The mechanism of metabolic *N*-oxidation of phentermine and chlorphentermine to their hydroxylamino- and nitroso-compounds

After *in vitro* studies using liver homogenates and also after *in vivo* studies in animals and man with phentermine (I, R = H) and chlorphentermine (I, R = Cl), the hydroxylamines II, R = H and Cl and the nitroso-compounds III, R = H and Cl were isolated (Beckett & Bélanger, 1974a, b). However, in solution and during usual analytical techniques, II is rapidly oxidized to III and, if the solutions are alkaline, then the nitro compound (IV) is also produced (see Fig. 1).

The amounts of the nitroso-compound (III) relative to those of the hydroxylamine present in the *in vitro* studies indicated that chemical change from the metabolically produced hydroxylamine (II) alone could not account for the results. The possibility of metabolic oxidation of the metabolically produced hydroxylamine II to give III was considered; however, the separate incubation of II with liver homogenates did not yield III. On the other hand, fine suspensions of III were rapidly reduced to II by fortified hepatic fractions which indicated that possibly the hydroxylamine II was a metabolic reduction product of the metabolic oxidation of I, namely of the nitroso compound III.

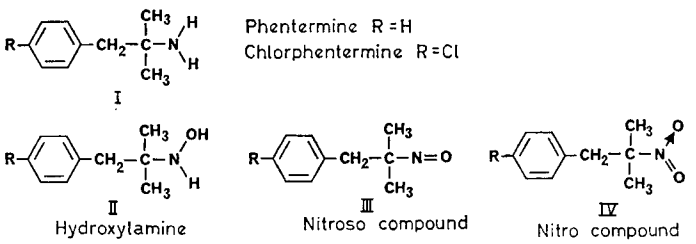

FIG. 1. The structure of phentermine and chlorphentermine and some of their *N*-oxygenated products.

To clarify the situation, we have studied the kinetics of formation of II and III upon incubation of phentermine (I) with hepatic fractions from rabbits for up to 60 min. Microsomes gave curves which indicated that II and III were formed independently; much more nitroso (III) than hydroxylamine (II) was present in the solutions. Likewise results using 10 000 *g* fractions indicated independent formation of II and III but reduction of III to II also occurring; at the later stages of incubation more II than III was present in the solution. Little metabolism occurred using the soluble fraction but both II and III were present.

The independent direct formation of the nitroso compound (III) and the hydroxylamine (II) indicates the final involvement of one oxygen atom in the immediate precursor of II and two in the case of III but it seemed more logical to consider a common intermediate rather than involve two distinct metabolic pathways to these two *N*-oxygenated products.

N-Oxidation and α-*C*-oxidation involve separate metabolic routes (Gillette, Davis & Sasame, 1972; Arrhenius, 1971; Beckett, 1971) in which flavoprotein electron transfer is involved in both (Ziegler & Pettit, 1964, 1966) but in which cytochrome P-450 is involved in the latter but not the former. Proposals about the mechanism of oxygen involvement and electron transfer have been made but definite evidence is lacking.

The present results with phentermine and chlorphentermine throw further light on the mode of involvement of oxygen in *N*-oxidation. The separate formation of *C*-nitroso and hydroxylamino groups from aliphatic primary amines devoid of

H-atoms on the α-*C*-atom is explicable in terms of the reactions outlined in Fig. 2; use is made of the principles outlined by Arrhenius to explain *N*-oxide formation from aromatic tertiary amines.

The proposals are as follows. The amine first forms a complex (Fig. 2, 1) with flavoprotein and molecular oxygen in reaction 1 which is followed by the transfer of one electron from the *N*-lone pair to the flavoprotein to give a complex (II) involving a *N*-free radical cation, a flavoprotein radical and oxygen. Reaction 3 then involves the transfer of one electron from the flavoprotein radical to the oxygen molecule to form an oxygen radical/flavoprotein anion complex associated with the nitrogen radical cation in the complex (III) (see Fig. 2); the rate of dissociation of this complex is considered to control whether it leads to either a nitroso or hydroxylamino compound.

