
**Regular Article**

*Endogenous Uremic Substances are not Involved in the Reduced Hepatic Extraction of Metoprolol in Bilateral Ureter-Ligated Rats*†

Masato TAGUCHI, Misato URAI, Shigehiro TAIRA, Hiroko TANABE, and Yukiya HASHIMOTO*

Graduate School of Pharmaceutical Sciences, University of Toyama, Toyama, Japan

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**Summary:** The hepatic extraction of metoprolol is reduced in rats with bilateral ureter ligation (BUL)-induced renal failure. The aim of the present study was to evaluate the effect of uremic substances on the hepatic metabolism of metoprolol in rats with BUL. The metabolic rate in the liver microsomes of BUL rats was similar to that in sham rats, and there was no significant difference between sham and BUL rats in the effect of the supernatant of liver homogenates on the metabolism. The rate of metabolism in the liver microsomes in the presence of the plasma of BUL rats was also similar to that in the presence of the plasma of sham rats. These findings indicated that uremic substances which accumulate in BUL rats do not directly inhibit the activity of CYP2D2, which is responsible for the metabolism of metoprolol in the rat liver.

**Key words:** metoprolol; renal failure; bilateral ureter-ligated rats; uremic substance

**Introduction**

The intestinal absorption of orally administered propranolol is essentially complete, with no metabolism of this drug occurring in the gut.1,2) After the oral administration of propranolol, the liver is the principal site of extensive pre-systemic and systemic metabolism, and less than 1% of the intact drug is found in the urine.1,3) However, Bianchetti et al.4) showed that the area under the concentration-time curve for orally administered propranolol in renal failure patients not on hemodialysis is 7- to 8-fold higher than that in healthy volunteers. The pharmacokinetics of propranolol has been extensively investigated using uranyl nitrate-induced renal failure in rats.5,6) These studies showed increased bioavailability and reduced hepatic first-pass extraction of propranolol in rats with uranyl nitrate-induced renal failure, although the precise biochemical and/or physiological mechanism for the decreased presystemic clearance is unclear.7,8)

Because changes in governmental regulations regarding the production of radioactive substances have made uranyl nitrate less available, we investigated the mechanisms responsible for the increased bioavailability of propranolol in rats with cisplatin-induced renal failure.9) The hepatic intrinsic clearance of propranolol was not significantly altered in rats with renal failure as compared with control rats. However, hepatic first-pass extraction of propranolol was dose-dependent and saturable in both renal failure and control rats, and the initial rate of absorption of the drug from the intestine was significantly greater in rats with renal failure than in control rats. Accordingly, the increased bioavailability of propranolol in rats with cisplatin-induced renal dysfunction is mainly a result of the increased initial absorption rate in the intestine followed by the partial saturation of hepatic first-pass metabolism.9)

The mechanism responsible for the increased bioavailability of propranolol in bilateral ureter-ligated (BUL) rats is different from that in the rats with cisplatin-induced renal failure.2,10) That is, Laganière and Shen investigated the pharmacokinetics of intravenously and orally administered propranolol in BUL rats, and showed that the bioavailability is increased in BUL rats as compared with control rats.2) They reported that the gastrointestinal absorption of propranolol is not altered in BUL rats as compared with control rats. We also investigated the pharmacokinetics of propranolol and metoprolol in BUL rats, and confirmed that the rate of intestinal absorption of these drugs was only slightly greater than that in control rats.10) Therefore, the absorption rate-dependent decrease in the hepatic first-pass clearance of these drugs due to saturation kinetics was marginal in BUL rats. On
the other hand, we found that the blood concentrations of propranolol and metoprolol following intra-portal infusion were significantly higher in BUL rats than control rats.\textsuperscript{10} Therefore, the increased bioavailability of propranolol and metoprolol in BUL rats was attributed to diminished hepatic first-pass metabolism. The activity of CYP2D2, which is responsible for the metabolism of propranolol and metoprolol in the rat liver, was not altered by BUL, whereas the rate at which NADPH was generated in the liver cytosolic fraction was lower in BUL than control rats.\textsuperscript{10-12} Accordingly, we speculated that the decrease in the hepatic metabolic activity and extraction of propranolol and metoprolol in BUL rats is at least partly due to the reduced generation of NADPH in the liver.

