MOLECULAR ORBITAL CALCULATIONS AND NICOTINE METABOLISM: A RATIONALE FOR EXPERIMENTALLY OBSERVED METABOLITE RATIOS

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SUMMARY

The results of molecular orbital calculations and molecular modelling studies on nicotine are reported. It is shown that the product ratio of nicotine metabolism can be directly related to HOMO electron densities on the relevant hydrogen atoms associated with oxidation sites in *S*-nicotine. In addition, molecular modelling of nicotine within the putative active site of CYP2A6, the enzyme most closely associated with nicotine metabolism, indicates that the substrate is orientated for oxidation at the 5′-position via a combination of hydrogen bonding and π-π stacking interactions. Alternative routes of metabolism may require rotation of the pyrrolidine ring system and could, therefore, involve a degree of re-orientation of the nicotine molecule which is energetically less favourable than the modelled interaction indicating formation of cotinine via 5′-oxidation.

KEY WORDS

molecular orbital calculations, molecular modelling, nicotine, metabolism, cytochrome CYP2A6, cotinine

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INTRODUCTION

Nicotine is a dibasic alkaloid present in tobacco and a number of other plants. The world-wide production of tobacco is approximately six million metric tonnes annually (data prepared by USDA/FAS/ COTS, 2000) containing on average 2% nicotine (based on a range of between 1.05% and 2.91%). This equates to a world production of nicotine in excess of 120 million kg annually. The bulk of this nicotine is present in tobacco products, i.e. cigarettes, cigars, pipe tobacco and snuff, which are utilised by individuals according to their desires and social habit. Some nicotine is destroyed by pyrolysis during the smoking of tobacco products; some is transferred to the atmosphere, where it may be destroyed by photo-decomposition or by micro-biological degradation, and some is transferred to the person using the product.

The transfer of nicotine to the user can be via direct absorption, as in chewing tobacco and snuff in the buccal or nasal cavities, or via tobacco smoke where it is predominantly absorbed by the lungs, although some may be released into the atmosphere with exhaled breath. In the former cases, nicotine is rapidly circulated in the body and undergoes metabolism in various organs but, primarily, the liver. The metabolism of nicotine is extremely complex (/1,2/ and references therein) and under certain circumstances can give rise to carcinogenic *N*-nitroso compounds /3,4/.

During mammalian nicotine metabolism, initial electron abstraction produces a nicotine aminium radical /5/, which is further converted to three isomeric iminium ions by hydrogen abstraction. These are then converted to cotinine, A (Fig. 1, pathway a) /6/, nornicotine, B (Fig. 1, pathway b) /7/, and 4-(3-pyridyl)-4-oxo-*N*-methylbutylamine, C (Fig. 1, pathway c) /8/, probably by cytosolic aldehyde oxidase /9/. These pathways are not utilised to an equal extent, however, as is discussed later in the Results and Discussion section.

Further details of nicotine occurrence, biosynthesis, pharmacology, biotransformation, chemistry and utilisation can be found in the monograph edited by Gorrod and Jacob /10/. Pathways b and c can lead to carcinogenic *N*-nitrosamines /3,11/ so that it was considered important to establish the factors which bias nicotine towards oxidation at these sites.

