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SHORT COMMUNICATION

Reduced Hepatic Expression of CYP7A1 and CYP2C13 in Rats with Spontaneous Hyperlipidaemia

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ABSTRACT. A strain of hyperlipidaemic Sprague–Dawley (HSD) rat was compared with normal Sprague–Dawley (SD) rats for expression of cholesterol 7α-hydroxylase activity (CYP7A1) and other cytochrome P450 (P450) enzymes in liver. Hepatic microsomal CYP7A1 activity in male HSD rats was 2–3-fold lower than in male SD rats with CYP7A1 apoprotein levels being similarly reduced. CYP7A1 expression was subject to diurnal variation in HSD rats as found in SD rats. Treatment of HSD rats with cholestyramine caused an increase in hepatic microsomal cholesterol 7α-hydroxylase activity of 3.3-fold compared with a 3.5-fold increase in SD rats with similar changes in apoprotein levels. These results indicate that the lower activity in HSD rats is not due to a defect in the catalytic activity of the enzyme, regulation affecting diurnal variation or regulation through bile acid feedback inhibition. No difference between hepatic microsomal methoxyresorufin-*O*-demethylase, benzoxyresorufin-*O*-debenzylase or chlorzoxazone 6-hydroxylase activities in SD and HSD rats was found, nor was there any difference in the levels of CYP1A2, CYP2D1, CYP2E1, CYP3A1, CYP3A2 or NADPH cytochrome P450 reductase determined by immunoblotting using specific anti-peptide antibodies. However, unlike in male SD rats, CYP2C13 was absent in male HSD rats and this was associated with a two-fold reduction in testosterone 6β-hydroxylase activity. In conclusion, while HSD rats do not have a general reduction in P450 levels, they do lack CYP2C13 and have lowered cholesterol 7α-hydroxylase activity, as a result of a reduced level of expression of the enzyme. BIOCHEM PHARMACOL **56**;2:253–257, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. cholesterol 7α-hydroxylase; cytochrome P450; CYP7A1; CYP2C13; cholestyramine; diurnal variation; spontaneously hyperlipidaemic rat

Atherosclerosis and coronary heart disease are the leading causes of stroke and fatality in many areas of the world and hyperlipidaemia, particularly elevated cholesterol levels, has been shown to be one of the major causative factors leading to these conditions [1, 2]. Cholesterol homeostasis is maintained by the activity of two key hepatic microsomal enzymes, HMG CoA§ reductase, which catalyses the formation of mevalonic acid and is the rate limiting step in cholesterol synthesis [3], and cholesterol 7α-hydroxylase, a P450 enzyme (CYP7A1), which is the first and rate-limiting enzyme in the conversion of cholesterol into bile acids [4]. The secretion of bile acids is a major route for removal of cholesterol from the body [4].

Studies of the biochemistry of hypercholesterolaemia in human are hampered by difficulties in obtaining suitable samples of liver. Therefore, models of hypercholesterolaemia in animals have been widely used [5–7]. This is achieved by either feeding animals with a cholesterol-rich diet or using animals that are genetically predisposed to this condition, e.g. the Watanabe heritable hyperlipidaemic rabbit. Recently, a strain of SD rat that exhibits hyperlipidaemia when fed a diet containing a normal lipid content has been described [8]. These HSD rats have raised serum total cholesterol levels and reduced hepatic drug-metabolising capacity [8]. Specifically, the activities of aryl hydrocarbon hydroxylase, flavone containing monooxygenase and uridine diphosphate glucuronyl transferase were lower in HSD rats of both sexes, while aniline hydroxylase, aminopyrine *N*-demethylase and glutathione S-transferase were lower in female HSD rats. If the reduction in P450 activity is a general phenomenon, then it is possible that CYP7A1 may also be reduced in HSD rats leading to a decrease in the metabolism of cholesterol to bile acids and consequently an elevation of serum cholesterol. In this study, the expression of CYP7A1 and other P450 enzymes was determined in SD and HSD rats using specific anti-peptide antibodies and P450 substrate selective assays.

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§ *Abbreviations:* HMG CoA, hydroxy-3-methylglutaryl-CoA; HSD, hyperlipidaemic Sprague–Dawley; P450, cytochrome P450; SD, Sprague–Dawley.

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MATERIALS AND METHODS

Treatment of Animals, Preparation of Hepatic Microsomal Fractions

Male SD (Nippon Crea) and male HSD rats were housed in the Department of Pharmacology, St. Marianna University, Japan, and fed a standard laboratory chow (CE-2, Nippon Crea) or the same diet supplemented with 5% (w/w) cholestyramine. Animals were maintained under a normal 12 hr light–dark cycle. At 8 weeks of age groups of rats were killed in the morning (10 a.m.) or at night (10 p.m.) to study diurnal variation. The livers were perfused *in situ* with ~10 ml of ice-cold 0.25 M of potassium phosphate buffer pH 7.25 containing 0.15 M of KCl, 1 mM of EDTA and 5 mM of dithiothreitol and snap frozen in liquid nitrogen. Each liver was homogenised and the microsomal fraction prepared by differential ultracentrifugation as described previously [9].

