Vol. 128, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1985 Pages 312-316

REMARKABLE SUBSTRATE-INHIBITOR PROPERTIES OF NICOTINE ENANTIOMERS TOWARDS A GUINEA PIG LUNG AROMATIC AZAHETEROCYCLE N-METHYLTRANSFERASE

Kenneth C. Cundy, Peter A. Crooks* and C. Steven Godin

Division of Medicinal Chemistry and Pharmacognosy
College of Pharmacy, University of Kentucky,
Lexington, Kentucky 40536-0053

Received February 25, 1985

The kinetics of nicotine methylation by guinea pig lung homogenates has been investigated. An interesting stereospecificity has been observed for nicotine enantiomers. R-(+)-Nicotine is a substrate K_m=1.42 x 10^{-5}M for an SAM-dependent guinea pig lung aromatic azaheterocycle N-methyltransferase, whereas S-(-)-nicotine acts as a competitive inhibitor (K_i=6.25 x 10^{-5}M) of the N-methylation of its antipode.

The *in vivo* N-methylation of nicotine to N-methylnicotinium ion was first demonstrated by McKennis (1), in the dog. In vitro studies (2) have shown that this biotransformation is catalyzed by an S-adenosylmethionine (SAM)-dependent aromatic azaheterocycle N-methyltransferase which is widely distributed in tissues. Recent studies from this laboratory (3,4) have demonstrated that in vivo methylation of nicotine in the guinea pig is stereospecific for the R-(+)-enantiomer. This communication reports on the substrate properties of S-(-)- and R-(+)-nicotine enantiomers against guinea pig lung aromatic azaheterocycle N-methyltransferase.

MATERIALS AND METHODS

[^{3}H-NCH$_3$]-S-(-)-Nicotine (specific activity 68.6 Ci/mmole) and [^{3}H-NCH$_3$]-R-(+)-nicotine (specific activity 76.5 Ci/mmol) were obtained from New England Nuclear, Boston, MA). The optical purity of these radionuclides was demonstrated to be better than 99% (5). R-(+)-Nicotine was prepared from S-(-)-nicotine by the method of Bowman et al. (6); S-(-)-nicotine was obtained from Aldrich (Milwaukee, WI). Both nicotine samples were vacuum distilled prior to experimentation.

Dialyzed guinea pig lung homogenates were prepared as previously described (2). Protein content was determined by the method of Lowry et al. (7).

*To whom all correspondence should be addressed.

0006-291X/85 $1.50

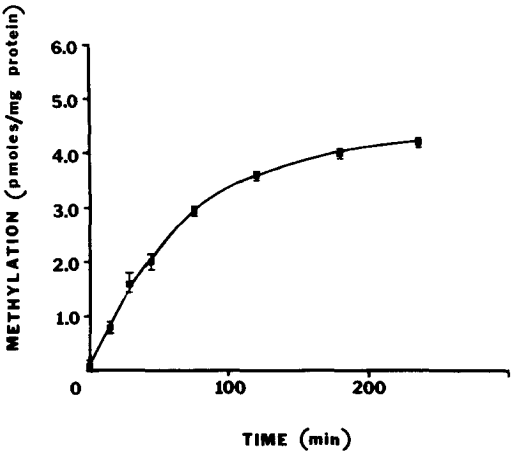

Fig. 1 Time course of in vitro N-methylation of R-(+)-nicotine by dialyzed guinea pig lung homogenate (See Materials and Methods for experimental details). Each point is the mean of three determinations ± S.D.

Kinetic experiments. All experiments were carried out in triplicate. Nicotine methylation was demonstrated to be linear over the time course chosen for the assay (see Fig. 1), and was linear with respect to protein concentration over the range 4.9-19.6 mg/ml (see Fig. 2). [^{3}H-NCH$_3$]-R-(+)-Nicotine (3 µCi) was diluted with unlabelled R-(+)-nicotine to afford concentrations ranging from 1.25 µM to 1.24 mM. Incubation mixtures were prepared containing the following in a total volume of 250 µl; homogenate (125 µl 10 mg/ml protein), SAM (125 nmol), substrate solution (60 µl) and 0.1M phosphate buffer, pH 7.4 (to 250 µl). Appropriate controls were prepared for each substrate concentration, in which homogenate was boiled for 2 minutes prior to use. All samples were pre-incubated at

