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N-Methylation of nicotine enantiomers by human liver cytosol

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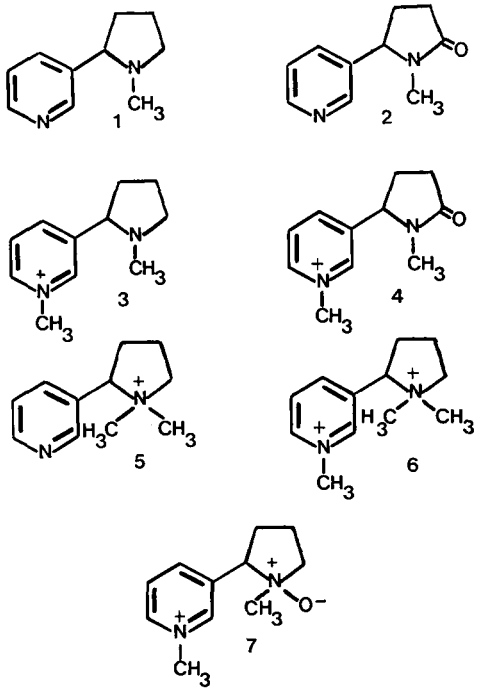
Abstract—Incubation of human liver cytosol with either *R*-(+)-[^{3}H-N'CH$_3$]nicotine or *S*-(−)-[^{3}H-N'CH$_3$]nicotine results in the formation of the corresponding *N*-methyl quaternary ammonium metabolite. A substrate stereoselectivity was observed in that the turnover number for the methylation of the *S*-(−)-isomer was 0·25 pmol mg^{-1} protein h^{-1}, whereas that for the *R*-(+)-isomer was 2·11. The latter substrate exhibited an apparent K_m value of 20·1 μM. Nicotine *N*-methylation appears to be species-dependent, since rat liver homogenates contained no 'nicotine *N*-methyltransferase' activity, whereas with guinea-pig liver homogenates, a substrate specificity for only *R*-(+)-nicotine was observed.

A lesser known route of metabolism of nicotine (1) is the methylation pathway, which was first noted by McKennis et al (1963), and which has been investigated more recently (Cundy & Crooks 1983; Cundy et al 1984, 1985a, b; Sato & Crooks 1985; Pool et al 1986). The earlier work by McKennis et al (1963) showed that in the dog, *N*-methylnicotinium ion (3) is a major urinary metabolite of the natural *S*-(−)-nicotine isomer. The unnatural *R*-(+)-isomer, which is not a constituent of tobacco leaf, but which has been detected in cigarette smoke condensate (Klus & Khun 1977), was not examined in that study. It has also been established that *N*-methylcotininium ion (4) is a major urinary metabolite of cotinine (2) (of unspecified stereochemistry) in man (McKennis et al 1963). Studies in the guinea-pig have shown a remarkable stereospecificity for the in-vivo and in-vitro *N*-methylation of *R*-(+)-nicotine by a cytosolic, *S*-adenosylmethionine-dependent methyltransferase which has high activity in liver, lung, spleen, and brain (Cundy & Crooks 1985; Cundy et al 1985b).

We have now undertaken a preliminary study to determine 'nicotine *N*-methyltransferase' activity in human liver cytosol. The results of this study are now presented.



Materials and methods

Materials. *S*-(−)-[*N'*-methyl-^{3}H]Nicotine (sp. act. 68·6 Ci mmol^{-1}) and *R*-(+)-[*N'*-methyl-^{3}H]nicotine (sp. act. 76·5 Ci mmol^{-1}) were purchased from New England Nuclear (Boston, MA, USA). The radiochemical purity of these tritiated nicotines was determined before experimentation, by cation exchange high performance liquid chromatography (Cundy & Crooks 1984). Each sample assayed at greater than 98% radiochemical purity and greater than 99% optical purity. All chromatographic standards were obtained as described by Pool et al (1986).

A human liver sample was obtained from an adult male who died as a result of congestive heart failure. The sample was removed shortly after death, and exhibited normal liver function, as determined by the following liver function tests: bilirubin content, serum glutamate oxaloacetate transaminase activity (SGOT), serum glutamate pyruvate transaminase activity (SGPT), and lactate dehydrogenase activity (LDH). Guinea-pig liver tissue was obtained from male Dunkin-Hartely guinea-pigs (500 ± 51 g) supplied by Hilltop Lab Animals, Scottdale, PA. Rat liver tissue was obtained from Sprague-Dawley rats (250 ± 30 g) supplied by Charles River, Boston, MA.

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Incubation conditions. Dialysed liver homogenates were prepared as described previously (Cundy et al 1985a). All incubations were performed in 6 × 50 mm disposable glass tubes sealed with rubber septa. Incubation mixtures contained 62·5 μL liver cytosol (0·49 mg protein), 56·25 μL of an aqueous solution containing 0·25 μCi of either *R*-(+)-[*N'*-methyl-^{3}H]-nicotine (sp. act. 76·5 Ci mmol^{-1}) or *S*-(−)-[*N'*-methyl-^{3}H]-nicotine (sp. act. 68·6 Ci mmol^{-1}), *S*-adenosyl-L-methionine (SAM) (6·25 μL of a 1·8 mM solution; final concentration 90 μM), and sufficient buffer to give a total incubation volume of 125 μL. Liver cytosol, SAM and buffer were preincubated for 10 min at 38 °C, and the reaction initiated by addition of substrate. Incubations were carried out in duplicate for increasing increments of time over a 2 h incubation period. Reactions were quenched by addition of 125 μL of 0·25 M borate buffer adjusted to pH 10·0 with aqueous NaOH solution. Control incubations were also run with heat-inactivated cytosol (2 min at 100 °C). For the determination of the K_m for *R*-(+)-nicotine, concentrations of *R*-(+)-nicotine ranging from 40 to 0·05 μM were used, with the SAM concentration fixed at 110 μM. Incubations were carried out at 38 °C for 30 min. The methyltransferase activity of the human cytosolic preparation was determined to be viable, using [G-^{3}H]histamine as a substrate for histamine *N*-methyltransferase activity, utilizing a recently described analytical procedure (Godin & Crooks 1986). All quenched incubations were frozen at −20 °C before analysis.

