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Characterization of liver microsomal cytochrome P450 from rats treated with muscone (3-methylcyclopentadecanone)

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Muscone (3-methylcyclopentadecanone) has been used recently in the treatment of coronary disease in China [1]. It is also a component in many perfumes and a product usually made from the musk glands of male musk deer. Previous studies have demonstrated a phenobarbital (PB)-type induction pattern of the hepatic drug metabolizing enzymes after muscone treatment [1, 2]. In addition we have shown that the serum dimethadione (DMO)/trimethadione (TMO) ratios at 2 hr after oral administration of TMO as a model compound to estimate hepatic drug-oxidizing capacity were significantly increased in the rats treated with muscone *in vivo* [2]. Recently, we indicated that four forms of cytochrome P450 (P450), P450 PB-1 (P450 IIIA2), P450 PB-2 (P450 IIC6), P450 PB-4 (P450 IIB1) and P450 PB-5 (P450 IIB2), were inducible in rat hepatic microsomes with PB and that testosterone 6β- and 6β-hydroxylation activities which are mainly catalysed by P450 PB-1 and P450 PB-4, respectively, were also inducible [3, 4]. Furthermore the amount of P450 UT-2 (P450 IIC11) decrease by starvation and PB-treatment together with decrease of testosterone 2α-hydroxylation activity [4, 5], and P450 DM (P450 IIE1) was induced by starvation or treatment with ketone [5, 6].

In this study, we investigated changes in testosterone hydroxylation activities of hepatic microsomes and alterations of amounts of P450s, P450 PB-1, PB-2, PB-4, PB-5, UT-2, and DM by treatment with muscone to confirm that muscone, one of the ketone, is a PB-type inducer or a ketone-type inducer.

Materials and Methods

Chemicals. Muscone, PB and streptozotocin (STZ) were purchased from Ogawa & Co. Ltd (Tokyo, Japan), Wako Pure Chemical Industries (Osaka, Japan) and the Sigma Chemical Co. (St Louis, MO, U.S.A.), respectively.

Animals and dosing. Adult male Wistar rats weighing 200–240 g were obtained from Doken (Ibaraki, Japan). Muscone was dissolved in corn oil and administered by intraperitoneal (i.p.) injection at a dose of 150 mg/kg for 3 days. The rats in the control group received corn oil only for 3 days. PB (80 mg/kg) dissolved in saline was also given by i.p. injection for 3 days. Diabetes was induced in rats by intravenous (i.v.) injection with STZ (65 mg/kg) freshly dissolved in 0.01 M citrate. The rats were killed at 6 weeks after induction of diabetes.

Biochemical analyses. Preparation of microsomes [2], purification of P450 and preparation of antibody were performed by methods described previously [6–8]. Characterization of antibodies used in this study was described elsewhere [5]. The immunoblotting and immunochemical quantitation [7], and the measurement of testosterone hydroxylase activity were also performed as described previously [9, 10]. The designations given to the rat hepatic P450s described in this study can be related to the standardized gene designation [11].

Results and Discussion

Typical levels of P450 UT-2, PB-1, PB-2, PB-4/5 and DM in muscone- and PB-treated microsomes after Western blotting are shown in Table 1. P450 PB-1, PB-2, and PB-4/5 were induced 1.1-, 1.9- and 13.8-fold with muscone and 1.5-, 1.9- and 46.5-fold with PB, respectively. On the other hand, P450 UT-2 was present in the hepatic microsomes of muscone and PB-treated rats at lower levels. The P450 DM levels with muscone was not changed but reduced 0.6-fold with PB. Imaoka *et al.* [4] have reported

Table 1. Alteration in the content of cytochrome P450 isozymes of rat hepatic microsomes