Rapid dissociation (reaction 4) will produce the zwitterion IV and free flavoprotein; proton rearrangement will then occur (reaction 5) to give the hydroperoxide (V) from which water will be eliminated (reaction 6) to yield the nitroso compound VI (see Fig. 2).

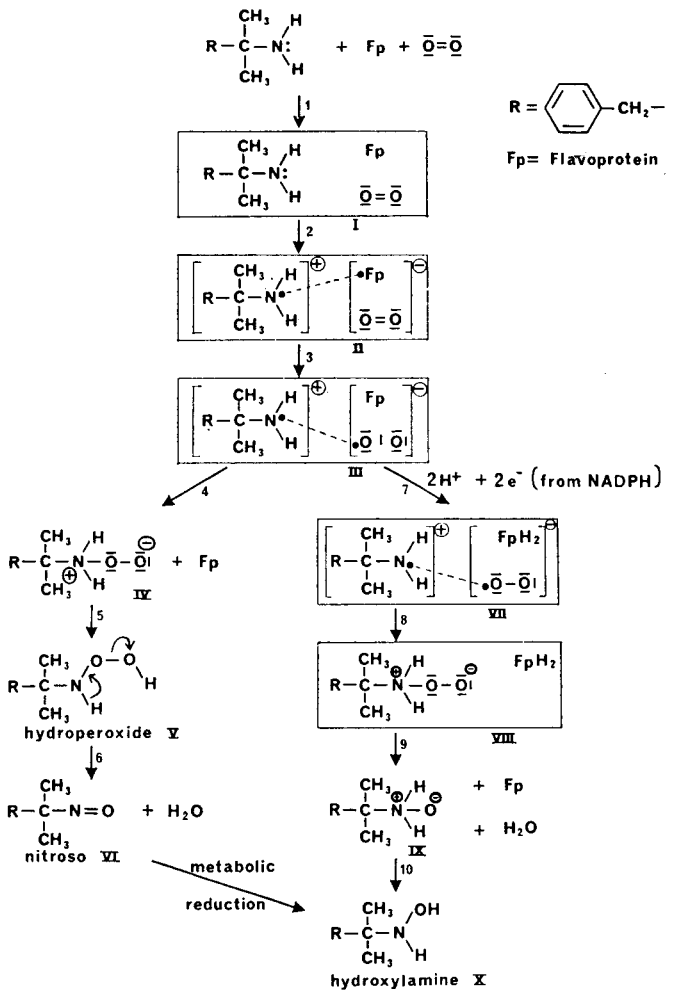

FIG. 2. Scheme for the metabolic *N*-oxidation of the primary amine, phentermine, to a complex which gives both the hydroxamine and the *C*-nitroso compound.

On the other hand, if there is not rapid dissociation of the components of complex III, the flavoprotein in the complex can be reduced by transfer of two electrons from NADPH (reaction 7) to form the radical complex (VII) containing FpH_2. The N and O free radicals will interact to give the zwitterion as a complex VIII with FpH_2; the latter can then reduce the zwitterion and dissociation follows (reaction 9) to yield flavoprotein, one molecule of water and the *N*-oxide (IX) which undergoes proton rearrangement (reaction 10) to yield the hydroxylamine (X).

The rate of dissociation of the complex III (see Fig. 2) therefore controls whether two or one oxygen atoms are present on the *N*-atom in the structures before the final step yielding nitroso (VI) and hydroxylamines (X) respectively.

Increase in steric features about the *N*-atom will tend to favour dissociation of complex III and thus an increase in the proportion of structure with two oxygen atoms (route 4) over those with one oxygen atom (route 7) in the penultimate structure before the final product of metabolic *N*-oxidation of primary and possibly secondary aliphatic amines; current investigations will test these extrapolations from the main theory.

In the case of phentermine (I, R=H) and chlorphentermine (I, R=Cl) (Fig. 1) the nitroso compounds (VI) are also reduced by fortified hepatic fractions to the hydroxylamines (X) (Fig. 2) under conditions of incubation in which the amines (I) are being oxidized to the nitroso compounds (VI) as indicated in Fig. 2.

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