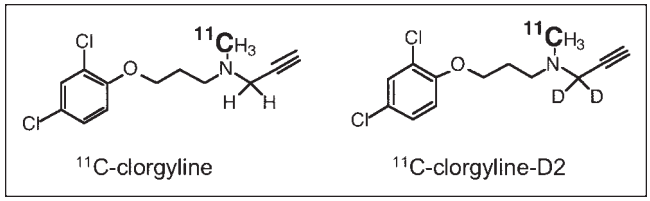

FIGURE 1. Structures of ^{11}C-clorgyline and ^{11}C-clorgyline-D2. The C—H (C—D) bond in the methylene carbon of the propargyl group is the one that is cleaved in the rate-contributing step of MAO-catalyzed oxidation.

Regions of Interest (ROIs)

Emission data were corrected for attenuation and reconstructed using filtered backprojection. Time frames from dynamic images taken from 0 to 60 min were summed. Planes were added in groups of 2 to obtain 16–30 planes for identification and placement of ROIs. ROIs were drawn directly on the PET scans using an atlas for reference (*15*) as described previously (*10*) and then projected to the dynamic scans to obtain concentration of ^{11}C versus time and expressed as a percentage of the total injected dose per cubic centimeter.

Data Analysis

Time–activity curves for the arterial plasma and for different organs were compared for ^{11}C-clorgyline and ^{11}C-clorgyline-D2 to identify organs that showed the reduction in binding-rate characteristic of the deuterium isotope effect. The uptake (% dose/cm^3) averaged over the time period 27.7–52.5 min was compared for ^{11}C-clorgyline and ^{11}C-clorgyline-D2 for the smokers and the values were compared with those obtained for the group of nonsmokers studied previously. MAO A was quantified using a 3-compartment model similar to the one used previously to estimate MAO A in brain and in peripheral organs in nonsmokers (*7,10*). Briefly, we estimated K_1, the plasma-to-organ transfer constant, which is related to blood flow; k_2, which is related to the back-transfer of tracer from organ to plasma; and k_3, the kinetic constant describing the rate of binding to MAO A. MAO A activity is not determined directly but is inferred from the model term k_3 (*10*).

Statistical Analysis

The averaged organ uptakes at plateau (27.5–52.5 min), the plasma integrals at 52.5 min, and the model terms K_1, k_3, and k_2/k_3 were compared for ^{11}C-clorgyline versus ^{11}C-clorgyline-D2 for smokers and for the nonsmokers using a paired-samples t test (*16*). These measures were also compared for the nonsmokers versus the smokers for both ^{11}C-clorgyline and ^{11}C-clorgyline-D2 using an

unpaired-samples *t* test. Normality of the data was examined via the Shapiro–Wilk test.

RESULTS

Radiotracer Uptake and Deuterium Isotope Effect

There was a significantly smaller area under the plasma time–activity curve over a 52-min period for ^{11}C-clorgyline versus ^{11}C-clorgyline-D2 (11,766 ± 1,591 and 13,764 ± 2,109 Bq · mL^{-1} · min; $P = 0.0001$), probably reflecting the slower MAO A–catalyzed oxidation of ^{11}C-clorgyline-D2. This same pattern was seen in the nonsmokers (*10*) and in studies of MAO B, in which the ^{11}C-L-deprenyl/^{11}C-L-deprenyl-D2 pair was compared (*9,17*). In addition to differences between ^{11}C-clorgyline versus ^{11}C-clorgyline-D2 for the plasma integrals, the time–activity curves for heart, lungs, and kidneys showed a decreased uptake in the plateau phase of the uptake curve (27.5–52.5 min after injection) with deuterium substitution supporting binding to MAO A in the rate-contributing step similar to prior studies in nonsmokers (Table 2). Though the pattern of uptake in the spleen was consistent with irreversible binding to MAO A, we did not observe an isotope effect, probably because of the very low uptake and poor counting statistics. There was no reduction in uptake with deuterium substitution for the liver similar to results in nonsmokers. Moreover, the time–activity curves for the liver showed a slow accumulation of ^{11}C over time (data not shown), differing from the typical rapid initial uptake, short-term clearance, and plateau at later times that characterize the influx of ^{11}C into the heart, lungs, kidneys, and spleen.