	Content of cytochrome P450				
Microsomes	UT-2	P450 PB-1	P450 PB-2	P450 PB-4/5	P450 DM
UT	406.0 ± 6.4	145.0 ± 7.8	105.0 ± 7.1	14.0 ± 5.0	68.5 ± 9.2
MS	358.5 ± 47.4	163.5 ± 0.7	195.0 ± 25.5*	192.5 ± 7.8†	68.3 ± 21.2
PB	292.0 ± 46.7	213.5 ± 16.3*	198.5 ± 21.9*	650.5 ± 123.7*	43.3 ± 7.1*

Hepatic microsomes (0.5–2 μg of protein) were analysed by Western blotting. Levels of cytochrome P450s were assayed by densitometry of nitrocellulose immunoblotted from SDS–polyacrylamide gel. Measurement was done with duplicates of two to four different preparations of microsomes. The values are expressed as mean ± SD of pmol of cytochrome P450/mg of microsomal protein. Total cytochrome P450 was measured by the reduced-CO spectral method [3].

UT, untreated; MS, muscone-treated; PB, phenobarbital-treated; DM, streptozotocin-treated.

* $P < 0.05$, † $P < 0.01$.

Table 2. Alteration in testosterone hydroxylation activity of rat hepatic microsomes

Microsomes	Testosterone hydroxylation activity							
	6α-OH	15α-OH	7α-OH	6β-OH	16α-OH	16β-OH	2α-OH	2β-OH
UT	—	—	—	2.39 ± 0.26	1.51 ± 0.04	0.08 ± 0.04	1.04 ± 0.26	0.31 ± 0.01
MS	—	—	—	3.52 ± 0.13*	2.29 ± 0.52	0.54 ± 0.01†	0.93 ± 0.28	0.49 ± 0.01†
PB	—	—	—	5.93 ± 0.83*	2.52 ± 0.13†	1.46 ± 0.17†	0.50 ± 0.04	0.95 ± 0.09*

Measurements were made from two to four different preparations of microsomes in duplicate, and the values are expressed as mean ± SD in nmol of product/min/mg of microsomal protein. Values less than 0.08 nmol/min/mg protein are expressed as "—".

The abbreviations refer to the position of testosterone hydroxylation.

UT, untreated; MS, muscone-treated; PB, phenobarbital-treated.

* $P < 0.05$, † $P < 0.01$.

that P450 PB-4 and PB-5 are the major PB-inducible forms of P450 and P450, PB-1 and PB-2 were weakly inducted with PB. We have found in agreement with other authors that none of these forms were induced in the liver of rats treated with MC (MC treatment data not shown). Our results with muscone agreed with the PB-inducible pattern [4]. In addition acetone- or alcohol-inducible form (P450 IIE1) of P450 did not induce with muscone treatment [6]. The testosterone hydroxylation activity of both muscone- and PB-treated hepatic microsomes is shown in Table 2. The 2β- and 6β-hydroxylation activity were increased 1.6- and 1.5-fold with muscone, and 3.1- and 2.5-fold with PB, respectively. The 16β-hydroxylation activity of testosterone was strongly induced 6.8- and 18.3-fold with muscone and PB, respectively. On the contrary, testosterone 2α-hydroxylation activity was suppressed by muscone and PB treatment. Our results are in accordance with those of Imaoka *et al.* [4].

These results suggest that the induction type of muscone treatment is very similar to that induced by PB, mainly affecting PB-4/5 but with lesser magnitude than PB. This data supports and extends our previously published findings with muscone [1, 2]. Therefore, care must be taken in the decrease of therapeutic effects when is given with muscone in the clinical use.

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Determination of the *in vivo* antigen–antibody affinity constant from the redistribution of desipramine in rats following administration of a desipramine-specific monoclonal antibody

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Although there are many *in vitro* procedures for determining the affinity of antibodies for antigen, little attention has been given to the possibility that the effective binding constant *in vivo* may be amenable to calculation. This communication describes such a procedure and illustrates its use by analysis of the redistribution of desipramine (DMI) in rats following administration of a monoclonal antibody specific for this tricyclic antidepressant (anti-TCA).

Materials and Methods

Animal studies. The animal studies have been described