Another possible explanation for the diminished hepatic extraction of drugs is the presence of uremic toxins during renal failure.\textsuperscript{13} Several reports have suggested possible effects of uremic substances on hepatic drug metabolizing activity.\textsuperscript{6,14} Yoshitani \textit{et al.}\textsuperscript{14} reported that the unbound clearance of losartan in rat liver microsomes was significantly lower in the presence of 10\% uremic serum obtained from uranyl nitrate-treated and BUL rats than in the presence of control serum. Terao and Shen\textsuperscript{6} also reported that endogenous substances in the blood of uranyl nitrate-induced uremic rats are capable of inhibiting the hepatic extraction of propranolol in perfused liver. However, it was not clear whether the endogenous uremic substances in BUL rats inhibited CYP2D2 directly. We have believed that further systematic study is necessary to determine the inhibitory effect of endogenous uremic substances on drug metabolizing activity.\textsuperscript{10,12}

The present study was conducted to evaluate the effect of endogenous uremic substances on the drug metabolism in hepatic microsomes of BUL rats. Propranolol highly binds to plasma albumin and \textit{a}1-acid glycoprotein, and the unbound fraction of propranolol is only 6.1\% in normal rat plasma.\textsuperscript{5} The \textit{a}1-acid glycoprotein is an acute-phase reactant, and increases in stressful situations such as inflammation.\textsuperscript{15} In addition, a surgical operation induces a significant increase in the plasma binding of propranolol due to an elevated plasma concentration of \textit{a}1-acid glycoprotein.\textsuperscript{15} Therefore, propranolol may not be a useful model drug in order to evaluate the effect of endogenous uremic substances in the blood on the drug metabolism in liver microsomes. In contrast, the affinity of metoprolol for plasma proteins is much lower than that of propranolol, and only 8–12\% of metoprolol binds to plasma protein in rats.\textsuperscript{16,17} Therefore, we thought that metoprolol is a more useful agent with which to investigate the effects of the liver cytosolic fraction and/or blood plasma on the metabolism of drugs in liver microsomes.

Materials and Methods

Materials: Metoprolol tartrate was purchased from ICN Biochemicals (Aurora, OH, USA). NADPH, bovine serum albumin, bovine serum \textit{\gamma}-globulin, and human serum albumin (fraction V) were purchased from Nacalai Tesque (Kyoto, Japan). Rat serum albumin was obtained from Sigma-Aldrich (St. Louis, MO, USA) or Merck-Calbiochem (Darmstadt, Germany). Rat serum globulin (cohn fraction II, III) was obtained from Sigma-Aldrich. Human serum \textit{\gamma}-globulin was obtained from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of the highest grade available.

Animals and preparation of BUL rats: The animal experiments were performed in accordance with the Guidelines for Animal Experiments of University of Toyama. Male Wistar rats (230–300 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan), and housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. BUL was performed according to the method of Giacomini \textit{et al.}\textsuperscript{18}, and the rats were used for experiments 28 h after the operation. Briefly, the abdominal cavity was opened under pentobarbital anesthesia, and a double ligature was placed on each ureter isolated.\textsuperscript{10,12} In all experiments, sham-operated rats served as controls.