Cholesterol Determination

Serum total cholesterol was measured enzymatically using the Cholesterol E-Test® (Wako Pure Chemical Industries). Microsomal unesterified cholesterol was measured using an enzymatic assay kit supplied by Sigma.

Immunoblotting

Immunoblotting of microsomal samples was performed as described previously [10] using enhanced chemiluminescence to detect peroxidase activity and polyclonal rabbit anti-peptide antibodies directed against CYP7A1 [11], CYP1A2 [12], CYP2E1 [13] and CYP3A1 and CYP3A2 [14]. Antibodies directed against CYP2C13, CYP2D1 and cytochrome P450 reductase were produced by immunising rabbits with the peptides Arg-Phe-Ile-Pro-Leu, Arg-Glu-Gln-Gly-Leu and Leu-Asp-Val-Trp-Ser, respectively by methods described previously [13, 15]. The intensity of immunoreactivity was quantified by densitometry using an LKB Ultrascan XL laser densitometer.

P450 Activity Determinations

Cholesterol 7α-hydroxylase [11], methoxyresorufin *O*-demethylase [16], benzyloxyresorufin *O*-debenzylase [16], chlorzoxazone 6-hydroxylase activity [17, 18] and testosterone 6β-hydroxylase [19] activities were measured as described previously.

RESULTS AND DISCUSSION

Measurement of serum total cholesterol confirmed that HSD rats were hypercholesterolaemic. The level measured in the morning was 3.2-fold higher in HSD rats (5.42 ± 0.24 mmol/l, N = 5) compared with SD rats (1.69 ± 0.07 mmol/l, N = 5). Similarly, the level measured at night was 2.8-fold higher in HSD rats (4.87 ± 0.16 mmol/l, N = 5) compared with SD rats (1.74 ± 0.05 mmol/l, N = 5).

Hepatic microsomal cholesterol 7α-hydroxylase activity was found to be 2–3 fold lower in HSD rats compared with that in SD rats (Fig. 1a). As the measurement of cholesterol 7α-hydroxylase *in vitro* utilizes cholesterol present in the microsomal fraction as substrate, it is possible that variation in cholesterol levels may affect enzyme activity. However, there was no difference between hepatic microsomal cholesterol levels, which were 66.3 ± 3.7 and 72.4 ± 5.4 nmol/mg protein in SD and HSD rats, respectively ($P > 0.05$, 4 degrees of freedom, N = 3 in each group, using rats killed in the morning). The lower cholesterol 7α-hydroxylase activity in HSD rats was accompanied by a proportionate decrease in the level of CYP7A1 apoprotein (Fig. 1a). Overall, a strong positive correlation ($r = 0.93$, $P < 0.01$) was found between enzyme activity and apoprotein levels. Therefore, the decrease in cholesterol 7α-hydroxylase activity in HSD rats is due to reduced protein expression and not a defect in the activity of the CYP7A1 enzyme.

A dysfunction in one of the various independent levels of regulation [4, 20–24] leading to a reduction in the expression of CYP7A1 was considered. It was found that the diurnal variation in CYP7A1, known to occur in normal rats, was also apparent in HSD rats (Fig. 1a). Further, administration of cholestyramine to sequester circulating bile acids and prevent feedback inhibition of CYP7A1 expression caused a 3.5-fold increase in cholesterol 7α-hydroxylase activity in SD rats and a similar 3.3-fold increase in HSD rats (Fig. 1b). CYP7A1 expression may also be controlled through steroid and thyroid hormones acting on response elements located in the upstream region of the CYP7A1 gene [25, 26]. Interestingly, Watanabe *et al.* [8] have shown that testosterone levels in male and estradiol levels in female HSD rats are lower than in SD rats and this may affect CYP7A1 expression.

As CYP7A1 expression is low and as a deficiency in drug metabolising enzymes was indicated previously [8], a more extensive investigation into the levels of hepatic microsomal P450 enzymes in HSD rats was undertaken. It was found that methoxyresorufin *O*-demethylase, benzyloxyresofin O-debenzylase and chlorzoxazone 6-hydroxylase activities, which are selective for CYP1A2 [27], CYP3A1/2 [28] and CYP2E1 [17], respectively, were similar in SD and HSD rats (data not shown). Correspondingly, there was no difference in the apoprotein levels of CYP1A2, CYP3A1, CYP3A2 or CYP2E1 (Fig. 2). Also, no difference between the levels of CYP2D1, or NADPH cytochrome P450 reductase in SD and HSD rats was found (Fig. 2). In contrast, CYP2C13 was undetectable in HSD rats (Fig. 2). CYP2C13 catalyses, amongst other reactions, the 6β-hydroxylation of testosterone [29, 30] and this activity was found to be two-fold lower in HSD rats compared with SD rats (22.8 ± 3.5 and 46.3 ± 5.9 pmol/min/mg protein, respectively, $P < 0.01$, 8 degrees of freedom, N = 5 in each group). CYP3A1 and CYP3A2 also catalyse testosterone