Comparison of Plasma Time–Activity Curves and Plasma Integrals in Nonsmokers Versus Smokers

The plasma time–activity curves showed lower values during the first few minutes for the nonsmokers versus the smokers. This is illustrated in Figure 2, where the first 5 min of the plasma time–activity curve (corrected for the presence of labeled metabolites) is plotted for smokers and for nonsmokers for ^{11}C-clorgyline and ^{11}C-clorgyline-D2. There was also a significantly smaller area under the plasma time–activity curve over a 52-min period for smokers versus nonsmokers for both ^{11}C-clorgyline and ^{11}C-clorgyline-D2. For ^{11}C-clorgyline, values were 13,394 ± 1,369 and 11,581 ± 1,850 Bq · mL^{-1} · min for nonsmokers and smokers, respectively ($P = 0.023$), and, for ^{11}C-clorgyline-D2, values were 16,243 ± 1,887 and 13,431 ± 2,220 Bq · mL^{-1} · min for nonsmokers and smokers, respectively ($P = 0.042$). The difference between the nonsmokers and the smokers occurs mainly in the first few minutes of the time–activity curves and is not driven by the metabolite correction, which is minimal at early time points (Fig. 2A).

TABLE 2
Organ Uptake (% Injected Dose/cm^3) at Plateau (Average of Time Points Between 27.5 and 52.5 Minutes After Injection) for Heart, Lungs, and Kidneys

Organ (tracer)	Nonsmoker uptake (% dose/cm^3)	Smoker uptake (% dose/cm^3)
Heart (H)	0.0063 ± 0.0006	0.0058 ± 0.0007
Heart (D)	0.0039 ± 0.0002	0.0033 ± 0.0007
Lungs (H)	0.0039 ± 0.0012	0.0037 ± 0.0009
Lungs (D)	0.0021 ± 0.0007	0.0021 ± 0.0006
Kidneys (H)	0.0106 ± 0.0011	0.0109 ± 0.0028
Kidneys (D)	0.0056 ± 0.0013	0.0058 ± 0.0019
Spleen (H)	0.0027 ± 0.001	0.002 ± 0.0007
Spleen (D)	0.0022 ± 0.0012	0.0022 ± 0.0007

Values for ^{11}C-clorgyline are indicated as (H) and values for ^{11}C-clorgyline-D2 are indicated as (D). There were significant differences in uptake at plateau for heart, lungs, and kidneys (but not spleen) for ^{11}C-clorgyline vs. ^{11}C-clorgyline-D2 ($P < 0.001$) but there were no differences in uptake at plateau for nonsmokers vs. smokers for either ^{11}C-clorgyline or ^{11}C-clorgyline-D2.

Comparison of Organ Uptake for Labeled Clorgyline in Nonsmokers Versus Smokers

There was no difference in ^{11}C uptake at plateau for heart, lungs, or kidneys for the nonsmokers versus the smokers (Table 2). However, even though nonsmokers and smokers did not differ in uptake at plateau in the lungs, the rate of clearance of ^{11}C from the peak value was slower for the smokers than for the nonsmokers for both ^{11}C-clorgyline and ^{11}C-clorgyline-D2 (7.2 vs. 3.5 min; Fig. 2B).

Comparison of Model Terms K_1, k_3, and k_2/k_3 in Nonsmokers Versus Smokers

The plasma time–activity curves (input function) and the organ time–activity curves were used to estimate K_1 and k_3 for nonsmokers and smokers, respectively. There was no difference between nonsmokers and smokers in the average values of the plasma-to-organ transfer constant K_1 for heart. There was a significant elevation of K_1 in the kidneys ($P = 0.02$) in the smokers for ^{11}C-clorgyline but not ^{11}C-clorgyline-D2. There was a significant elevation in K_1 in the lungs for ^{11}C-clorgyline ($P = 0.002$) and a trend for ^{11}C-clorgyline-D2 ($P = 0.06$), indicating greater blood flow and permeability of the tracer in lung tissue in smokers (Table 3).

The model term k_3, which represents the irreversible trapping of radiotracer in tissue, was used as an index of MAO A activity. There was no difference in k_3 between nonsmokers and smokers for the heart and kidneys, which is consistent with the fact that there was no difference in uptake at plateau for these 2 groups. However, k_3 was significantly reduced in the lungs in the smokers relative to a group of nonsmokers studied previously. The reduction was ~50% for the model term k_3. This reduction in MAO A was seen for both ^{11}C-clorgyline and ^{11}C-clorgyline-D2 (Table 3).