Preparation of liver microsomal and cytosolic fractions: Liver microsomal and cytosolic fractions were prepared as previously reported.\textsuperscript{10-12} Briefly, the liver was removed after 24 h of fasting, and homogenized with 4 volumes of ice-cold 1.15\% potassium chloride dissolved in 50 mM phosphate buffer (KCl-PB) (pH 7.4) using a Potted-Elvejem homogenizer. The homogenate was centrifuged at 9,000 g for 20 min, and the supernatant was transferred and centrifuged again at 105,000 g for 60 min. The supernatant after the 105,000 g centrifugation (SUP) was collected as the hepatic cytosolic fraction, and the precipitate (microsomal fraction) was re-suspended in a volume of ice-cold KCl-PB equivalent to 3 times the original weight of the liver. The microsomal suspensions and SUP were stored at –85\(^\circ\)C until used.

Metabolism of metoprolol in liver microsomes: The reaction mixture consisted of 50 \textmu L of microsomal suspension (0.2 mg of protein), 50 \textmu L of NADPH solution (final concentration: 1 mM), and 350 \textmu L of Krebs-Henseleit bicarbonate buffer (KHB) containing sodium chloride (115 mM), potassium chloride (4.7 mM), calcium chloride (2.5 mM), monobasic potassium phosphate (1.2 mM), magnesium sulfate (1.2 mM), and sodium bicarbonate (25 mM). The reaction mixture was preincubated for 5 min at 37\(^\circ\)C. The reaction was started by adding 50 \textmu L of a metoprolol solution (final concentration: 5 \mu M or 30 \mu M), and was allowed to run
for 50 min at 37°C. The $K_m$ value of recombinant CYP2D2 for the metabolism of metoprolol was reported to be 3.53 $\mu$M. Therefore, we used the metoprolol concentration of 5 $\mu$M and 30 $\mu$M in the present study. The reaction was stopped by adding 1 mL of ice-cold glycine buffer (pH 10.6), in order to quantitate metoprolol. The amount of metoprolol metabolized was calculated by subtracting the amount remaining in the reaction mixture from the amount applied, and the rate of metabolism was expressed as the percentage metabolized.

To evaluate the effect of endogenous substances in BUL rats on the metabolic activity, 350 $\mu$L of KHBB (70% of reaction mixture) was replaced with the same volume of uremic SUP or plasma obtained from BUL rats. In addition, to evaluate the effect of a high concentration of $K^+$ on the metabolism of metoprolol in the liver microsomes, the incubation was also performed with KCl-PB instead of KHBB. To evaluate the effect of purified proteins on the metabolic activity of the liver microsomes, protein (4% albumin or 2% globulin) dissolved in KHBB was added to the reaction mixture.

**Binding of metoprolol to protein:** The binding of metoprolol to protein in the reaction mixture was evaluated using a centrifugal ultrafiltration method. The reaction mixture consisted of 50 $\mu$L of microsomal suspension (0.2 mg of protein), 350 $\mu$L of KHBB, and 100 $\mu$L of a metoprolol solution (final concentration: 5 $\mu$M), which was kept at room temperature for a 10-min equilibration period. The unbound metoprolol in the reaction mixture was separated by centrifugation (1,000 g for 10 min) using an ultrafiltration device equipped with a YMT membrane (Centrifree®, Millipore, Bedford, MA, USA). To evaluate the effect of plasma proteins on the unbound fraction of metoprolol, 350 $\mu$L of KHBB in the reaction mixture was replaced with the same volume of a 4% albumin solution or a 2% globulin solution.

**Analytical methods:** The total protein concentration in plasma was measured using a kit purchased from Wako Pure Chemical Industries (Osaka, Japan). The protein concentration in the microsomal suspensions and SUP were determined using Bio-Rad protein assay dye reagent with bovine serum albumin as a protein standard (Bio-Rad, Munich, Germany). The metoprolol concentration was measured with an HPLC-based method as reported previously.

**Data analysis:** Values are expressed as the mean ± S.E. for $n$ animals. The statistical significance of differences between mean values was tested using an unpaired $t$-test provided that the variances of the groups were similar. If this was not the case, the Mann-Whitney $U$-test was applied. Multiple comparisons were performed using Scheffé's test following a one way ANOVA provided that the variances of the groups were similar. If this was not the case, a Scheffé-type test was applied following Kruskal-Wallis analysis. $P$ values of less than 0.05 were considered to be significant.