Analysis of incubates. Thawed incubates were centrifuged at 10 000*g*, and the supernatant analysed directly by high performance liquid radiochromatography using procedures previously developed in our laboratory (Pool et al 1986). These methods allowed for the analysis of five potential *N*-methylated metabolites of nicotine [i.e., *N*-methylnicotinium ion (NMN, 3), *N'*-methylnicotinium ion (N'MN, 5), *N,N'*-dimethylnicotinium ion (DMN, 6), *N*-methylcotininium ion (NMC, 4) and *N*-methyl-*N'*-oxonicotinium ion (NMNO, 7)]. In each analysis, the extent of nicotine methylation in cytosol incubates was determined as a percentage of the recovered radioactivity. The values were then adjusted, based upon mg of protein mL^{-1}, to give a value of pmoles of product formed per mg of protein per hour.

Results and discussion

The results obtained from human liver cytosol incubations showed clearly that both nicotine enantiomers were *N*-methylated exclusively to the corresponding *N*-methylnicotinium ion (3) by a 'nicotine-*N*-methyltransferase' enzyme. No other *N*-methylated products were observed. Interestingly, a marked stereoselectivity was demonstrated; the *R*-(+)-isomer was a much better substrate for the liver methyltransferase system than its optical antipode. *R*-(+)-Nicotine exhibited an apparent K_m of 20·1 μM against human liver 'nicotine *N*-methyltransferase'.

Turnover values for each substrate at 30 min were 2·11 and 0.25 pmol product (mg protein)$^{-1}$ h^{-1} for the *R*-(+)-isomer and the *S*-(−)-isomer, respectively. The *N*-methylation of *S*-(−)-nicotine was linear throughout the whole of the time course examined, whereas *R*-(+)-nicotine *N*-methylation was linear only up to 60 min. We attribute this to the build-up of significant amounts of *S*-adenosylhomocysteine formed as a product in the latter enzymic reaction. *S*-Adenosylhomocysteine has been shown to be a potent feed-back inhibitor of *S*-adenosylmethionine-dependent methyltransferase reactions (Coward & Crooks 1979) and will inhibit the *N*-methylation of *R*-(+)-nicotine in guinea-pig lung cytosolic preparations (Cundy et al 1985c).

Studies were also carried out to compare the nicotine *N*-methyltransferase activities in liver cytosol from man, guinea-pig and rat. These latter two animals species are widely used as experimental models in cigarette smoking studies. The results clearly indicated that the *N*-methylation of nicotine enantiomers by liver cytosol is species-dependent. Rat liver cytosol did not *N*-methylate either of the nicotine enantiomers, whereas guinea-pig liver cytosol exhibited an absolute stereospecificity for the *R*-(+)-isomer (turnover value = 1·61 pmol product (mg protein)$^{-1}$ h^{-1}); however, the methyltransferase activity was somewhat less than that observed in human liver cytosol.

These in-vitro results are consistent with in-vivo data (Cundy et al 1985b; Nwosu 1987), which demonstrate that in the rat, no urinary *N*-methylated products of either *S*-(−)- or *R*-(+)-nicotine are observed, whereas in guinea-pig, only *N*-methylated urinary metabolites of *R*-(+)-nicotine are observed. The above data may be of relevance in choosing a suitable animal model for smoking studies. It would appear from our results, that the rat, a widely used animal smoking model, is not comparable with man in the way that it biotransforms nicotine, and although the guinea-pig is capable of *N*-methylating *R*-(+)-nicotine, unlike man, it does not *N*-methylate *S*-(−)-nicotine, the major alkaloidal component of tobacco smoke. In fact, previous studies have shown that *S*-(−)-nicotine is a potent inhibitor of *R*-(+)-nicotine *N*-methylation in guinea-pig tissue cytosols (Cundy & Crooks 1985; Cundy et al 1985c). Thus, it would be of interest, in this regard, to undertake a more extensive species-screening study to identify an animal model more comparable with man in the way that it biotransforms the nicotine molecule.

Conclusions. These studies have demonstrated for the first time that human liver cytosol contains a methyltransferase system capable of *N*-methylating both enantiomers of nicotine to their corresponding *N*-methylnicotinium ions. A marked stereoselectivity was observed, in that the *R*-(+)-isomer was a much better substrate for the methyltransferase enzyme than the *S*-(−)-isomer. *N*-Methylnicotinium ion is a pharmacologically active quaternary ammonium derivative of nicotine (Euler & Persson 1970; Hedqvist 1970; Bhattacharya 1968) and may be of some significance in the toxicological effects that result from nicotine exposure. In this respect, the above data will be of obvious value in studies dealing with the metabolic fate and toxicology of nicotine in man.

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