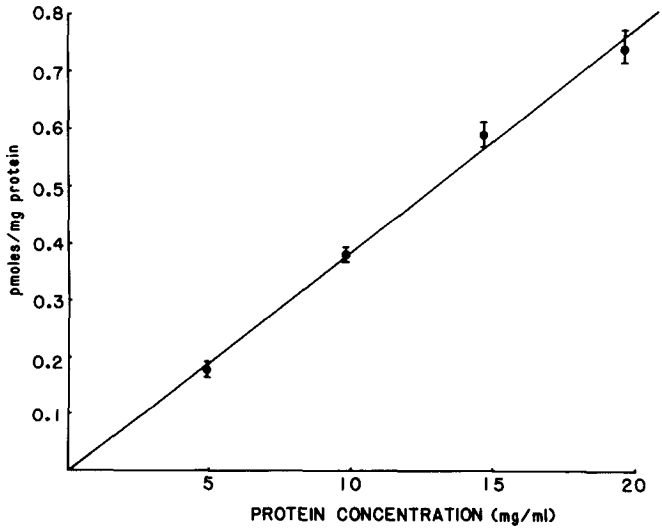

Fig. 2 Linearity of R-(+)-nicotine N-methylation after fifteen minutes incubation at 37°. The abscissa indicates the protein concentration in individual incubates; the ordinate indicates the percent methylation observed expressed as pmoles of product formed per mg. protein. Each point is the mean of three observations ± S.D.

37°C for 10 minutes before additon of substrate solutions. Incubation was then continued at 37°C for 15 minutes. After this time, the samples were immediately frozen at -20°C prior to analysis.

Individual incubates were analysed directly by high performance cation-exchange liquid radiochromatography, as described previously (8), and the initial velocities of nicotine N-methylation (nmoles/mg protein/hr) determined. These values were then used in the calculation of apparent K_m and V_{max} for R-(+)-nicotine. An identical experiment was performed using [^{3}H-NCH$_3$]-S-(-)-nicotine and unlabelled S-(-)-nicotine in place of the corresponding R-(+)-enantiomeric material

The effect of S-(-)-nicotine on the methylation of the R-(+)-enantiomer was examined by the following procedure. [^{3}H-NCH$_3$]-R-(+)-Nicotine (3 μCi) was diluted with unlabelled R-(+)-nicotine to give concentrations ranging from 1.25 μM to 1.25 mM. Unlabelled S-(-)-nicotine was added to each of the substrate solutions to give 30 μM S-(-)-nicotine. Incubation mixtures were prepared which contained the following in a total volume of 250 μl; homogenate (125 μl), SAM (125 nmol), substrate-inhibitor solution (60 μl) and 0.1 M phosphate buffer, pH 7.4 (to 250 μl). Appropriate controls were prepared in which homogenate was boiled for 2 minutes prior to use.

An identical experiment was performed in which the S-(-)-nicotine concentration was 60 μM. All samples were pre-incubated for 10 minutes at 37°C prior to addition of substrate/inhibitor solutions. Incubation was continued for 15 minutes at 37°C, and incubates then treated as described above.

RESULTS

Fig. 3 illustrates the Lineweaver-Burke plot obtained from the methylation of R-(+)-nicotine to N-methylnicotinium ion by guinea pig lung aromatic azaheterocycle N-methyltransferase, using SAM as co-factor. R-(+)-Nicotine exhibited an apparent K_m of 14.2 μM, with V_{max} = 1.85 nmoles/mg/hr. Under similar conditions, S-(-)-nicotine was not a substrate for the above N-methyltransferase. However, the S-(-)-enantiomer did inhibit the N-methylation of its optical antipode in a competitive manner, exhibiting a K_i of 62.5 μM (see Fig. 4).

Other characteristics of the N-methyltransferase enzyme are given in Table 1. S-Adenosyl-L-homocysteine (SAH), a reaction product, was a potent competitive inhibitor of R-(+)-nicotine methylation. The endogenous pyridine analogue, nicotinamide was not a substrate for the above enzyme, but was a weak competitive inhibitor of R-(+)-nicotine methylation.