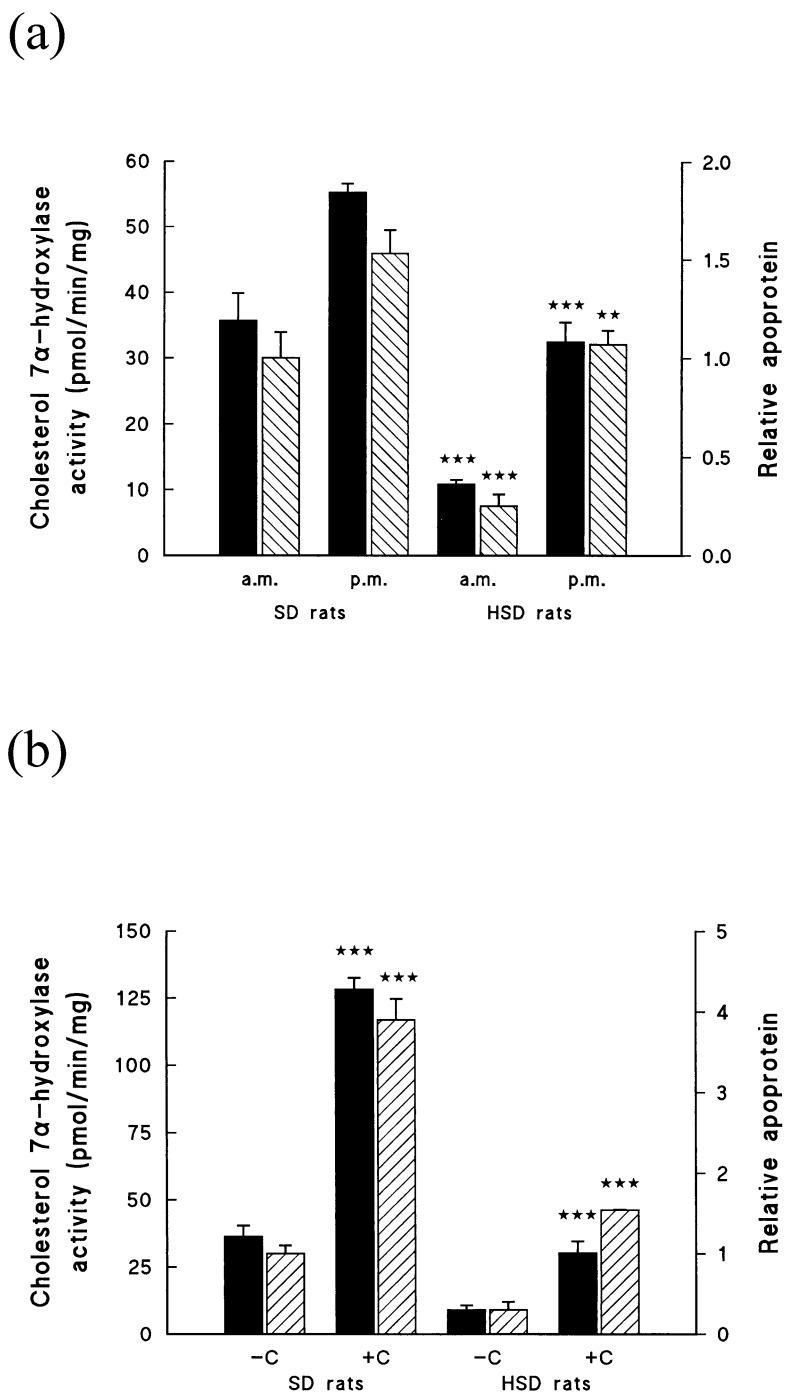

FIG. 1. Hepatic microsomal cholesterol 7α-hydroxylase activity and CYP7A1 apoprotein levels in SD and HSD rats. Groups of rats aged 8 weeks were maintained on a normal diet and killed at either 10 a.m. or 10 p.m. (a) Cholesterol 7α-hydroxylase activity (solid bars) and CYP7A1 apoprotein levels, shown as relative to the amount in SD rats killed at 10 a.m. (hashed bars). Statistical differences between SD and HSD rats are indicated. (b) The effect cholestyramine treatment on SD and HSD rats in groups of rats maintained on a normal diet (−C) or a diet supplemented with 5% (w/w) cholestyramine for 2 weeks (+C). All rats were killed at 10 a.m. and cholesterol 7α-hydroxylase activity (solid bars) and CYP7A1 apoprotein levels, shown as relative to the amount in SD rats fed a normal diet (hashed bars), were measured. Statistical differences between cholestyramine treated and rats fed a normal diet are indicated. The results shown are mean values ± SEM (N = 5) ($P < 0.01$, ***$P < 0.001$, Student's *t*-test, 8 degrees of freedom).**

