NICOTINE: METABOLIC PATHWAYS AND METABOLIC MYSTERIES
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Introduction
Nicotine is a simple bicyclic alkaloid present in a number of plants including fruits and vegetables and
is the major alkaloid present in *Nicotiana tobacum*. During the smoking of tobacco nicotine is
transferred to smoke and is absorbed by the body via the lungs. Exhaled smoke and side-stream smoke
also contain nicotine and it is sometimes used as an insecticide, so that it can be assumed that virtually
everyone is exposed to this ubiquitous compound. Nicotine contains two nitrogen centres with pKa's
of about 8.2 and 3.4, and is extremely soluble in water, organic solvents and lipids. Nicotine can exist
as two stereoisomers with the S-(−)-nicotine being the natural product although some racemisation to
the R-(+) isomer occurs during the smoking process and humans are therefore exposed to both
isomers although the former always predominates. Nicotine can undergo both Phase 1 (oxidative) and
Phase 2 (conjugation) reactions in mammalian systems and certain metabolites may be involved in
oxidation/reduction cycling.

Phase I Oxidation at Aliphatic Carbon
Oxidation of nicotine occurs at the 2' & 5' position and the methylene function. The reactions involve
intermediate isomeric iminium ions, formed by CYP450 isozymes, which are further oxidised by
aldehyde oxidase. It appears that the extent of initial oxidation of nicotine at a specific site is related
to the electronic characteristics of the individual carbon atoms. These oxidations yield 4-(3-pyridyl)-4-oxo-N-methylbutylamine as a minor product, cotinine as the major product and nornicotine.

Phase I Oxidation at Aliphatic Nitrogen
Nicotine is oxidised by a microsomal flavin containing enzyme (FMO3) to both cis and trans-
icotine-1'-N-oxides: the ratio of the two stereoisomers formed depending upon enzyme source and
incubation conditions but with the trans isomer always predominating. The isomeric N-oxides can be
reduced back to nicotine by micro-organisms in the gastrointestinal tract and by cellular fractions
from various organs.

Further Oxidation of Primary Metabolites
Nornicotine is converted to norcotinine both in vivo and in vitro as the major metabolite and the open
chain compound 4-(3-pyridyl)-4-aminobutyric acid, has been isolated from the urine of dogs receiving
nornicotine. The pyrrolidine nitrogen of nornicotine is subject to sequential oxidation to give
norcotinine -$\Delta^{1h}$ (3)$\alpha$-nitrone via 1'-N-hydroxynornicotine utilising the microsomal flavin containing
amine oxidase.

Cotinine is oxidised to both cis and trans-3'-hydroxycotinine with the latter predominating; to 5'-hydroxycotinine which exists in equilibrium with 4-(3-pyridyl)-4-oxo-N-methylbutyramide and to both
cotinine and 5'-hydroxycotinine-N-oxide. These oxidations are mediated via CYP isozymes. During in
vitro studies on the metabolism of cotinine using hepatic preparations from a variety of species a new
metabolite characterised as N-hydroxymethylnorcotinine was detected. This compound was
metabolically stable and did not produce norcotinine under any of the incubation conditions studied.
This was surprising as norcotinine has been reported as a metabolite of both nicotine and cotinine in vivo and poses a question as to the route of its formation which will be discussed.

**Further Metabolism of Pyridylbutyric Acid Derivatives**

4-(3-Pyridyl)-4-oxo-N-methylbutyramide is hydrolysed to 4-(3-pyridyl)-4-oxo-butyric acid, which is the same compound as that derived from the hydrolysis of the norcotinine metabolite, 4-(3-pyridyl)-4-oxo-butyramide. It is possibly also formed by the deamination of the nornicotine metabolite 4-(3-pyridyl)-4-aminobutyric acid. Thus this compound would be a common intermediate on three metabolic pathways, all derived from nicotine.

4-(3-Pyridyl)-4-oxo-butyric acid is reversibly reduced to the corresponding alcohol, 4-(3-pyridyl)-4-hydroxybutyric acid which cyclises to 5-(3-pyridyl)-tetrahydrofuran-2-one. This latter compound seems to be off the major pathway of nicotine metabolism but can be converted back to the two precursor pyridylbutyric acids, which are thought to enter the fatty acid metabolic pathway and ultimately be converted to pyridylacetic acid as the end product of nicotine metabolism. This pathway raises many unanswered questions which will be discussed.

**Other Pathways of Oxidative Nicotine Metabolism**

During studies on the excretion of radiolabelled nicotine in man a compound was detected with a rather long half life; the authors claimed that this metabolite was norcotinine-\(\Delta^4\)-(5)-enamine however its characteristics did not seem to fit in with those of related compounds; this was investigated and our observations will be discussed.

**Phase 2 Conjugation Reactions: Methylolation**

It has long been known that nicotine and cotinine can undergo methylation reactions to form highly polar water soluble quaternary compounds by methylation of the pyridine nitrogen. In early work these compounds were isolated from the urine of dogs receiving either S-nicotine or S-cotinine or from a non-smoking man who received a large dose of cotinine. This is in contrast to more recent work where it was found that in the guinea pig only the R-nicotine isomer was methylated. Methylation of nornicotine, as well as the isomeric 1'-N-oxides was also observed after the administration of R-nicotine to this species: no methylated derivatives of S-nicotine were detected. In other species there have been sporadic and sometimes conflicting reports of the methylation of nicotine and its metabolites which will be discussed.

**Phase 2 Conjugation Reactions: Glucuronidation**

A more recent observation has been the recognition of the glucuronic acid conjugates of nicotine, cotinine and 3-hydroxycotinine. In the former compounds the glucuronic acid moiety is inked via the pyridine nitrogen to form a quaternary compound. 3'-Hydroxycotinine is conjugated via the hydroxyl group. Whilst it may have been expected that the quaternary N-glucuronides would be formed by a common enzyme acting on both substrates, this has not proved to be the case and recent results will be discussed.

**Conclusions**

This review of the metabolism of nicotine has shown that it is extremely complex and that many areas remain “mysteries” with regard to either the intermediates or the enzymology involved. These will be discussed and suggestions made as to their solution.