The ratio k_2/k_3 is the ratio of the efflux of unbound tracer from tissue to the rate of irreversible binding of tracer to tissue. In terms of quantification, low values (<1) of k_2/k_3 indicate a flow-limited situation, where the rate of irreversible binding (which in this case is an index of MAO A activity) may be underestimated because of limited radio-

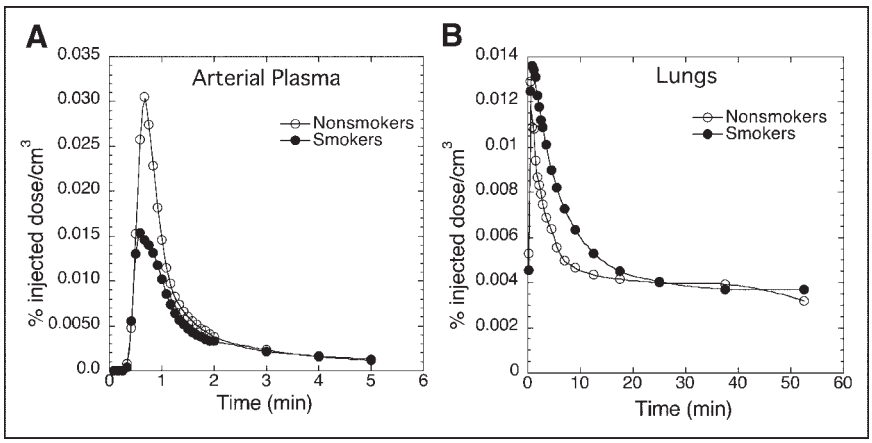

FIGURE 2. Time–activity curves for nonsmokers and smokers for ^{11}C-clorgyline for arterial plasma for the first 5 min after injection corrected for the presence of labeled metabolites (A) and for lungs over a 52-min experimental period (B). Note that smokers have reduced arterial input as well as slower lung clearance of ^{11}C. A similar pattern is seen for ^{11}C-clorgyline-D2 (*31*). (Adapted with permission of (*31*).)

tracer delivery to tissue. As expected, all of these ratios are greater for ^{11}C-clorgyline-D2 than for ^{11}C-clorgyline because deuterium substitution would be expected to reduce binding to tissue but not delivery to tissue. The ratio k_2/k_3 for lungs is significantly greater for smokers than for nonsmokers, reflecting the reduction in k_3 for smokers (Table 3). The high values for this ratio for ^{11}C-clorgyline-D2 and even for ^{11}C-clorgyline (except for heart) facilitate quantification.

DISCUSSION

The effects of smoking on human health are enormous; yet, little is known about the pharmacologic effects of smoking on the human body apart from the effects of nicotine. In this study we found that lung MAO A is reduced by about 50% in smokers relative to nonsmokers. Robust lung MAO A is of particular importance in human health because of the protective role that the lung plays in the deactivation of locally released norepinephrine and circulating vasoactive amines, such as norepinephrine, dopamine, and serotonin (*18,19*). In this regard, we note that postmortem studies (*20*) as well as human PET studies (*10,21*) have documented high lung MAO A activity in humans. For these reasons, we can speculate that, in the absence of compensatory mechanisms, reduced lung MAO A in smokers may compromise their ability to regulate the concentration of circulating vasoactive substances, such as norepinephrine, dopamine, and serotonin. Supporting this is a study in heavy smokers reporting that smokers have significantly lower plasma concentrations of MAO-induced catecholamine metabolites (plasma 3,4-dihydroxyphenylglycol, 3,4-dihydroxyphenylacetic acid, and 3,4-dihydroxylphenylalanine) than nonsmokers, but both groups have similar levels of plasma norepinephrine (*22*).

Low lung MAO A in the smokers is also relevant in the context of the fact that nicotine in tobacco smoke mediates the exocytotic release of norepinephrine, thereby increasing levels of circulating norepinephrine and epinephrine (*23*). This occurs via nicotine-induced activation of nicotinic acetylcholine receptors located on peripheral postganglionic sympathetic nerve endings as well as the adrenal medulla (*24*). Taken together, nicotine-induced elevations in circulating norepinephrine and reduced lung MAO A may act synergistically to enhance circulating norepinephrine levels, thereby contributing to some of the central and peripheral effects of smoking.

