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THE INHIBITION OF INDUCTION OF MICROSOMAL MONOOXYGENASE ACTIVITY BY 1,3-DIAMINO-2-PROPANOL, AN INHIBITOR OF ORNITHINE DECARBOXYLASE

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SUMMARY: The p.o. administration of 1,3-diamino-2-propanol, an indirect inhibitor of ornithine decarboxylase, to rats and mice inhibited in a dose-dependent manner the induction of cytochrome P-450, benzo(a)pyrene hydroxylase, and 7-ethoxycoumarin O-deethylase by phenobarbital, β-naphtoflavone or Clophen C, a mixture of polychlorinated biphenyls. The results are consistent with the hypothesis that the induction of ornithine decarboxylase is a necessary step in the induction of microsomal monooxygenases.

The induction of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17, ODC) in response to the activation of cyclic 3',5'-AMP-dependent protein kinase has been suggested to be a key event in liver growth and in the subsequent induction of drug-metabolizing enzymes (1). A single injection of phenobarbital, Aroclor 1254 or 3-methylcholantrene has been shown to elicit the following sequence of events: 1) increased cAMP concentration and/or activation of cAMP-dependent protein kinase, 2) an increase in ODC activity and 3) induction of drug-metabolizing enzymes (1,2).

Repeated injections of 1,3-diaminopropane, an indirect inhibitor of ODC prevented the prereplicative accumulation of putrescine and spermidine (3,4) and produced a profound inhibition of the stimulation of liver DNA synthesis normally occurring after the partial resection of the liver (4,5). Piik et al. showed that a close analog of 1,3-diaminopropane, 1,3-diamino-2-propanol, administered orally inhibits completely

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ODC activity in livers of partially hepatectomized rats (6). If ODC induction is indeed a prerequisite for the induction of drug-metabolizing enzymes, then the inhibition of ODC induction by 1,3-diamino-2-propanol should also inhibit the induction of drug-metabolizing enzymes.

MATERIALS AND METHODS:

Chemicals: 1,3-diamino-2-propanol was purchased from Fluka AG (Buchs, Switzerland). β-Naphtoflavone was obtained from Pfalz & Bauer Inc. (Flushing, NY, USA), Clophen C from Bayer (Leverkusen, West Germany) and phenobarbital from E. Merck (Darmstadt, West Germany). The lingonberry juice was purchased from a local grocery. All other chemicals were of the highest purity commercially available.

Animals and treatments: Male Wistar rats (120-160 g) and male and female mice of both the aryl hydrocarbon responsive (C57Bl/6) and aryl hydrocarbon non-responsive (DBA/2) strains (20-30 g) were used in this study. The animals were fed standard rodent chow. Drinking water was replaced during the actual experiments by lingonberry juice to mask the taste of diaminopropanol. Various doses of diaminopropanol were given orally to the animals, the controls receiving the vehicle alone. 24 hours after the beginning of diaminopropanol administration the animals were injected with either β-naphtoflavone (100 mg/kg i.p. in corn oil) or Clophen C (200 mg/kg i.p. in corn oil). The aryl hydrocarbon non-responsive mice received phenobarbital (250 mg/litre p.o.) for 7 days. Diaminopropanol was continued until the killing of animals. The mice were killed by cervical dislocation and the rats by decapitation, the livers of the animals were removed, chilled immediately on ice, weighed, and homogenized in 4 vol. of 0.1 M sodium potassium phosphate buffer, pH 7.4. The homogenate was used for enzymatic assays. Determination of monooxygenase activities were all performed on samples from individual livers. The livers from each group of 6 mice were pooled for measuring the cytochrome P-450 content in microsomes.

Analytical methods: 7-Ethoxycoumarin O-deethylase activity was measured as described by Aitio (7). Benzo(a)pyrene hydroxylase activity was determined by the method of Nebert and Gelboin (8). Cytochrome P-450 content was measured according to Omura and Sato (9). Microsomal protein concentrations were determined according to Lowry *et al.* (10), using bovine serum albumin as a standard.

RESULTS: In the first experiment with C57Bl/6 mice (table 1) the effect of diaminopropanol administration on the induction of monooxygenase activities by β-naphtoflavone was studied. Diaminopropane alone caused a significant decrease in benzo(a)pyrene

TABLE 1: Effect of 1,3-diamino-2-propanol (DAP) on mouse hepatic 7-ethoxycoumarin O-deethylase and benzo(a)pyrene hydroxylase activities and cytochrome P-450 content after the administration of β-naphtoflavone (BNF).

