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# SUICIDAL INACTIVATION OF MICROSOMAL CYTOCHROME P-450 BY HYDRAZONES.

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ABSTRACT

Various phenylhydrazones $R_1R_2C$=N-NHPh, are good inhibitors of microsomal 4-nitroanisole demethylase and 7-ethoxycoumarin dealkylase. Upon metabolic oxidation, they lead to destruction of cytochrome P-450 and to formation of a stable σ- phenyl cytochrome P-450-Fe(III)-Ph complex.

## INTRODUCTION.

Hydrazine derivatives have often been used as enzyme inhibitors, but have been found to cause various toxic effects. Phenylhydrazine is oxidized by rat liver microsomes leading to a stable cytochrome P-450-metabolite complex which accounts at least in part for the inhibitory effects of this hydrazine on monooxygenase activities (1). It has been recently shown that, in a more general manner, the oxidation of hydrazines ($RNHNH_2$) by rat liver microsomes leads to the formation of σ-alkyl (or aryl) cytochrome P-450-Fe(III)-R complexes as well as to some destruction of this cytochrome (2). This communication reports that hydrazones $R_1R_2C$=N-NHR are good inhibitors of microsomal cytochrome P-450 and compares their effects with those of the corresponding hydrazines $RNHNH_2$.

## MATERIALS AND METHODS.

All hydrazones were prepared according to usual procedures and were stored under nitrogen. Liquid hydrazones were purified by distillation under reduced pressure and solid phenylhydrazones were purified by recristallisation from deaerated methanol : $R_1R_2C$=NNHPh : $R_1$,$R_2$ = cyclohexyl, mp : 75°C ; $R_1$ = Ph, $R_2$=$CH_3$ mp : 105°C ; $R_1$ = $R_2$ = $CH_3$ bp : 113°C (3.5 mmHg) ; $R_1$=n-hexyl, $R_2$=H, bp : 150°C (2 mmHg). Hepatic microsomes were prepared from male Sprague Dawley rats (about 175-200 g) as described previously (3). Feeding was stopped 12h before death. Phenobarbital was given in the drinking water (0.1 %) for 5 days. Concentrations of protein and cytochrome P-450 were determined according to Lowry and coworkers (4) and Omura and Sato (5), respectively. Spectral changes were measured as difference spectra using an Aminco DW2 UV-visible spectrometer. The activities of 4-nitroanisole O-demethylase were assayed by the method described by Netter (1μM cytochrome P-450, 0.75 mM 4-nitroanisole, 0.2 mM NADPH)(6).

## RESULTS.

Inhibitory effects of hydrazones on microsomal 4-nitroanisole O-demethylase : The effects of four phenylhydrazones $R_1R_2C$=NNHPh, (1 :$R_1$,$R_2$=cyclohexyl ; 2 :$R_1$=n-$C_6H_{11}$, $R_2$=H ; 3 :$R_1$=Ph, $R_2$=Me ; 4 : $R_1$=$R_2$=Me) on the O-dealkylation of paranitroanisole by liver microsomes from phenobarbital-pretreated rats was studied under different conditions.

Without preincubation : The cyclohexanone phenylhydrazone 1 with the NADPH-containing microsomal system, inhibits the 4-nitroanisole demethylation in a non-competitive manner. Moreover the following $K_i$-values (from Dixon plots) were found for the four tested hydrazones, 1 :$10^{-5}$M ; 2 :$10^{-5}$M ; 3 :$2.5X10^{-5}$M ; 4 :$5X10^{-5}$M. The hydrazones deriving from cyclohexanone or 2-hexanone are also potent inhibitors of the 7-ethoxycoumarin dealkylase activity of cytochrome P-450 ($IC_{50}$ 2 and 10 µM).

Preincubation of the NADPH-containing microsomes with the cyclohexanone phenylhydrazone 1 leads to a marked increase of its inhibitory effects, indicating that part of the inhibition is irreversible in nature. For instance, a six-fold molar excess of this hydrazone over cytochrome P-450 is sufficient to inhibit 50 % of 4-nitroanisole demethylase activity after 5 min preincubation.

Interaction of phenylhydrazones with cytochrome P-450 in the presence of NADPH. The phenylhydrazones used, 1, 2, 4 have two effects on cytochrome P-450 upon incubation with rat liver microsomes in the presence of NADPH : they produce a difference spectrum characterized by a Soret peak at 480 nm and cause fast destruction of cytochrome P-450. For instance, 10eq. (relative to cytochrome P-450) of 1 leads to a maximum formation of the 480 nm-absorbing complex within 1 min. (about 5 % of starting cytochrome P-450) and causes a 60 % loss of the ability of starting cytochrome P-450 to form a CO complex.

Since the difference spectra derived from phenylhydrazine (1,2) and phenylhydrazones are almost identical and both characterized by a redshifted Soret peak at 480 nm, it is very likely that the cytochrome P-450 complexes derived from phenylhydrazones are also σ-phenyl-Fe(III) complexes(eq. 1).

$$\begin{matrix} R_1 \\ R_2 \end{matrix}\!\!>\!C{=}N\text{-}NHPh \;+\; Cyt.P\text{-}450\text{-}Fe^{III} \xrightarrow[O_2]{NADPH} Cyt.P\text{-}450\text{-}Fe^{III}\text{—}Ph \;(\lambda_{max}{=}480nm) \qquad (eq.\ 1)$$

However, although phenylhydrazine and phenylhydrazones produce the same 480 nm absorbing complexes, they exhibit different reactivity characteristics : (i) cyclohexanone phenylhydrazone leads to a faster appearance of the σ-phenyl cytochrome P-450 complex than phenylhydrazine ($t_{½}$ respectively of 6 and 60s with NADPH 0.2 mM, P-450 3 µM and hydrazone or hydrazine 30 µM), (ii) under aerobic conditions but in the absence of NADPH, $PhNHNH_2$ leads to a slow formation of the σ-phenyl complex whereas the cyclohexanone phenylhydrazone leads only to some destruction of cytochrome P-450.

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