TABLE 3
Values for K_1, k_3, and k_2/k_3 for Nonsmokers (NS) and Smokers (S) with *P* Values Indicating Significant Differences Between Smokers and Nonsmokers

Organ (tracer)	K_1 (NS)	K_1 (S)	*P* value	k_3 (NS)	k_3 (S)	*P* value	k_2/k_3 (NS)	k_2/k_3 (S)	*P* value
Heart (H)	0.40 ± 0.11	0.42 ± 0.06	ns	0.10 ± 0.02	0.11 ± 0.03	ns	1.1 ± 0.25	0.96 ± 0.34	ns
Heart (D)	0.38 ± 0.09	0.59 ± 0.33	ns	0.032 ± 0.004	0.03 ± 0.011	ns	3.33 ± 0.7	6.6 ± 5.6	ns
Lungs (H)	2.25 ± 0.57	4.29 ± 1.26	0.002	0.087 ± 0.019	0.038 ± 0.013	0.0001	4.9 ± 1.3	9.9 ± 2.8	0.001
Lungs (D)	2.31 ± 0.85	3.7 ± 1.54	0.06	0.032 ± 0.007	0.0154 ± 0.009	0.002	12.0 ± 6.1	26.6 ± 16.5	0.05
Kidneys (H)	0.84 ± 0.20	1.24 ± 0.33	0.02	0.24 ± 0.08	0.18 ± 0.06	ns	1.17 ± 0.32	1.97 ± 0.65	ns
Kidneys (D)	0.85 ± 0.13	1.02 ± 0.23	ns	0.07 ± 0.013	0.063 ± 0.012	ns	5.12 ± 0.56	5.10 ± 1.8	ns
Spleen (H)	1.2 ± 0.29	0.98 ± 0.44	ns	0.026 ± 0.015	0.015 ± 0.006	0.07	14.9 ± 7.4	16.3 ± 7.0	ns
Spleen (D)	0.79 ± 0.26	1.17 ± 0.16	0.002	0.014 ± 0.008	0.013 ± 0.007	ns	21.1 ± 11.7	21.6 ± 7.7	ns

ns = not significant.
Values for ^{11}C-clorgyline are indicated as (H) and values for ^{11}C-clorgyline-D2 are indicated as (D). Units for K_1 and k_3 are mL · min^{-1} · g^{-1}.

Systemically administered nicotine has been reported to increase brain serotonin in the rat (*25*). However, nicotine alone did not change plasma serotonin or 5-hydroxyindoleacetic acid in the rat, though there was a synergistic elevation of serotonin levels in plasma when nicotine administration was combined with a stressor (foot shock) (*26*). Racke et al. (*27*) also reported that smokers have an elevated platelet serotonin relative to nonsmokers. Because serotonin is metabolized specifically by MAO A, the possibility that the breakdown of circulating serotonin is compromised when lung MAO A is partially inhibited needs to be considered.

Though lung MAO A activity is reduced by about 50% in smokers relative to nonsmokers, MAO A activity in heart and kidneys was not affected. In this respect, the effect of smoking on peripheral MAO A is less widespread than the effect on peripheral MAO B, for which we observed a significant reduction in heart, kidneys, and spleen as well as the lungs (*9*). The more localized effect of tobacco smoker exposure on MAO A relative to MAO B could reflect differences in the concentrations of the MAO subtypes or differences in the distribution and inhibitory potencies of the MAO A and B inhibitors in smoke. The lung would receive the largest exposure to MAO B–inhibitory compounds in smoke, which is consistent with a high degree of inhibition. We note that nicotine does not inhibit MAO A and B (*28*) and that extracts of smoke have been reported to inhibit lung MAO in rats (*29*). In addition, MAO inhibitor compounds have been isolated from tobacco plants (*30*).