**Results**

Protein concentrations in plasma, microsomal suspensions, and SUP: The total protein concentration in the plasma of BUL rats (4.79 ± 0.01 g/dL) was similar to that in sham rats (5.38 ± 0.04 g/dL). The protein concentration in the microsomal suspensions of BUL rats (4.32 ± 0.18 mg protein/mL) was again similar to that in sham rats (4.64 ± 0.17 mg protein/mL). The protein concentration in the SUP of BUL rats (12.2 ± 0.1 mg protein/mL) was also similar to that in sham rats (12.1 ± 0.4 mg protein/mL).

Effect of SUP on the metabolism of metoprolol in liver microsomes: Figure 1 shows the time course of the rate at which 5 $\mu$M metoprolol was metabolized in rat liver microsomes in the absence and presence of SUP. There was no significant difference in the metabolic rate between the liver microsomes of sham and BUL rats (Fig. 1A). After the addition of SUP to the reaction mixture, there was no significant difference between sham and BUL rats in the metabolic rate of metoprolol (Fig. 1B). Figure 2 shows the time course of the rate at which 30 $\mu$M metoprolol was metabolized in rat liver microsomes in the absence and presence of SUP (70% of reaction mixture). The metabolic rate in the liver microsomes of BUL rats at 10 min was statistically different from that in sham rats, because the variances of the measurement were small. However, there was only a marginal difference between sham and BUL rats in the mean rate of metabolism of metoprolol (Fig. 2A).
After the addition of SUP to the reaction mixture, there was no significant difference between sham and BUL rats in the rate at which metoprolol was metabolized (Fig. 2B). These results indicate that the metabolic activity of the liver microsomes in BUL rats is similar to that in sham rats, and that any uremic substance present in the cytosolic fraction of BUL rats does not directly inhibit the metabolism of metoprolol in the liver microsomes.

**Effect of endogenous uremic substances in plasma on the metabolism of metoprolol in liver microsomes:** We examined whether the uremic plasma of BUL rats can inhibit the hepatic metabolism of metoprolol. Figure 3 shows the effects of plasma (70% of reaction mixture) on the metabolism of metoprolol in the liver microsomes. The percentage of metoprolol metabolized in the liver microsomes of sham rats was 52% after the 50-min reaction period, and up to 77% and 82% in the presence of the plasma obtained from sham and BUL rats, respectively (Fig. 3A). Similarly, the percentage metabolized in the liver microsomes of BUL rats was increased approximately 1.3- and 1.4-fold by the addition of the plasma obtained from sham and BUL rats, respectively (Fig. 3B). These findings suggest that some endogenous substances in plasma enhance the metabolism of metoprolol in the liver microsomes, but that the endogenous uremic substances, which accumulate in the plasma of BUL rats, do not affect the metabolic activity of P450 directly.

**Effect of high K⁺ concentration on the metabolism of metoprolol in liver microsomes:** The rate at which metoprolol was metabolized in the liver microsomes was increased by the addition of SUP prepared from BUL and normal rats (Figs. 1 and 2). In order to characterize this mechanism, we evaluated the effect of a high concentration of K⁺, which was included in SUP, on the microsomal metabolism of metoprolol. Table 1 shows the metabolism of metoprolol in 500 μL of reaction mixture after the addition of 350 μL of KHBB (5.9 mM K⁺) or KCl-PB (254 mM K⁺). The percentage of metoprolol metabolized in the liver microsomes of sham rats after the addition of KCl-PB was 1.4-fold that after the addition of KHBB. The percentage metabolized in the liver microsomes of BUL rats after the addition of KCl-PB was 1.3-fold that after the addition of KHBB (Table 1). Therefore, the high concentration of K⁺ in the SUP appeared to be one of the factors affecting the metabolism of metoprolol in the liver microsomes (Figs. 1 and 2).

