METABOLIC INTERCONVERSIONS BETWEEN IMIPRAMINE, ITS N-OXIDE, AND ITS DESMETHYL DERIVATIVE IN RAT TISSUES IN VITRO

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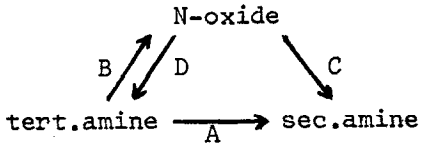
Summary: Imipramine-N-oxide (IPNO) is reduced to imipramine (IP) and to a minor degree demethylated to desmethylimipramine (DMI) by tissue homogenates or blood in the absence of added cofactors. IP remains unchanged in this system. Both processes depend on extramicrosomal enzymic and/or catalytic systems which can be simulated by ferrous ion model systems. Liver in vitro systems are described which allow the separate study of 1) demethylation and N-oxidation of IP, 2) reduction and demethylation of IPNO, 3) sum of all four metabolic reactions.

Several authors have reported contrasting data in respect to the question whether N-oxides are by-products (McMahon et al. 1964, Kuntzman et al. 1967) or intermediary products (Fish et al. 1956, Ziegler et al. 1964) in the N-demethylation of tertiary amine drugs. One or more of the following reactions (named A,B, C,D in this paper) have been described by many authors in the study of several substrates:



Previous studies with the tertiary amine, imipramine (IP) (N-3(-dimethylaminopropyl)-iminodibenzyl hydrochloride), imipramine-N-oxide (IPNO), and desmethylimipramine (DMI) by Bickel et al. (1966a,b, 1967, 1968) have led to the detection of the following reactions:

1) Liver microsomes + cofactors: A, B. (D negligible)
2) Extrahepatic microsomes + cofactors: (A,B,D negligible)
3) Tissue homogenates without cofactors: D. (No A and B)
4) In vivo (rat, guinea pig): A, B, D

This report contains the finding of reaction C as well as information on the optimum conditions for C and D in contrast to the liver-microsomal reactions A and B.

Materials and methods: Male Wistar rats (200-300 g) were used. The hydrochlorides of IP, DMI, and IPNO had a purity of >99.5% and did not contain detectable traces of other IP metabolites. Most determinations of IP metabolites were carried out with the thin layer chromatographic method described by Bickel and Weder (1968) and have been confirmed by the use of a solvent extraction and spectrofluorometric method to be published later. Unless otherwise stated the buffers and cofactors were used as indicated by Bickel and Baggiolini (1966a). The following heavy metal salts were used: Fe^{++}- and Fe^{+++}-ammonium sulfates, $CuSO_4$, $CoCl_2$, $MnCl_2$ and $ZnSO_4$.

Results and discussion: As shown in table 1 IPNO is both reduced to IP (reaction D) and demethylated to DMI (reaction C) by homogenates of various tissues in the absence of added cofactors. The presence of the biological material is essential. IP remains unchanged in these systems which indicates that A and B-C are separate reactions. C and D also occur in homogenates of organs from which blood was removed by perfusion with saline.

Table 1. Reduction and demethylation of imipramine-N-oxide by tissue homogenates

Tissue		% of 2 μmoles IP	% of 2 μmoles DMI
Kidney	(2)	57	6
Lung	(2)	27	2
Blood	(4)	22	1
Heart	(2)	22	2
Small intestine	(3)	20	4
Liver	(9)	19	4
Spleen	(3)	17	3
Brain	(2)	7	2
Skeletal muscle	(2)	3	1
Abdominal fat	(6)	2	0
Subcutaneous fat	(2)	1	0
Skin	(2)	0	0

2 μmoles IPNO in 5 ml homogenate (1 g tissue). N_2, 37°C, 1 hour. In brackets number of experiments.

Table 2. Reduction and demethylation of imipramine-N-oxide under various conditions

Tissue	Atmosphere	Pretreatment		% of 2 μmoles IP	% of 2 μmoles DMI
Liver	air	-	(11)	5	1
Liver	air	100°C, 15 min.	(7)	3	0
Liver	N_2	-	(12)	10	1
Liver	N_2	100°C, 15 min.	(6)	10	1
Blood	air	-	(8)	4	0
Blood	air	100°C, 15 min.	(6)	5	0.5
Blood	N_2	-	(12)	6	0
Blood	N_2	100°C, 15 min.	(6)	5	0.5

2 μmoles IPNO in 5 ml homogenate or blood (0.25 g tissue). 37°C, 1 hour. In brackets number of experiments.

The conditions for the reactions C and D have been investigated using liver homogenate and blood respectively. Table 2 shows that these reactions do not require O_2 and are only partially heat labile. In blood the reaction equilibrium is reached

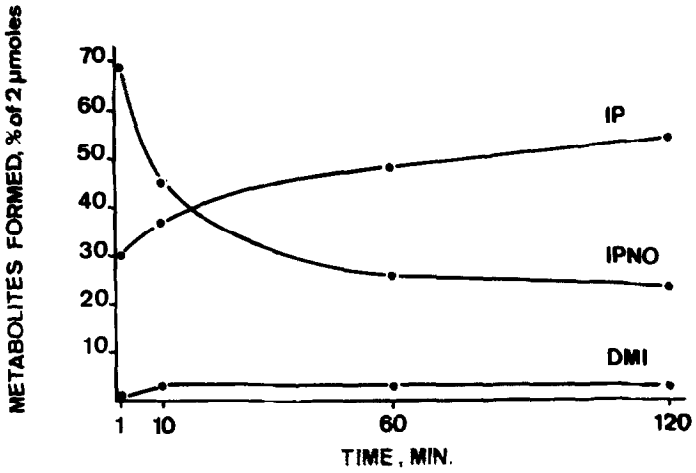


Figure 1. Time course of reduction and demethylation of imipramine-N-oxide by liver homogenate.
Livers perfused free of blood. 2 µmoles IPNO in 5 ml homogenate (1 g liver), pH 7.4, N_2, 37°C.