In addition to having reduced MAO A, which is represented by the model term k_3, the smokers differed in the time–activity curve for the radiotracer in the arterial plasma with the area under the curve for the smokers being significantly lower than the area under the curve for the nonsmokers. This represents a lower input from blood to the different organs in the body for the smoker. As can be seen for the plasma time–activity curves for ^{11}C-clorgyline (Fig. 2A), the major difference between nonsmokers and smokers occurs during the first few minutes after the radiotracer is injected. We speculate that the lower concentration of the tracers in the arterial plasma during the first few minutes after injection is accounted for by the short-term retention of the tracer in the lungs, which are exposed to all of the venous blood and, consequently, to the entire radiotracer bolus (Fig. 2B).

We do not know the extent to which smokers (relative to nonsmokers) show this general pattern of reduced radiotracer concentration in arterial plasma during the first few minutes after an intravenous injection. However, we have compared the arterial plasma input function in smokers versus nonsmokers for the MAO B radiotracers (^{11}C-L-deprenyl and ^{11}C-L-deprenyl-D2) and find the same general patterns for the first few minutes after injection (*31*). This leads us to speculate that lower drug concentration in arterial plasma for the first few minutes after an intravenous administration in smokers versus nonsmokers may occur for other chemical compounds and that drugs and other substances that are introduced into the bloodstream by the intravenous route may achieve higher tissue concentrations in nonsmokers versus smokers during the first few minutes after administration. For example, Rose et al. (*32*) have reported that arterial concentrations of nicotine in smokers after intravenous nicotine administration are 10 times lower than would be expected if nicotine were absorbed as rapidly as has generally been assumed. These authors also comment on the data of Evans et al. (*33*) for intravenous cocaine administration to cocaine abusers, where peak cocaine levels in arterial plasma were an order of magnitude lower than would be expected. They speculate that the lower arterial plasma concentration of nicotine and cocaine may reflect the distribution of these compounds in lung tissue. On the basis of our observations of differences between smokers and nonsmokers, it is possible that the lower arterial concentrations may also be due to difference in lung tissue integrity in the smoker versus the nonsmoker.

Smoking is known to induce inflammation, oxidative stress, and epithelial damage (*34*). However, the specific mechanism contributing to the longer lung retention and the reduced arterial plasma concentration of substances that we and others have observed is not known. Nonetheless, for substances of abuse, this could significantly modulate the behavioral effects of the drug during the crucial first few minutes after administration in the smoker versus the nonsmoker and, thus, it is possible that the behavioral response to drugs administered intravenously may differ between the smoker and the nonsmoker. We note that there is a high comorbidity between cigarette smoking and abuse of other substances. For therapeutic drugs that are administered intravenously, reduced plasma concentration may alter the short-term drug delivery in the smoker relative to the nonsmoker. However, it is important to note that the major effect is in the first few minutes, after which the plasma concentrations for the smoker and the nonsmoker become equal.

The reduction in k_3 (model term which is proportional to MAO A activity) was surprising given that there was no difference in uptake at plateau ($>$30 min after injection) for nonsmokers and smokers (Table 2). However, a comparison of the shape of the lung time–activity curve for the nonsmokers versus the smokers reveals a slower decline of radioactivity from the peak to plateau, indicating that the lungs retain the tracer over the first few minutes. For example, the half-times for clearance of ^{11}C from peak uptake were 3.5 min for nonsmokers and 7.2 min for smokers. The uptake at plateau represents both MAO A bound and unbound components. The longer retention of ^{11}C in the lung for the smoker relative to the nonsmoker and the binding of the radiotracer to MAO A both contribute to the plateau uptake and, thus, the contribution of radiotracer binding to MAO A is smaller in the smoker. We have performed a detailed analysis of the sensitivity of model terms and have determined that our estimate of k_3 is accurate and that

smokers do indeed have lower values of k_3 than nonsmokers (*31*). The physiologic basis for slower radiotracer clearance in the smokers' lungs is not known. However, it is a phenomenon that does not occur in nonsmokers. It may represent a general breakdown of the integrity of the lung tissue and merits further mechanistic study to determine whether it is related to some of the pulmonary disorders associated with smoking. Others have reported anomalous radiotracer accumulation in lungs in smokers (*35*).

The transfer of the tracers from plasma to lung, K_1, which is a model value that contains terms for blood flow and the permeability surface area of the capillaries (*36*), was also higher for the smokers. We saw a similar elevation in lung K_1 in smokers in whom we measured MAO B (*9*). The lower radiotracer concentration in the arterial plasma and the similar uptake of radiotracer in the lungs lead to the higher values for K_1 for ^{11}C-clorgyline and ^{11}C-clorgyline-D2.