**Effect of albumin and globulin on the metabolism of metoprolol in liver microsomes:** The rate at which metoprolol was metabolized in the liver microsomes was increased by the addition of plasma (Fig. 3), and there

![Fig. 2. Metabolism of 30 μM metoprolol in rat liver microsomes in the absence (A) and presence (B) of SUP.](image)

Open circles represent the liver microsomes of sham rats, and closed circles represent the liver microsomes of BUL rats. SUP obtained from sham rats was added to the liver microsomes of sham rats. SUP obtained from BUL rats was added to the liver microsomes of BUL rats. Each symbol and bar represents the mean ± S.E. (n = 4). *p < 0.01 compared with microsomes of sham rats.

![Fig. 3. Effect of plasma on the metabolism of metoprolol in liver microsomes of sham (A) and BUL (B) rats.](image)

Plasma obtained from sham or BUL rats was added to the liver microsomes. The final concentration of metoprolol was 5 μM. Each column and bar represents the mean ± S.E. (n = 4,5). **p < 0.01 compared with microsomes alone.
was a report that the metabolism of phenytoin in human liver microsomes was enhanced in the presence of bovine albumin, which is a major protein, as well as globulin in plasma.\(^{20}\) Accordingly, we evaluated the effect of albumin and globulin on the microsomal metabolism of metoprolol. Table 2 shows the unbound fraction and the metabolic rate of metoprolol in the reaction mixture. No significant change was observed in the unbound fraction of metoprolol by the addition of the albumin and globulin solutions (Table 2). On the other hand, the percentage of metoprolol metabolized in the liver microsomes of sham rats was significantly decreased by the addition of human albumin, rat albumin, human globulin, bovine globulin, and rat globulin solutions. These results indicated that the increased metabolic rate of metoprolol in the presence of plasma (Fig. 3) was not due to the increased concentration of albumin and globulin in the reaction mixture (Table 2).

### Discussion

The hepatic extraction of metoprolol is reduced in BUL rats as compared with normal rats.\(^{10}\) In the present study, we investigated the effect of endogenous uremic substances on the hepatic metabolism of metoprolol in BUL rats. Our results suggested that uremic substances which accumulate in BUL rats do not directly inhibit the activity of CYP2D2, which is responsible for the metabolism of propranolol and metoprolol in the rat liver (Figs. 1, 2 and 3).

**Table 1.** Effect of a high K\(^+\) concentration on the metabolism of metoprolol in rat liver microsomes

<table>
<thead>
<tr>
<th>Liver microsomes</th>
<th>% metoprolol metabolized</th>
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<tbody>
<tr>
<td></td>
<td>KHBB</td>
</tr>
<tr>
<td>Sham</td>
<td>55.5 ± 1.2</td>
</tr>
<tr>
<td>BUL</td>
<td>60.7 ± 4.4</td>
</tr>
</tbody>
</table>

KHBB (5.9 mM K\(^+\)) or KCl-PB (254 mM K\(^+\)) was added to the liver microsomes obtained from sham and BUL rats. The final concentration of metoprolol was 5 \(\mu\)M. Values are expressed as the mean ± S.E. \((n = 5)\). *\(p<0.05\) compared with KHBB added.