6β-hydroxylation [19] and both SD and HSD rats process similar levels of these enzymes. Therefore, it would appear that in SD rats the 6β-hydroxylation of testosterone is catalysed by CYP2C13, CYP3A1 and CYP3A2, and in HSD rats by CYP3A1 and CYP3A2. The difference in enzyme activity between the two strains of rat represents the contribution of CYP2C13 to the reaction. CYP2C13 also catalyses aryl hydrocarbon hydroxylase activity [30], and the reduced levels of this enzyme may explain the decrease of aryl hydrocarbon hydroxylase activity in male

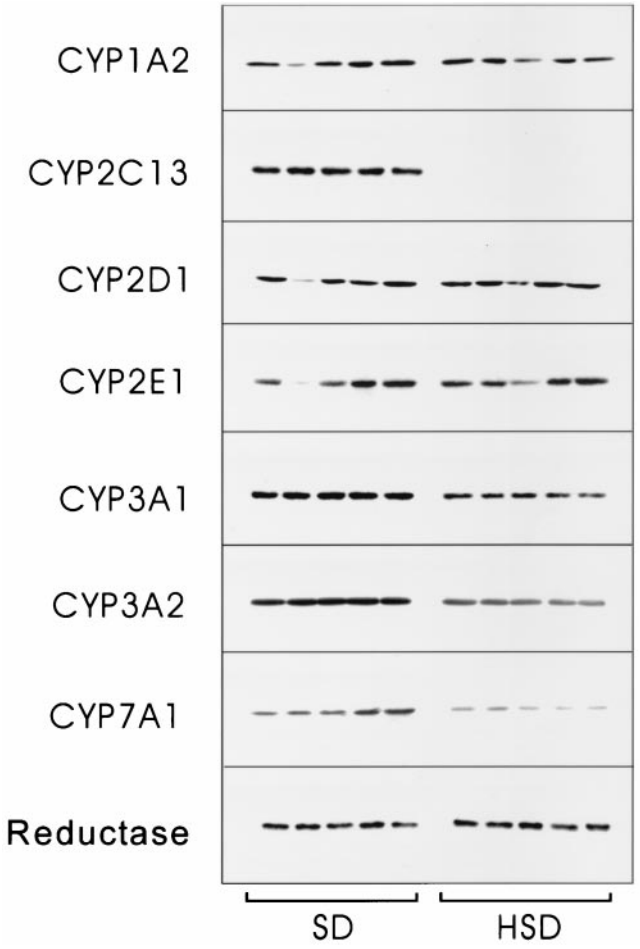

FIG. 2. Immunoblots showing the relative levels of P450 enzymes in SD and HSD rats. Rats were maintained on a normal diet and at 8 weeks of age were killed at 10 a.m. For each group, samples of 20 μg of microsomal protein were loaded onto SDS-polyacrylamide gels and immunoblotting was performed as described in the text. Immunoblots were incubated with antiserum against CYP1A2 (diluted 1:4000), CYP2C13 (diluted 1:4000), CYP2D1 (diluted 1:4000), CYP2E1 (diluted 1:2000), CYP3A1 (diluted 1:16,000), CYP3A2 (diluted 1:4000), CYP7A1 (diluted 1:4000) and NADPH cytochrome P450 reductase (diluted 1:8000).

HSD rats reported previously [8]. CYP2C13 is expressed in males but not females, and this is determined by the higher level of serum androgens normally present in males during development [31]. Adult male HSD rats have a low level of serum testosterone [8]. A similar low level during the perinatal period may suppress CYP2C13 expression.

In conclusion, HSD rats have lower hepatic microsomal cholesterol 7α-hydroxylase activity than SD rats, as a result of a reduced level of expression of the enzyme. At present the reason for this is unknown, but defects in the catalytic activity of the enzyme or its regulation through diurnal and bile acid feedback mechanisms are excluded. Future studies should address the relationship between hypercholesterolaemia and lower hepatic microsomal cholesterol 7α-hydroxylase activity, the effect on CYP7A1 mRNA levels, the involvement of HMG-CoA reductase and the response of HSD rats to a cholesterol-rich diet. Although most of the P450 enzymes studied here were not affected, showing that the attenuated expression of CYP7A1 is not as a result of a general decrease in P450 activity, male HSD rats were shown to lack CYP2C13 possibly as a result of lowered serum testosterone levels.

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