Treatment	7-ethoxycoumarin O-deethylase (nmol/min per g of liver)	Benzo(a)pyrene hydroxylase (nmol/min per g of liver)	Cytochrome P-450 (nmol/mg of microsomal protein)
Control	17.90 ± 5.87	13.41 ± 2.91	1.34
DAP 50 mM	17.29 ± 3.40	5.28 ± 3.34	1.20
BNF	30.08 ± 2.59	37.06 ± 3.78	1.62
DAP 10 mM+BNF	32.08 ± 2.99	43.98 ± 9.62	1.65
DAP 50 mM+BNF	23.20 ± 5.67 [a]	26.15 ± 12.61	0.83

The mice received lingonberry juice alone (controls) or containing 10 mM or 50 mM 1,3-diamino-2-propanol for 48 hours. After 24 hours some of the animals were injected with β-naphtoflavone (100 mg/kg i.p. in corn oil). Liver samples were prepared and enzyme assays carried out as described in "Materials and methods". Each group consisted of 6 animals. The results are expressed as the mean ± SEM.

[a] Significantly different compared to the induced value ($p<0.05$)

hydroxylase activity, but had no effect on other activities. The pretreatment with β-naphtoflavone increased different monooxygenase activities up to 276 per cent (aryl hydrocarbon hydroxylase). The 10 mM concentration of diaminopropanol in drinking water had no effect on the induction, whereas 50 mM decreased both cytochrome P-450 content (50 per cent decrease from the induced value) and monooxygenase activities (about 26 per cent).

In the second experiment with DBA/2 mice (table 2) the effect of diaminopropanol on the phenobarbital induction was studied. Concentrations of diaminopropanol of 50 and 100 mM were too toxic to mice and only the lowest concentration of 10 mM could be studied during this one-week experiment. As seen, diaminopropanol inhibited the induction of cytochrome P-450 (50 per cent decrease from the induced value), benzo(a)pyrene hydroxy-

TABLE 2: Effect of 1,3-diamino-2-propanol (DAP) on mouse hepatic 7-ethoxycoumarin O-déethylase and benzo(a)pyrene hydroxylase activities and cytochrome P-450 content after the administration of phenobarbital (PhB).

Treatment	7-ethoxycoumarin O-deethylase (nmol/min per g of liver)	Benzo(a)pyrene hydroxylase (nmol/min per g of liver)	Cytochrome P-450 (nmol/mg of microsomal protein)
Control (6)	18.04 ± 3.85	19.83 ± 4.37	0.99
PhB (6)	36.58 ± 4.48	25.96 ± 3.84	1.88
DAP 10 mM + PhB (4)	22.31 ± 7.49 [a]	16.02 ± 4.70 [b]	0.95

The mice received lingonberry juice alone (controls) or containing 250 mg/l of phenobarbital or phenobarbital and 10 mM 1,3-diamino-2-propanol for 7 days. The number of animals in each group is given in parenthesis. The results are expressed as the mean ± SEM.

[a] Significantly different compared to the induced value ($p<0.01$)

[b] Significantly different compared to the induced value ($p<0.02$)

lase (38 per cent) and 7-ethoxycoumarin O-deethylase (39 per cent). Apparently diaminopropanol prevented completely the induction caused by phenobarbital.

In the third experiment with male Wistar rats (table 3) the effect of diaminopropanol on the induction of monooxygenases by Clophen C was studied. Diaminopropanol alone decreased benzo(a)-pyrene hydroxylase activity, but had no effect on other monooxygenases. The Clophen-inducible enzyme levels decreased when the concentration of diaminopropanol in drinking water was increased.

In all experiments, the extent of induction was usually lower than what has been earlier observed in our laboratory. This "suppression" of inducibility may be related to the sugar-containing vehicle (50 per cent lingonberry juice) and is reminiscent of the"glucose effect" observed in many laboratories.

TABLE 3: Effect of 1,3-diamino-2-propanol (DAP) on rat hepatic 7-ethoxycoumarin O-deethylase and benzo(a)pyrene hydroxylase activities and cytochrome P-450 content after the administration of a polychlorinated biphenyl (PCB).

