Notes

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Studies on The Metabolic N-Demethylation. VII.[1] Effect of *p*-Hydroxyphenobarbital on the Oxidative Demethylation in Rat Liver

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In part III[3] of this series, the present authors reported that there appeared two maximum activation of demethylation around 24—40 and 60—70 hours after pretreatment with phenobarbital when 3-methyl-monomethylaminoazobenzene (3-methyl-MAB), methylbarbital and cyclobarbital (EHB) were used as substrates. It might be considered that the one peak may be caused by the direct induction by pretreated phenobarbital, and the other may appear by the influence of either some metabolites or the hormonal effect which is affected by phenobarbital.

Miller, *et al.*[4] reported that N-hydroxy-2-acetylaminofluorene, a major metabolite of 2-acetylaminofluorene in the rat, was more active than the parent amide in producing tumors in the liver, mammary gland, small intestine and the ear duct of the rat.

Takabatake and Ariyoshi[5] reported that the pretreatment with 3-OH-EHB (5-ethyl-5-(3-hydroxy-1-cyclohexenyl)barbituric acid), one of the major metabolite of cyclobarbital, increased the formation of 3-OH-EHB and decreased that of 3-*keto*-EHB (5-ethyl-5-(3-oxo-1-cyclohexenyl)barbituric acid) from EHB in the rat. Horita[6] reported that the incubation of modaline sulfate (5×10^{-5}M) with NADPH and rat liver microsomes resulted in the rapid formation of highly active monoamine oxidase inhibitor. So it became interest to examine whether the metabolite of phenobarbital was playing as the more potent activator on the oxidative demethylation or not.

In this experiment, effect of *p*-hydroxyphenobarbital, the main metabolite of phenobarbital in animals, on the oxidative demethylation in rat liver was investigated. It was previously reported[7] that methylbarbital and meperidine were respectively useful substrates for measuring the degree of activation and inhibition of oxidative demethylation respectively, so these two substrates were employed in the following experiments.

The results were shown in Table I. When meperidine was used as a substrate, the activation of demethylation caused by the pretreatment of *p*-hydroxyphenobarbital was similar to the extent in phenobarbital-treated rats, but in the case of the demethylation of methylbarbital, the activating ratio in *p*-hydroxyphenobarbital treated rats was about one-fourth as much as that in phenobarbital treated rats.

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1) Patr VI: K. Minegishi, Y. Kuroiwa, and *late* S. OKui, *Chem. Pharm. Bull.* (Tokyo), **16**, 1649 (1968).
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Table I. Increased Activity of the Oxidative Demethylation in Rat Liver pretreated with Phenobarbital and *p*–Hydroxyphenobarbital

Pretreatment	Substrate	Metabolized (%)[a]	Ratio of activation[b]
None	methylbarbital	6.2± 1.8	1.00
	meperidine	54.2±11.9	1.00
Phenobrabital	methylbarbital	37.2± 7.1	6.00
	meperidine	68.8±10.7	1.26
p–Hydroxyphenobarbital	methylbarbital	8.4± 2.8	1.35
	meperidine	66.1± 9.8	1.22

a) $\text{metabolized }(\%)=\frac{\text{formed formaldehyde }(\mu\text{moles})}{\text{substrate }(\mu\text{moles})}\times 100$

b) $\text{ratio of activation}=\frac{\text{activity after pretreatment}}{\text{control activity}}$

Each rat was intraperitoneally administered with a dose of 15 mg per kg body weight of phenobarbital or *p*–hydroxyphenobarbital twice a day for 3 days.

Each reaction mixture containing 4 ml of $9000\times g$ supernatant, 100 μmoles of nicotinamide 100 μmoles of semicarbazide, 50 μmoles of $MgCl_2$, 5 μmoles of substrate and 1 ml of 0.3 M phosphate buffer (pH 7.4) was made up to a final volume of 10 ml with distilled water, and incubated for 1 hr at 37°.

Toki, *et al.*[8] reported that *p*–hydroxyphenobarbital was a less effective stimulator on the duration of hexobarbital hypnosis; the effect of *p*–hydroxyphenobarbital was about one–fourth that of phenobarbital. The data in our experiment concerning the demethylation of methylbarbital coincided with their data. Also the blood level of corticosterone in the rat with pretreating of phenobarbital have not affected significantly.[9]

Therefore, it might be considered that both the main metabolite and corticoid level in blood is not so much related to the appearance of two maximum peaks in the previous report.[3]

Experimental

Materials——Phenobarbital and meperidine were commercially obtained. Methylbarbital was synthesized by the method of Butler and Bush.[10] *p*–Hydroxyphenobarbital was prepared from the urine of phenobarbital–treated rabbit and, at same time, synthesized chemically by the method of Butler.[11]

Animals and Pretreatment of Drugs——Male Wistar rats weighing 60—100 g were used for the supply of liver tissue. Animals were intraperitoneally administered with a dose of 15 mg per kg body weight of phenobarbital or *p*–hydroxyphenobarbital twice a day for 3 days. Animals were decapitated 24 hr after the last administration of the drugs.

Determination of Enzyme Activity——Methylbarbital and meperidine were used as substrates. A typical reaction mixture of oxidative demethylation was shown in Table I. Activities of the oxidative demethylation of methylbarbital and meperidine were determined by the estimation of the amount of formed formaldehyde which was trapped with semicarbazide. The procedural details were previously reported.[7]

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