A number of organic acids such as hippuric acid, indoxyl sulfate, and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid are markedly accumulated in the plasma of uremic patients.\(^{21–23}\) Recently, Yoshitani et al. reported that the uremic serum obtained from BUL rats inhibited the microsomal metabolism of losartan.\(^{14}\) That is, they demonstrated that unbound clearance of losartan in normal rat liver microsomes in the presence of uremic serum (only 10% of reaction mixture) obtained from BUL rats was significantly lower than that in the presence of control serum. In addition, they showed that indoxyl sulfate (final concentration 1 mM) significantly inhibited the metabolism of losartan (10 \(\mu\)M) in a competitive manner in rat hepatic microsomes.\(^{10}\) Losartan is metabolized to an active metabolite (EXP3174) in rats and humans.\(^{24,25}\) CYP2C9 is involved in the conversion of losartan to EXP3174 in human liver microsomes.\(^{26}\) Though the rat CYP2C subfamily is involved in the metabolism of a variety of acidic compounds, the CYP isoform responsible for the metabolism of losartan in rats has not been identified.\(^{14}\) The findings reported by Yoshitani et al. suggested that the plasma concentration of indoxyl sulfate in BUL rats may be high enough to inhibit the CYP isoform responsible for the metabolism of losartan. On the other hand, the metabolic rate of metoprolol in the presence of BUL rat plasma was similar to that in the presence of sham rat plasma, even though a large volume of plasma (70% of reaction mixture) was added to the liver microsomes (Fig. 3). We had assumed that the concentration of endogenous uremic substances might be higher in the liver tissue than in the plasma. However, the metabolic rate of metoprolol in the presence of BUL rat SUP (70% of reaction mixture) was also similar to that in the presence of sham rat SUP (Figs. 1 and 2). The results suggested that the organic acids accumulated in BUL rats do not affect the activity of CYP2D2, which is mainly responsible for the metabolism of cationic drugs.

Terao and Shen\(^6,8\) reported that the presence of an endogenous inhibitor(s) in uremic blood was responsible for the reduced hepatic extraction of \(l\)-propranolol in

**Table 2.** Effects of albumin and globulin on the metabolism of metoprolol in rat liver microsomes

<table>
<thead>
<tr>
<th></th>
<th>Unbound fraction (%)</th>
<th>Metabolic rate (%)</th>
</tr>
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<tbody>
<tr>
<td>Microsomes alone</td>
<td>80.5 ± 1.5 (8)</td>
<td>54.8 ± 2.0 (10)</td>
</tr>
<tr>
<td>+ 4% human albumin solution</td>
<td>79.3 ± 3.3 (4)</td>
<td>27.0 ± 2.5** (5)</td>
</tr>
<tr>
<td>+ 4% bovine albumin solution</td>
<td>80.7 ± 5.0 (4)</td>
<td>43.5 ± 1.9 (5)</td>
</tr>
<tr>
<td>+ 4% rat albumin solution</td>
<td>73.9 ± 1.3 (4)</td>
<td>16.1 ± 5.0* (5)</td>
</tr>
<tr>
<td>+ 2% human globulin solution</td>
<td>86.9 ± 8.3 (4)</td>
<td>38.9 ± 3.3 (5)</td>
</tr>
<tr>
<td>+ 2% bovine globulin solution</td>
<td>88.9 ± 2.8 (4)</td>
<td>29.8 ± 1.5** (5)</td>
</tr>
<tr>
<td>+ 2% rat globulin solution</td>
<td>83.7 ± 4.4 (4)</td>
<td>30.6 ± 4.6** (5)</td>
</tr>
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</table>

Plasma protein (albumin and globulin) dissolved in KHBB was added to the liver microsomes of sham rats. Values are expressed as the mean ± S.E. Values in the parenthesis indicate number of experiment. *\(p<0.05\) and **\(p<0.01\) compared with microsomes alone.
rats with uranyl nitrate-induced acute renal failure. That is, when livers isolated from control rats were perfused with blood from control rats, the extraction ratio of l-propranolol (E₈) was 0.974. Similarly, when livers isolated from rats with acute renal failure were perfused with blood from control rats, the E₈ was 0.970. On the other hand, when livers isolated from control rats were perfused with blood from rats with acute renal failure, there was a significant decrease in the E₈ to 0.927. These findings suggested that the concentration of endogenous uremic substances in uranyl nitrate-induced renal failure rats is high enough to inhibit the CYP2D2 activity. However, it is also possible that an endogenous inhibitor(s) in uremic blood did not inhibit the activity of CYP2D2 directly, but affect the metabolism of l-propranolol by an indirect mechanism. Indeed, we previously found that the decrease in hepatic metabolic activity and the extraction of propranolol and metoprolol in BUL rats was at least partly due to a reduction in the generation of NADPH in the liver. In addition, the mechanism responsible for the decrease in the production rate of NADPH in BUL rats was a decrease in the concentration of endogenous substrate(s) and/or increase in the concentration of endogenous inhibitor(s) for the pentose phosphate pathway.