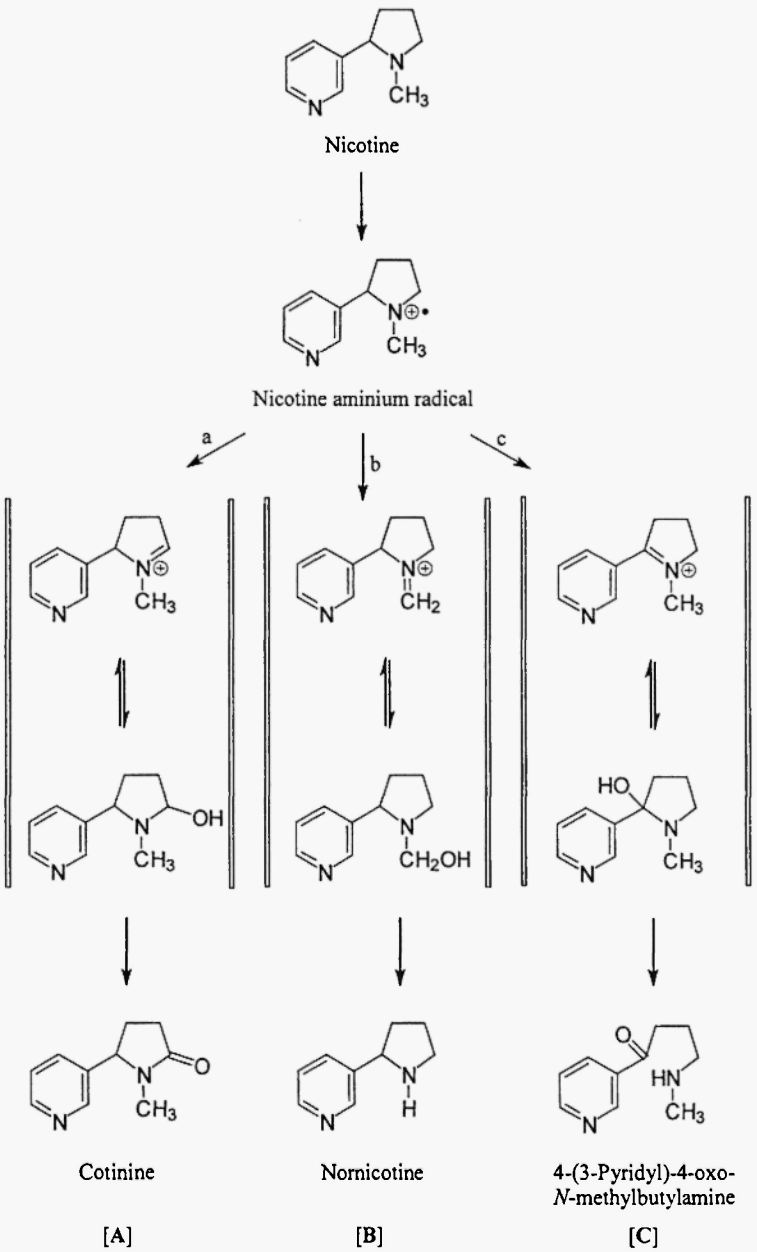

Fig. 1: Pathways (routes a, b and c) of nicotine metabolism in *Homo sapiens* mediated by P450 enzymes such as CYP2A6 (see text for details).

In an attempt to ascertain the physicochemical factors which predispose molecular sites to iminium ion formation and hence to either pathway a, b or c, we carried out molecular orbital (MO) studies on these intermediates. In addition, the recently available crystal structure of the mammalian P450 CYP2C5 /12/ facilitated the construction of a homology model of CYP2A6, the enzyme most closely associated with nicotine metabolism /11,13,14,15,16,17/. Consequently, molecular modelling of the CYP2A6 active site interaction with nicotine was also carried out in order to investigate possible orientations of the substrate which may be consistent with the metabolic information, such as the preference for 5′-oxidation.

METHODS

Molecular orbital (MO) calculations via the AM1 procedure were carried out on the three cationic intermediates postulated as precursors for the three main products of nicotine metabolism, namely, cotinine, nornicotine, and 4-(3-pyridyl)-4-oxo-*N*-methylbutyramine, together with the nicotine aminium radical cation.

The nicotine substrate was docked interactively within a homology model of CYP2A6 generated from the CYP2C5 crystal structure /18/ on the basis of optimal hydrogen-bonded and π-π stacking contacts with active site amino acid residues. The MO calculations (via AM1 method) involved full geometry optimization of the structures and normal convergence parameterization as implemented in the MOPAC package within the Sybyl Molecular Modelling System (Tripos Associates, St. Louis, MO). All electronic structural calculations and modelling studies were conducted on a Silicon Graphics Indigo^2 Impact 10000 graphics workstation operating under UNIX.

RESULTS AND DISCUSSION

Studies on the metabolism of nicotine in various biological systems have been extensive and extended over many years, yet many chemical species, including metabolic intermediates, the enzymology of their formation and control mechanisms, remain to be elucidated. Early studies indicated that cotinine was a major metabolite *in vivo* and *in vitro* /19,20/ (A; Fig. 1, pathway a) and it has been calculated

that about 70% of nicotine metabolism proceeds via this pathway /21/. Nornicotine (B; Fig. 1, pathway b) is formed to a lesser extent, although it has not been possible to calculate accurately its total contribution to nicotine metabolism due to difficulties in analysis and lack of understanding of its full metabolic pathway. It has only been found at about 0.3% in urine after intravenous administration of deuterium-labelled nicotine /22/ and at only 0.5% of the dose after infusion of the purified alkaloid /23/. This low excretion data could be due to rapid further metabolism of nicotine as it is known to be converted to norcotinine, a nitrone and an uncharacterised compound /24/. However, as Beckett *et al.* /25/ showed that nornicotine is excreted about 40% unchanged in human urine under fluctuating urinary pH conditions, this seems unlikely to be the whole explanation. At present, it is generally assumed that this pathway is utilised by about 1% during human nicotine metabolism.