Treatment	7-ethoxycoumarin O-deethylase (nmol/min per g of liver)	Benzo(a)pyrene hydroxylase (nmol/min per g of liver)	Cytochrome P-450 (nmol/mg of microsomal protein)
Control	11.41 ± 2.01	18.67 ± 5.62	0.86 ± 0.14
DAP 50 mM	11.70 ± 1.32	12.25 ± 2.80	0.83 ± 0.14
PCB	28.10 ± 2.07	49.67 ± 3.77	1.87 ± 0.18
DAP 10 mM+PCB	21.08 ± 1.97 [a]	46.37 ± 2.49	1.25 ± 0.37 [c]
DAP 50 mM+PCB	16.37 ± 2.56 [a]	37.76 ± 5.01 [b]	1.28 ± 0.18 [b]
DAP 100 mM+PCB	16.54 ± 1.56 [a]	18.84 ± 2.08 [a]	1.18 ± 0.52 [c]

The rats received lingonberry juice alone (controls) or 10 mM, 50 mM or 100 mM 1,3-diamino-2-propanol for 48 hours. After 24 hours some of the animals were injected with a polychlorinated biphenyl, Clophen C (200 mg/kg i.p. in corn oil). The number of animals in each group is 6. The results are expressed as the mean ± SEM.

[a] Significantly different compared to the induced value ($p<0.001$)
[b] Significantly different compared to the induced value ($p<0.01$)
[c] Significantly different compared to the induced value ($p<0.025$)

DISCUSSION: The present study indicates that peroral diaminopropanol to mice and rats produces an inhibition of the increase in monooxygenase activities after the administration of different inducers. Thus our findings seem to add further credence to the hypothesis that ODC is a key enzyme in the biochemical sequence resulting in the induction of microsomal drug-metabolizing monooxygenases (1). In the best studied experimental system of monooxygenase inducibility, the Ah locus-controlled inducibility of monooxygenase activities in inbred strains of mice (11), several enzymes including ODC segregate strictly with the Ah locus (12). In our study with the responsive strain (C57Bl/6), the induction of the mono-

oxygenase by β-naphtoflavone was inhibited by the simultaneous administration of the ODC inhibitor, suggesting a causal link.

Even though the results show that the administration of the ODC-inhibitor is associated with the inhibition of monooxygenase induction, it is difficult to exclude all secondary effects which possibly contribute to the action of diaminopropanol. However, there is evidence indicating that this effect may not be due to the general toxic effect: 1) the synthesis of RNA is not depressed by this drug and 2) the activities of S-adenosyl-L-methionine decarboxylase and thymidine kinase, both extremely sensitive indicators of unimpaired protein synthesis are not initially affected (6). The general condition of rats receiving diaminopropanol was good during the experiment. However, higher concentrations of diaminopropanol (50 and 100 mM) seemed to be toxic to the mice.

This study suggests that the use of ODC inhibitors may help to elucidate the biochemical sequences and the general mechanism of enzyme induction. On the other hand, the use of the hepatic monooxygenase induction as a model system may help to reveal the mechanism of action of ODC inhibitors and the role of polyamines in tissue growth and specific enzyme changes.

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REFERENCES:

1. Costa, M., Costa, E.R., Manen, C-A., Sipes, I.G. and Russel, D.H. (1976) Mol. Pharmacol. 12, 871-878.
2. Byus, C.V., Costa, M., Sipes, I.G., Brodie, B.B. and Russel, D.H. (1976) Proc. Nat. Acad. Sci. USA, 73, 1241-1245.
3. Pösö, H. and Jänne, J. (1976) Biochem. Biophys. Res. Commun. 69, 885-892.
4. Pösö, H. and Jänne, J. (1976) Biochem. J. 158, 485-488.
5. Kallio, A., Pösö, H. and Jänne, J. (1977) Biochim. Biophys. Acta, 479, 345-353.
6. Piik, K., Pösö, H. and Jänne, J. (1978) FEBS Lett. 89, 307-312.

7. Aitio, A. (1978) Anal. Biochem. 85, 488-491.
8. Nebert, D.W. and Gelboin, H.V. (1968) J. Biol. Chem. 243, 6242-6249.
9. Omura, T. and Sato, R. (1964) J. Biol. Chem. 239, 2470-2478.
10. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
11. Nebert, D.W. and Atlas, S.A. (1978) Human Genet. Suppl. 1, 149-160.
12. Nebert, D.W. and Oka, T. (1976) Proc. X Int. Congr. Biochem. p. 386.