We note that the measurement of MAO A in peripheral organs in vivo in humans is limited by the fact that we cannot measure liver and gut MAO A, which are both of major importance in regulating dietary amines and amines from other sources. Failure of these tracers in these organs is evidenced by their slow accumulation and lack of an effect of deuterium substitution. Thus, we cannot determine whether liver and gut MAO A are altered in smokers.

CONCLUSION

In summary, from this study we learned that there is a marked reduction of lung MAO A in smokers. The lung plays an important role in the deactivation of circulating vasoactive substances such as norepinephrine and serotonin. Because norepinephrine and serotonin are substrates for MAO A and are released by nicotine as well, the possibility that lung MAO A inhibition by smoke may combine with nicotine to influence local and circulating catecholamine levels and sympathetic tone and may contribute to some to the pulmonary effects seen in smokers needs to be considered. In addition, this study revealed that the concentration of radiotracer in the arterial plasma is significantly lower for the smoker versus the nonsmoker for the first few minutes after intravenous injection and that this appears to be caused in part by retention of the radiotracer in lungs. If this is generally true for other substances that are introduced into the bloodstream, then this needs to be considered as a variable that may contribute to different short-term behavioral responses to intravenously administered drugs for nonsmokers versus smokers.

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REFERENCES

1. Strolin Benedetti M, Tipton KF. Monoamine oxidases and related amine oxidases as phase I enzymes in the metabolism of xenobiotics. *J Neural Transm.* 1998;52 (suppl):149–171.
2. Shih JC, Chen K, Ridd MJ. Monoamine oxidase, from genes to behavior. *Ann Rev Neurosci.* 1999;22:197–217.
3. Johnston JP. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol.* 1968;17:1285–1297.
4. Knoll J, Magyar K. Some puzzling effects of monoamine oxidase inhibitors. *Adv Biochem Psychopharmacol.* 1972;5:393–408.
5. Youdim MB, Riederer P. Dopamine metabolism and neurotransmission in primate brain in relationship to monoamine oxidase A and B inhibition. *J Neural Transm.* 1993;91:181–195.
6. Anderson MC, Hasan F, McCrodden JM, Tipton KF. Monoamine oxidase inhibitors and the cheese effect. *Neurochem Res.* 1993;18:1145–1149.
7. Fowler JS, Volkow ND, Wang GJ, et al. Brain monoamine oxidase A inhibition in cigarette smokers. *Proc Natl Acad Sci USA.* 1996;93:14065–14069.
8. Fowler JS, Volkow ND, Wang GJ, et al. Inhibition of monoamine oxidase B in the brains of smokers. *Nature.* 1996;379:733–736.
9. Fowler JS, Logan J, Wang GJ, et al. Low monoamine oxidase B in peripheral organs in smokers. *Proc Natl Acad Sci USA.* 2003;100:11600–11605.
10. Fowler JS, Logan J, Wang GJ, et al. Monoamine oxidase A imaging in peripheral organs in healthy human subjects. *Synapse.* 2003;49:178–187.
11. Fowler JS, Logan J, Ding YS, et al. Non-MAO A binding of clorgyline in white matter in human brain. *J Neurochem.* 2001;79:1039–1046.
12. Belleau B, Moran J. Deuterium isotope effects in relation to the chemical mechanism of monoamine oxidase. *Ann NY Acad Sci.* 1963;107:822–839.
13. Fowler JS, Ding YS, Logan J, et al. Species differences in [^{11}C]clorgyline binding in brain. *Nucl Med Biol.* 2001;28:779–785.
14. MacGregor RR, Fowler JS, Wolf AP. Synthesis of suicide inhibitors of monoamine oxidase: carbon-11 labeled clorgyline, L-deprenyl and D-deprenyl. *J Labelled Compds Radiopharm.* 1988;25:1–9.
15. Dean D, Herbener TE. *Cross-Sectional Human Anatomy.* Philadelphia, PA: Lippincott Williams & Wilkins; 2000:8–59.
16. Ott RL. *An Introduction to Statistical Methods and Data Analysis.* 4th ed. Belmont; CA: Duxbury Press; 1992.
17. Fowler JS, Logan J, Wang GJ, et al. PET imaging of monoamine oxidase B in peripheral organs in humans. *J Nucl Med.* 2002;43:1331–1338.
18. Gillis CN, Pitt BR. The fate of circulating amines within the pulmonary circulation. *Annu Rev Physiol.* 1982;44:269–281.
19. Westwood NN, Bryan-Lluka LJ. Types A and B monoamine oxidase contribute to the metabolism of norepinephrine in perfused lungs of rats. *J Pharmacol Exp Ther.* 1993;267:815–821.
20. Saura J, Nadal E, van den Berg B, Vila M, Bombi JA, Mahy N. Localization of monoamine oxidases in human peripheral tissues. *Life Sci.* 1996;59:1341–1349.
21. Fowler JS, Logan J, Wang GJ, et al. Comparison of the binding of the irreversible monoamine oxidase tracers, [^{11}C]clorgyline and [^{11}C]l-deprenyl in brain and peripheral organs in humans. *Nucl Med Biol.* 2004;31:313–319.
22. Berlin I, Said S, Spreux-Varoquaux O, Olivares R, Launay J-M, Puech AJ. Monoamine oxidase A and B activities in heavy smokers. *Biol Psychiatry.* 1995;38:756–761.
23. Haass M, Kubler W. Nicotine and sympathetic neurotransmission. *Cardiovasc Drugs Ther.* 1997;10:657–665.
24. Walker JF, Collins LC, Rowell PP, Goldsmith LJ, Moffatt RJ, Stamford BA. The effects of smoking on energy expenditure and plasma catecholamine and nicotine levels during light physical exercise. *Nicotine Tobacco Res.* 1999;1:365–370.
25. Summers KL, Giacobini E. Effects of local and repeated systemic adminstration of (-)nicotine on extracellular levels of acetylcholine, norepinephrine, dopamine and serotonin in rat cortex. *Neurochem Res.* 1995;20:753–759.
26. Knofler R, Takada Y, Ihara H, Urano T, Takada A. Effects of nicotine and electric