Pathway c, leading to 4-(3-pyridyl)-4-oxo-*N*-methlybutylamine (C), is even more controversial as, until recently, little direct evidence for this pathway existed and its involvement in nicotine metabolism had been inferred from probable further metabolites /26/ or by trapping the intermediate iminium ion with cyanide /23,27/. More recently, Hecht and coworkers /11/ have shown that 2′-hydroxylation of nicotine occurs with both purified CYP2A6 and in human hepatic microsomes, and is in equilibrium with the 2′-iminium ion and 4-(3-pyridyl)-4-oxo-*N*-methylbutylamine. This compound is metabolised to 4-(3-pyridyl)-4-oxobutyric acid and sequentially to 4-(3-pyridyl)-4-hydroxybutyric acid. By determining these compounds in urine of subjects receiving nicotine it was calculated that approximately 13% of nicotine metabolism proceeded via this pathway compared to cotinine. From the above data, our working hypothesis was that pathways a, b and c were utilised in initial nicotine metabolism in a ratio of approximately 100:1:20%.

Table 1 represents the relevant results of MO calculations on the three cationic intermediates corresponding to the precursors of cotinine, nornicotine and 4-(3-pyridyl)-4-oxo-*N*-methylbutyramine, respectively. Based on these findings, it would appear that the product ratios should be in the order A>B>C on the basis of the partial charges (Q_H) on the relevant hydrogen atoms that may be abstracted from the nicotine aminium radical. Moreover, the molecular mechanics-minimized energies (E_{min}) are also lying in the same sequential order

TABLE 1

Data for nicotine metabolism

Pathway	Product	Q_H	E_{min}	H_f
a	Cotinine	0.1713	28.568	205.032
b	Nornicotine	0.1631	29.463	206.865
c	4-(3-Pyridyl)-4-oxo-*N*-methylbutyramine	0.1596	34.865	198.700

Q_H = Average partial net atomic charge on hydrogen atoms abstracted during the course of intermediate formation (in units of electronic charge, e).
E_{min} = Minimized energy of intermediate using AM1-calculated partial atomic charges (kcal.mole^{-1}).
H_f = MO-calculated enthalpy of formation of intermediate using the AM1 method (MOPAC).
Note: It would appear that the product ratios should be in the order A>B>C on the basis of the partial charges on the relevant hydrogen atoms which will be abstracted. The energies are also in the same order, although the heats of formation are at variance with the other two pieces of information.

as the hydrogen atom partial charges (average values). Clearly this does not correlate with the observed metabolic data. In addition, the calculated enthalpies of formation (H_f) appear to be at variance with the metabolic data and the MO data above, implying a stability in the order C>A>B.

We therefore extended our MO calculations to include HOMO electron densities, Q(HOMO), and determined the Q(HOMO) values of the nicotine structure leading to intermediates A, B and C, which gave electron densities of 0.0994, 0.0367 and 0.0637, respectively, for the relevant hydrogen atoms. These values show a clear linear relationship with the log of the metabolic ratios referred to above (Fig. 2) and appear to be the major determinant of the site of nicotine initial oxidation.

It has long been known that the C-oxidation of nicotine is mediated by the cytochrome P450 (CYP) family of enzymes (see /28/ and references therein), but few data are available as to the relationships between the regiospecificity of this process and the specific CYP

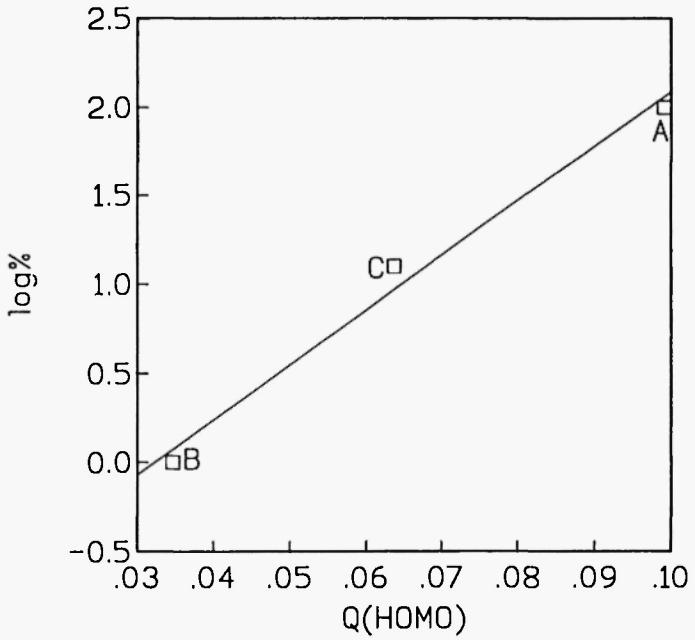

Fig. 2: A plot of log metabolite ratio versus Q(HOMO) for the relevant hydrogen atoms on the nicotine iminium ion (see text for details).

isozymes involved. In man, the conversion of nicotine to cotinine is thought to be predominantly carried out by CYP2A6, although CYP2D6 and other isozymes may be involved /29/. McCoy *et al.* /30/ showed that both isozyme 2 and 3b purified from rabbit liver were able to form cotinine and nornicotine, with the former metabolite predominating by fourfold. This latter observation is in line with that of Jones *et al.* /31/ who showed that $P450_{cam}$ also produced cotinine and nornicotine, although in this case the cotinine formed exceeded nornicotine about 100-fold. The recent publication of Hecht and coworkers /11/ also points to 2′-hydroxylation (c) and 5′-hydroxylation (a) pathways as representative of CYP2A6-mediated metabolism of nicotine.

