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Inhibition of drug demethylation by disulfiram *in vivo* and *in vitro*

(*Received* 27 *May* 1969; *accepted* 27 *June* 1969)

RAM is known as an inhibitor of liver aldehyde dehydrogenase[1, 2] and dopamine-ß-hydroxy- Recent clinical observation showed that disulfiram, when given to patients receiving other imultaneously, increased the serum levels of these drugs, e.g. that of diphenylhydantoin.[5] iphenylhydantoin is metabolized chiefly by *p*-hydroxylation to 5-(*p*-hydroxyphenyl)-5-phenyl-)in,[6] it was to be expected that disulfiram increases the serum levels of diphenylhydantoin by ing with its hydroxylation. In our respective experiments, however, disulfiram and its metabo-ıhyldithiocarbamate interfered with the determination of 5-(*p*-hydroxyphenyl)-5-phenyl)in according to the method of Folin–Ciocalteu[7] presumably by reducing the reagent and ıulating the presence of phenolic hydroxyl groups. Therefore, we studied the effects of disulfiram ; hydroxylation with amidopyrine and *p*-nitroanisole (*p*NA) as substrates. Amidopyrie is ely metabolized to 4-amidoantipyrine (4-AAP) part of which is acetylated to *N*-acetyl-4- N-demethylation). *p*NA is metabolized to *p*-nitrophenol[9] (*O*-demethylation).

ition of amidopyrine demethylation in vivo. In male Sprague–Dawley rats of 200–250 g body the left carotid artery was cannulated with polyethylene tubing according to[10] for repeated rawing. Disulfiram (1 g/kg) in an aqueous 1 % suspension of tragacanth was administered) these animals 15 hr prior to the i.p. application of amidopyrine (50 mg/kg). At 1, 3 and 6 hr ıidopyrine about 1·5 ml of blood was obtained from the cannula. 0·6 ml of plasma was used determination of total 4-APP. To minimize blood loss the sedimented erythrocytes were ed in the same volume of isotonic saline and reinjected through the catheter. Total 4-APP ermined according to[8] after hydrolysis of the acetylated 4-APP (0·5 N HCl, 30 min boiling ath) with slight modifications (12 ml $CHCl_3$, filtration through siliconized filter paper).

e 1 shows the concentration of total 4-APP in plasma of the controls and disulfiram treated normal rats total 4-APP levels increase to reach a maximum at about 3 hr and then decrease ly. In the disulfiram pretreated animals the 4-APP reaches a plateau at about 1 hr after amido- ınd does not change after that for further 5 hr. Apparently this inhibition of amidopyrine lation occurs only at certain dosage and time conditions. Oral application of 1 g/kg of m only 2 hr before amidopyrine did not lead to an *in vivo* inhibition of amidopyrine lation. This finding agrees with the observation that full effects of disulfiram on the disposi- thanol were not seen until 12 to 18 hr after its application to rabbits, at which time acetalde- els are greatly increased.[11] This is perhaps due to slow intestinal absorption or to the high fat y of disulfiram which prevents it from attaining sufficiently high concentrations in the liver

before the fat depots are saturated. On the other hand 500 mg/kg of disulfiram 15 hr before amidopyrine is not a sufficient dose to obtain *in vivo* inhibition. Since diethyldithiocarbamate is believed to be the active reduced form of disulfiram, it was also tried as an inhibitor. However, subcutaneous administration of 500 mg/kg of sodium diethyldithiocarbamate 30 min before amidopyrine did not cause an *in vivo* inhibition of amidopyrine demethylation.

Inhibition of amidopyrine demethylation in vitro. The effect of disulfiram on amidopyrine demethylation *in vitro* was studied with mouse liver microsomes stimulated by previous application of phenobarbital (four times 60 mg/kg) to the animals. Microsomes were prepared as described previously.[12]

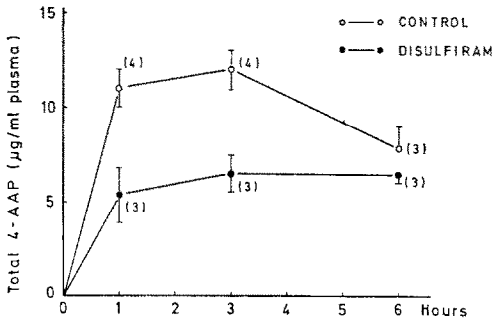

FIG. 1. Inhibition *in vivo* of *N*-demethylation of amidopyrine by disulfiram.
Plasma levels of total 4-AAP after intraperitoneal administration of 50 mg/kg of amidopyrine to control and disulfiram pretreated rats (1 g/kg 15 hr before amidopyrine). The values are mean values ± S.E.M. Numbers in parenthesis are numbers of animals. The 1 and 3 hr values are significantly different ($P < 0.05$).