footshock on peripheral serotonergic measures and on platelet aggregation in whole blood of rats. *Life Sci.* 1995;57:363–369.
27. Racke K, Schworer H, Simson G. Effects of cigarette smoking or ingestion of nicotine on platelet 5-hydroxytryptamine (5-HT) levels in smokers and nonsmokers. *Clin Invest.* 1992;70:201–204.
28. Carr LA, Basham JK. Effects of tobacco smoke constituents on MPTP-induced toxicity and monoamine oxidase activity in the mouse brain. *Life Sci.* 1991;48: 1173–1177.
29. Yu P, Boulton A. Irreversible inhibition of monoamine oxidase by some components of cigarette smoke. *Life Sci.* 1987;41:675–682.
30. Khalil AA, Steyn S, Castagnoli N. Isolation and characterization of monoamine oxidase inhibitor from tobacco leaves. *Chem Res Toxicol.* 2000;13:31–35.
31. Logan J, Fowler JS. Evidence for reduced arterial plasma input, prolonged lung retention and reduced lung monoamine oxidase in smokers. *Nucl Med Biol.* 2005;32:521–529.
32. Rose JE, Behm FM, Westman EC, Coleman RE. Arterial nicotine kinetics during cigarette smoking and intravenous nicotine administration: implications for addiction. *Drug Alcohol Depend.* 1999;56:99–107.
33. Evans SM, Cone EJ, Henningfield JE. Arterial and venous cocaine plasma concentrations in humans: relationship of route of administration, cardiovascular effects and subjective effects. *J Pharm Exp Ther.* 1996;279:1345–1356.
34. van der Vaart H, Postma DS, Timens W, ten Hacken NH. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. *Thorax.* 2004; 59:713–721.
35. Kagaya A, Fukuda H, Yoshida K, et al. Pulmonary kinetics of ^{13}N-ammonia in smoking subjects: a quantitative study using dynamic PET [in Japanese]. *Kaku Igaku.* 1992;29:1099–1106.
36. Logan J, Volkow ND, Fowler JS, et al. Effects of blood flow on [^{11}C]raclopride binding in the brain: model simulations and kinetic analysis of PET data. *J Cereb Blood Flow Metab.* 1994;14:995–1010.