Biochemical Pharmacology, Vol. 37, No. 13, pp. 2503–2506, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

ENHANCEMENT BY ALLOXAN-INDUCED DIABETES OF THE RATE OF METABOLIC ACTIVATION OF THREE PYROLYSATE CARCINOGENS VIA INCREASE IN THE P-448-H CONTENT IN RAT LIVER

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(Accepted 21 March 1988)

Heterocyclic amines are believed to be a causative factor in human cancer. To exert their carcinogenic effects, these chemicals, including Glu-P-1[+], IQ and MeIQx, require metabolic activation through their N-hydroxylations catalyzed by a 3-MC-inducible cytochrome P-450, P-448-H [2-4]. Although P-448-H is low, but detectable in untreated rats [2], the factor regulating the endogenous expression of this form is still unclear. Diabetes is known to affect the levels of hepatic drug-metabolizing activities probably through alterations in the hormonal level and/or other biological components. These results, together with the high susceptibility of hepatic cytochrome P-450 to endocrine factors [5], suggest the possibility that diabetes changes the levels of specific forms of cytochrome P-450 and has an effect on the activating capacity for carcinogens in the liver. Thus, we have studied the influence of diabetes on the level of pyrolysate activation.

Materials and Methods

Alloxan monohydrate was administered (170 mg/kg body weight) subcutaneously 10 days before sacrifice to male and female Sprague-Dawley rats (8 weeks old). Another group of rats with alloxan-induced diabetes was treated with insulin Novo R (25 units/kg body weight), six doses daily from day 4 after the alloxan administration. Isoniazid was dissolved in the drinking water as 0.1% (w/v) and given to a group of animals for 10 days before they were killed. Hepatic microsomes were prepared as reported previously [2]. The content of P-448-H was determined immunochemically as described previously [5]. Under the experimental conditions, P-448-H is clearly separated from P-448-L (P-450c) on nitrocellulose sheets and stained using an IgG fraction of a specific antibody for P-448-H.

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[+]**Abbreviations:** Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethyl imidazo[4,5-*f*]quinoxaline; 3-MC, 3-methylcholanthrene; Rev, revertants; and P-448-H, probably corresponds to P-450d (P-450IA2, recent nomenclature [1]) as determined by their spectral and catalytic properties, and the comparison in SDS gel [2].

The incubation mixture for the activation of pyrolysates consisted of 100 µg of microsomal protein, an NADPH generating system (1.6 mM NADP, 16 mM glucose-6-phosphate, 6 mM $MgCl_2$ and 0.2 I.U. of glucose-6-phosphate dehydrogenase), 50 mM KCl buffer (pH 7.4) and 0.2 mM Glu-P-1, IQ or MeIQx in a final volume of 200 µl. The mixture was incubated at 37° for 10 min, and an aliquot of the supernatant fraction after filtration was used to assess the mutagens as mentioned previously [6].

Results and Discussion

The rates of mutagenic activation of the three pyrolysates by hepatic microsomes are shown in Table 1. The number of revertants induced by a mutagenic metabolite of Glu-P-1 was 2.8-fold higher in microsomes prepared from alloxan-treated than from control rats, indicating that the activating capacity for Glu-P-1 is increased in the diabetic state. Similar results were also detected in the activation of IQ and MeIQx. Treatment of alloxan-induced diabetic rats with insulin decreased both the blood sugar level (191 ± 97 mg/dl compared to 412 ± 88 mg/dl) and the microsomal activating abilities of these pyrolysates. In diabetes, P-450j is reported to be increased [7]. However, the treatment of rats with isoniazid, which also enhances the hepatic P-450j level [8], had no significant effect on the mutagenic activation of pyrolysates, although microsomal aniline *p*-hydroxylation was 2.4-fold higher in isoniazid-treated than in control rats. These results suggest that a form(s) of cytochrome P-450 other than P-450j mediates mainly the mutagenic activation of pyrolysates in diabetic rats.