In the present study, the addition of SUP to liver microsomes resulted in a significant increase in the metabolism of metoprolol (Figs. 1 and 2). That is, the percentage of metoprolol metabolized in the liver microsomes of sham rats in the presence of the SUP of sham rats was approximately 1.5-fold higher than that in the absence of SUP. Similarly, the percentage metabolized in the liver microsomes of BUL rats was in the presence of the SUP of BUL rats was approximately 1.5-fold that in the absence of SUP (Figs. 1 and 2). The increase was at least partly attributable to the high concentration of K⁺ in KCl-PB, which was used for the preparation of SUP (Table 1). Expectedly, the percentage of metoprolol metabolized in the liver microsomes of sham rats after the addition of KCl-PB was 1.4-fold that after the addition of KHBB (Table 1). The precise molecular mechanism is still unclear; however, our finding is consistent with the findings of Tsoulis et al. and Gemzik et al. That is, the 16α-hydroxylation of estrone 3-sulfate catalyzed by cytochrome P450 in liver microsomes in the presence of high concentrations of K⁺ (40–300 mM) was about 1.6-fold that in the liver microsomes without K⁺ ion (control). Though testosterone 7α- and 2α-hydroxylase activity was attenuated at high concentrations of potassium phosphate, testosterone 6β-hydroxylase activity was enhanced.

The rate at which metoprolol was metabolized in the liver microsomes was increased by the addition of plasma (Fig. 3). This finding suggests that some endogenous substances in plasma would enhance the metabolism of metoprolol in the liver microsomes, because the electrolyte composition of KHBB used in the control can be similar to that of blood plasma. Unexpectedly, however, the metabolism of metoprolol in rat liver microsomes was significantly inhibited by the addition of albumin and globulin solutions (Table 2). Several investigators reported that plasma proteins (e.g. albumin and globulin) affect drug metabolizing activity in liver microsomes. Ishii et al. reported that human albumin and human globulin (α-globulin) respectively caused 35% and 71% inhibition of the 4-hydroxylation of debrisoquine in rat liver microsomes, but α₁-acid glycoprotein and human globulin (γ-globulin) had no effect. On the other hand, Tang et al. reported that the metabolism of tolbutamide, which is a substrate of CYP2C9, in human liver microsomes was enhanced in the presence of 0.25% bovine albumin but remained at only 35% of the control level at 1% bovine albumin. Baba et al. recently reported the effect of human albumin on the unbound Km values for tolbutamide, diclofenac and terfenadine metabolism in human liver microsomes. The addition of human albumin to the reaction mixture resulted in smaller unbound Km values for the metabolism of diclofenac and terfenadine by CYP2C9 and CYP3A4, respectively. In contrast, there was no significant change in the unbound Km value for the metabolism of tolbutamide by CYP2C9 in the presence of human albumin. At present, however, the biochemical and/or molecular mechanisms responsible for the change in drug metabolizing activity caused by plasma proteins are unclear. The plasma of both sham and BUL rats significantly enhanced the metabolism of metoprolol in rat liver microsomes (Fig. 3), whereas the purified plasma proteins inhibited the metabolism (Table 2). The finding suggested that some other endogenous substances in rat plasma enhance drug metabolism in liver microsomes. Because the purpose of this study was to evaluate the effect of endogenous uremic substances on drug metabolizing activity in BUL rats, we did not try to identify the substances which are present in the plasma of both normal and BUL rats and enhances the metabolism of metoprolol. However, there is limited information available concerning the endogenous substances responsible for triggering the metabolism of drugs. Komatsu et al. have recently reported that catalase enhances the microsomal oxidative activities of phenytoin. That is, the addition of purified catalase to the reaction mixture increased the rate of formation of a 3′,4′-dihydroxylated metabolite of phenytoin from 3′- and 4′-hydroxylated metabolites. In reconstituted systems containing CYP2C9, the formation of a 3′,4′-
dihyroxylated metabolite of phenytoin was also increased in catalase. However, in the course of the purification of this factor from human livers, increased rates of microsomal formation of 3',4'-dihydroxylated phenoxyin were obtained on the addition of other fractions eluted from the DEAE-Sephacel column. Komatsu et al. proposed that there is another factor that enhances the microsomal formation of 3',4'-dihydroxylated phenytoin.