It is clear that orientation of the nicotine molecule within the enzyme active site is also an important prerequisite to determining the position of oxidative metabolism via P450. Substrate rotation within the active site of the enzyme can enable the most energetically favourable pathway for metabolism to take place, although inter-molecular interactions with neighbouring amino acid residues may orientate and/or guide the substrate into position above the haem iron. Figure 3 shows how the nicotine molecule is able to fit within the putative active site of CYP2A6, where a combination of hydrogen bonding and π-π stacking interactions orientates the substrate for 5′-

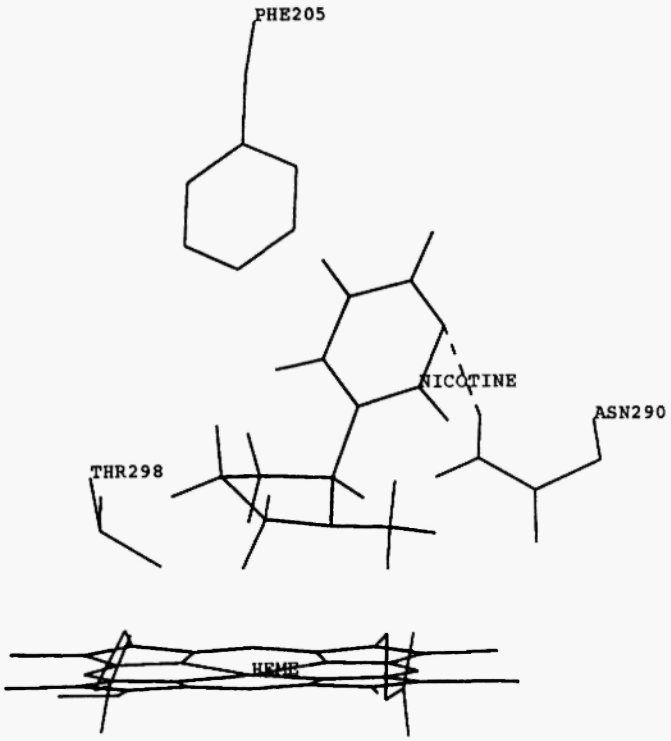

Fig. 3: A view of nicotine orientated for 5′-oxidation within the putative active site of CYP2A6. Hydrogen bonds are shown as dashed lines and amino acid residues are labelled according to the alignment with CYP2C5 /18/. In particular, π-π stacking occurs between the pyridine ring of nicotine and the side-chain of phenlalanine-205, whereas hydrogen bonding is between the pyridyl nitrogen atom and the side-chain of asparagine-290.

oxidation. It is possible, however, that rotation of the pyrrolidine ring of the substrate can give rise to metabolism at alternative sites, such as the *N*-methyl group or 2′-position, although it is likely that this is energetically less favourable than 5′-oxidation, and the electronic structural calculations presented here tend to support this view. The emerging picture from active site modelling and MO calculations is that the substrate binds to CYP2A6 via hydrogen bonding and π-π stacking (as shown in Fig. 3) which indicates 5′-oxidation of nicotine. However, rotation of the substrate's pyrrolidine ring can give rise to the other known metabolites although, on electronic grounds, the pathway yielding cotinine is the most favourable. In this respect, it is assumed that the aminium radical does not dissociate from the active site and that the steps leading to formation of each metabolite are sequential, as inferred from Figure 1.

CONCLUSIONS

The data presented indicate that the Q(HOMO) values correlate well with the overall observed metabolic picture as determined by a review of the literature. In addition, molecular modelling of the possible interaction between nicotine and CYP2A6 shows that there are favourable binding interactions (hydrogen bonding and π-π stacking) which predominantly orientate the substrate for metabolism at the 5′-position. Further work is needed to establish the exact enzymology of the cytochrome P450 isozymes involved in each pathway of initial oxidative nicotine metabolism by human liver and liver from experimental animals. This may allow determination of the factors influencing nicotine metabolism including observed differences in metabolism between various species and indicate those subjects at risk from toxicity due to nitrosamine formation /11,32/.

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