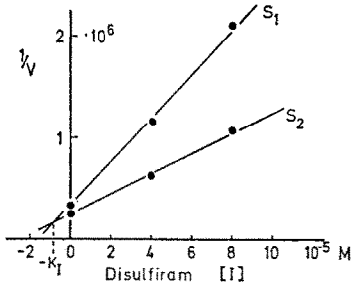

FIG. 2. Dixon diagram of the inhibition of the *O*-demethylation of *p*-nitroanisole by disulfiram. Formation of *p*-nitrophenol in moles/liter/minute. Abscissa: concentration of disulfiram. Ordinate: Reciprocal velocity of *p*-nitrophenol formation. Substrate concentrations: S_1: 1×10^{-4} M *p*NA S_2: 2×10^{-4} M *p*NA. Conditions of incubation as described in the text.

The incubation mixture contained 10^{-3} M amidopyrine, 10^{-1} M nicotinamide, $2.5\ 10^{-3}$ M glucose-6-phosphate, 0·5 Int. units of glucose-6-phosphate dehydrogenase, and 3×10^{-4} M NADP in a total volume of 2 ml 0·07 M phosphate buffer pH 7·4. The protein concentration (biuret) was 1·5 mg/ml. Disulfiram was dissolved in the same buffer in a concentration of 10^{-4} M. Incubation was carried out at 37° under air for 10 and 30 min. Under these conditions the *N*-demethylation of amidopyrine is markedly inhibited by disulfiram: After 10 min of incubation, 4·4 and 8.8×10^{-5} M disulfiram inhibited the 4-AAP formation by 47 and 65 per cent, respectively. After 30 min the corresponding values were 38 and 50 per cent respectively. In these experiments about 2×10^{-5} M 4-APP were produced during 30 min, indicating a rather slow rate of *in vitro* metabolism. Thus disulfiram presents itself to be an inhibitor of oxidative microsomal drug metabolism in a way similar to other previously known inhibitors such as SKF 525-A[13] or metyrapone.[14]

Inhibition of p-*nitroanisole demethylation* in vitro. The demethylation of *p*NA to *p*-nitrophenol can be measured directly in a cuvette at 405 mμ in a photometer, thus allowing kinetic experiments. The incubation mixture was essentially the same as for amidopyrine demethylation except that the buffer was adjusted to pH 7·85 and the protein content was 0·5 mg/ml. Reaction velocity was measured by reading the optical density every minute. The data obtained were treated according to Dixon[15] (Fig. 2). Inhibition of *p*NA demethylation by disulfiram according to the reciprocal velocity–inhibitor plot was found to be competitive in nature. The inhibitor constant was calculated to be $0{\cdot}8 \times 10^{-5}$ M. Sodium diethyldithiocarbamate also inhibits competitively, the K_I being about one order of magnitude greater than that for disulfiram ($1{\cdot}3 \times 10^{-4}$ M).

The described results indicate that disulfiram under certain conditions inhibits the demethylation of amidopyrine *in vivo*. Incubation experiments with mouse liver microsomes demonstrate that this inhibition seems to be due to a competitive interference with the microsomal drug oxidizing enzymes.

Acknowledgement—The work was supported by a grant from the Deutsche Forschungsgemeinschaft, Bad Godesberg.

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Biochemical Pharmacology, Vol. 18, pp. 2683–2688. Pergamon Press. 1969. Printed in Great Britain

A spectrophotometric method for the estimation of the carcinostatic agent, 5-aziridino-2,4-dinitrobenzamide (CB 1954), in biological fluids

(*Received* 9 *June* 1969; *accepted* 27 *June* 1969)

IT HAS recently been shown that 5-aziridino-2, 4-dinitrobenzamide (CB 1954) has a highly selective tumour growth inhibitory action on the transplanted Walker rat carcinoma 256.[1, 2] Further studies of