We have reported the increased intestinal absorption rate of several drugs in rats with experimental renal failure. That is, we found that the intestinal absorption rate of propranolol is significantly increased in rats with cisplatin- and also glycerol-induced renal dysfunction. We also reported that the intestinal absorption rate of tacrolimus is significantly increased in rats with cisplatin-induced renal failure, and that the intestinal absorption rate of ajmaline is significantly increased in rats with uranyl nitrate-induced renal failure. Moreover, Kimura et al. reported that the intestinal absorption rate of sulfanilic acid is increased in rats with HgCl2-induced renal dysfunction and in five-sixths nephrectomized rats. They also reported the increased intestinal absorption rate of many drugs (sulfanilic acid, procainamide ethobromide, cefadroxil, sulfaguanidine, sulfadiazine, quinine, salicylic acid, imipramine, cefadroxil, etc.) in glycerol-induced renal failure rats. These findings suggested that the intestinal barrier function is decreased by the reduced renal function, and that the absorption rate-dependent decrease in the hepatic first-pass clearance of propranolol due to saturation kinetics can be one of the most plausible mechanisms responsible for the increased bioavailability of propranolol in patients with renal failure. In fact, Lowenthal et al. reported that the mean serum concentration of propranolol at 30 minutes (the initial period of absorption) after single oral administration in patients with chronic renal failure was approximately 10-fold higher than that in healthy volunteers.

The BUL model offers the obvious advantage of avoiding the use of toxic agents that can potentially inflict injuries to extrarenal metabolic organs. In addition, reproducible azotemia and good survivability (over a 1- to 2-day period) are achieved, as compared to the other available surgical models of acute renal failure (e.g., bilateral nephrectomy). However, the intestinal absorption rate of propranolol and metoprolol was only slightly increased in BUL rats. The finding suggested that the damage to the intestinal barrier function caused by renal dysfunction may be small in rats with BUL within 28 h after the operation. Accordingly, BUL rats may not be a useful animal model in order to investigate the mechanism responsible for the alteration of intestinal barrier function due to renal dysfunction. On the other hand, the hepatic metabolism of propranolol and metoprolol was decreased in BUL rats, and the decreased hepatic NADPH generation was responsible for the decreased hepatic drug metabolism in BUL rats. The hepatic extraction of propranolol and/or metoprolol has not been investigated in rats with nephrectomy and glycerol-induced renal failure. Further systematic study will be needed to clarify whether the hepatic drug metabolism and the generation of NADPH are altered in experimental renal failure rats other than BUL rats, and to investigate the mechanism responsible for malfunction of the pentose phosphate pathway.

In conclusion, the activity of CYP2D2, which is responsible for the metabolism of propranolol and metoprolol in the rat liver, was not altered by BUL. The uremic substances which may accumulate in BUL rats did not directly inhibit the activity of CYP2D2. These findings suggested that the decrease in the hepatic extraction of propranolol and metoprolol in BUL rats is mainly due to the reduced generation of NADPH in the liver. This may provide new insight into the altered bioavailability of drugs during renal failure.

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