Table 1. Activation of three heterocyclic arylamine pyrolysates by microsomes of alloxan-, alloxan plus insulin- and isoniazid-treated male rat liver

Pyrolysates	Mutagenicity (X 10^{-3} Rev/mg protein)			
	Control	Alloxan-treated	Alloxan plus insulin-treated	Isoniazid-treated
Glu-P-1	13.8 ± 6.0 (100)	38.6 ± 8.2* (280)	17.6 ± 4.6 (127)	11.1 ± 2.0 (80)
IQ	7.2 ± 2.0 (100)	15.2 ± 4.6+ (211)	8.4 ± 3.4 (117)	11.4 ± 3.0 (158)
MeIQx	5.4 ± 1.6 (100)	12.6 ± 2.4* (233)	8.8 ± 3.8 (163)	6.5 ± 2.5 (120)

The data presented are the number of His^+ revertants of Salmonella typhimurium TA98 from experiments repeated twice (mean ± SD of five different animals), and the numbers in parentheses indicate the relative percents with respect to their controls. *,+ Statistically significant compared to the respective control: * $P < 0.01$ and + $P < 0.05$.

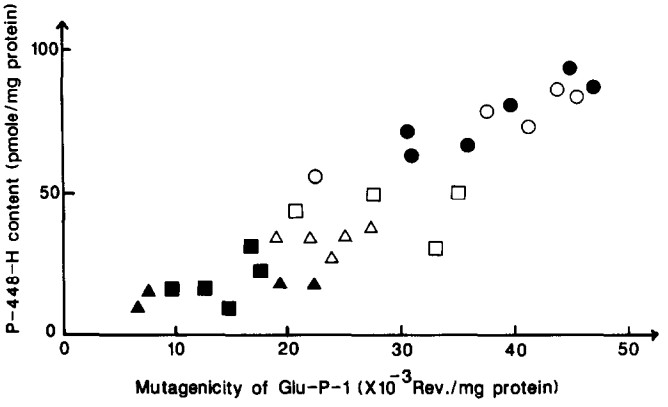

Fig. 1. Relation between P-448-H content and the mutagenicity of Glu-P-1 in rat hepatic microsomes. Key: male control (▲), female control (△), male treated with alloxan plus insulin (■), female treated with alloxan plus insulin (□), male treated with alloxan alone (●), and female treated with alloxan alone (○).

Heterocyclic arylamines have been shown to be activated mainly by 3-MC-inducible P-448-H in rats. Thus, to verify the role of P-448-H in the mutagenic activation of Glu-P-1 in alloxan-induced diabetic rats, the numbers of revertants induced were compared with the hepatic content of P-448-H (Fig. 1). A good correlation was observed between the mutagenic activating ability and the level of P-448-H in liver microsomes ($\underline{r}$ = 0.879).

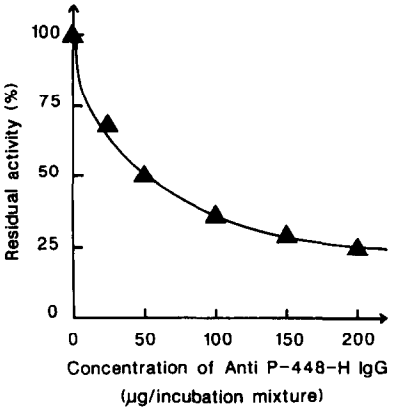

Fig. 2. Effect of anti-P-448-H IgG on the microsomal activation of Glu-P-1 by liver microsomes obtained from alloxan-treated rats (100 μg microsomal protein/incubation mixture).

The results were also confirmed by the effective inhibition of Glu-P-1 activation by anti-P-448-H IgG (Fig. 2). These results indicate that increased content of P-448-H is responsible for the enhanced capacity of mutagenic activation for heterocyclic arylamines in diabetic rat livers.

Acknowledgement: This work was supported by a grant-in-aid for cancer research from the Ministry of Health and Welfare, and a grant from the Ministry of Education, Sciences and Culture, Japan.

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