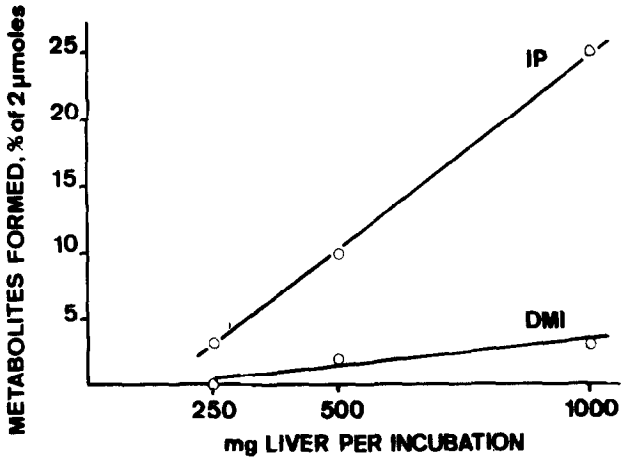


Figure 2. Reduction and demethylation of imipramine-N-oxide by various amounts of liver homogenate.
Livers from bled animals. 2 µmoles IPNO in 5 ml homogenate, pH 7.4, aerobic, 37°C, 1 hour.

within minutes or less; in (blood-free) liver (Fig.1) the reactions proceed after a similar initial jump. The amounts of IP and DMI formed are linearly dependent on the amount of blood or liver homogenate (Fig.2). The temperature dependence is more

Table 3. Reduction and demethylation of imipramine-N-oxide in heavy metal model systems

Ion		Chelating agent		% of 2 µmoles IP	% of 2 µmoles DMI
Fe^{++}	10^{-2} M	-	(10)	15	3
	10^{-3} "	-	(2)	2	0
	10^{-2} "	EDTA 10^{-2}, 10^{-3} M	(8)	65	30
	10^{-2} "	EDTA 10^{-4} M	(2)	8	2
	10^{-3} "	EDTA 10^{-2} M	(2)	20	4
	10^{-3} "	EDTA 10^{-3} M	(2)	8	1
	10^{-2} "	Citrate, oxalate 10^{-2}	(6)	50,25	40,10
Fe^{+++}	10^{-2} "	-	(3)	1	0
	10^{-2} "	EDTA 10^{-2} M	(2)	10	2
Cu,Co,Mn,Zn	10^{-2} "	± EDTA 10^{-2} M	(15)	0	0

$4 \cdot 10^{-4}$ M IPNO (2 µmoles). 37°C, 30 minutes, aerobic.
In brackets number of experiments.

pronounced with liver than with blood. No differences were observed using six different buffers, and little differences using citrate-borate-phosphate buffer in the pH range 5 - 10. Both reactions C and D are not inhibited by CO, SKF 525-A (10^{-4}M), or CN^- (10^{-3}M).

In liver homogenates most C- and D activity is found in the 9000·g-pellet or after dialysis in the undialyzable residue. In blood the total activity can be traced to the hemoglobin fraction. Corresponding amounts of pure HbO_2 behave exactly like whole blood. Reduced cytochrome c and other hemoproteins also show C- and D activity. This obvious heme catalysis can be simulated by using model systems containing ferrous ions in excess molar ratios (Table 3) instead of biological material. The results thus favor the hypothesis that in liver the C- and D reactions depend on both heme catalysis and enzymatic processes.

Table 4. Metabolism of imipramine-N-oxide, imipramine and desmethylimipramine in various liver systems in vitro

g wet weight	Fraction	Cofactors 1)	Substrate		% of 2 µmoles IP	IPNO	DMI	2-OH-2)
1.00	Homogenate	-	IPNO	(4)	25	72	3	0
1.00	"	-	IP	(4)	100	0	0	0
1.00	"	-	DMI	(2)	0	0	100	0
0.25	9000·g-SN 3)	+	IPNO	(3)	0	100	0	0
0.25	"	+	IP	(6)	41	15	33	11
0.25	"	+	DMI	(2)	0	0	97	3
1.00	Homogenate	+	IPNO	(3)	49	22	25	4
1.00	"	+	IP	(2)	75	0	23	2
0.25	Homogenate	+	IPNO	(2)	23	69	8	0
0.25	"	+	IP	(2)	51	5	37	7

1) NADPH generating system, 2) 2-hydroxy metabolites,
3) SN = supernatant.
2 µmoles substrate, pH 7.4, 5 ml total volume, aerobic, 37°C, 1 hour. In brackets number of experiments.

Table 4 shows the actions of the systems used for the separate study of the reactions A and B, the reactions C and D, and the reactions A,B,C,D. 0.25 g liver wet weight was found to be optimal for reaction B. Both reactions A and B are inhibited by heat pretreatment, N_2, CO, and SKF 525-A. In the above systems the reactions A,B,D and possibly C are also observed with amitriptyline and chlorpromazine and their N-oxides. Studies are in progress aiming at the evaluation of the reaction kinetics in vitro and in vivo.

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