DISCUSSION

The results from this study demonstrate a remarkable substrate specificity in the N-methylation of nicotine by a guinea pig lung N-methyltransferase, and also provides an interesting example of diametrically opposing activities of enantiomers towards a specific enzymic system. The product inhibition exhibited

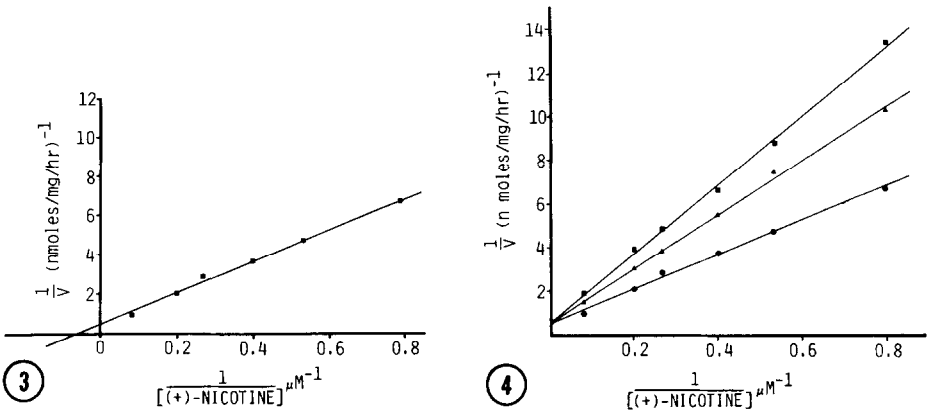

Fig. 3 Lineweaver-Burk plot for the determination of K_m for R-(+)-nicotine methylation using guinea pig lung homogenate (see (Materials and Methods for details). Each data point represents the mean of 3 determinations.

Fig. 4 Lineweaver-Burk plot showing inhibition of R-(+)-nicotine methylation by S-(-)-nicotine (see Materials and Methods for details). Each data point represents the mean of 3 determinations. (●), 0μM; (▲)30 M and (■)60μM S-(-)-nicotine.

by SAH, and the apparant K_m value for SAM, both confirm that the enzyme involved in the N-methylation of R-(+)-nicotine is an S-adenosylmethionine-dependent N-methyltransferase. The inability of nicotinamide to act as a substrate rules out the identity of the enzyme being nicotinamide-N-methyltransferase. Thus, an endogenous substrate for the above methyltransferase remains to be established.

It is of interest to note here that although the S-(-)-enantiomer is the natural isomer of nicotine, and consequently the form present in tobacco products, it has been observed that significant racemization of S-(-)-nicotine can occur in the pyrrolysis event during the smoking of a cigarette. Thus, the above

TABLE 1

Substrate and Inhibitor Properties of Guinea Pig Lung Aromatic Azaheterocycle N-Methyltransferase

Compound	Substrate (K_m)	Inhibitor (K_i)
R-(+)-nicotine	14.2 μM	-
S-(-)-nicotine	-	62.5 μM
S-Adenosyl-L-methionine	13.2 μM	-
S-Adenosyl-L-homocysteine	-	32.2 μM
Nicotinamide	-	417.8 μM

findings may be of relevence in providing further information on the toxicology of nicotine in tobacco products.

ACKNOWLEDGEMENT

This investigation was carried out with financial support from the Tobacco and Health Research Institute, Lexington, KY (Grant No. 4C018).

REFERENCES

1. McKennis, Jr., H., Turnbull, L.B., and Bowman, E.R. (1963) J. Biol. Chem. 238, 719-723.

2. Cundy, K.C., Godin, C.S. and Crooks, P.A. (1985) Biochem. Pharmacol., 34, 281-284.

3. Cundy, K.C., Godin, C.S. and Crooks, P.A. (1984) Drug Metab. Disposit. 12(6), 2103-2107.

4. Cundy, K.C., Sato, M. and Crooks, P.A. (1985) Drug Metab. Disposit., in press.

5. Cundy, K.C., and Crooks, P.A. (1983) J. Chromatogr. 281, 17-33.

6. Bowman, E.R., McKennis, Jr., H. and Martin, B. (1982) Synthetic Commun. 12, 871-879.

7. Lowry, O.H., Rosebrough, A.L.F. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

8. Cundy, K.C. and Crooks, P.A. (1984) J. Chromatogr. Biomed. Applic., 306, 291-301.

9. Klus, H. and Kuhn, H. (1977) Fachliche Mitt. Oesterr. Tabakregie 17, 331-336.