Intestinal Absorption of a β-Adrenergic Blocking Agent Nadolol. II.[1] Mechanism of the Inhibitory Effect on the Intestinal Absorption of Nadolol by Sodium Cholate in Rats

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The mechanism of the inhibitory effect of sodium cholate and its taurine and glycine conjugates on the intestinal absorption of nadolol was investigated in rats. It was found that the uptake of nadolol into the intestinal membrane was inhibited by sodium cholate and its conjugates, but the membrane permeability to nadolol was not affected. The inhibitory effect on nadolol absorption can be interpreted in terms of loss of thermodynamic activity of nadolol due to stable micelle formation, resulting in a decrease of the uptake into the intestinal membrane. Other β-blocking agents also form micellar complexes with sodium cholate, but their micelles are ten times less stable than that of nadolol. It is concluded that the stability of the micellar complex may be an important factor in the inhibition of intestinal absorption of nadolol. The micellar stability seems to be associated with not only the chemical nature of the bile salts but also that of the drug.

Keywords——nadolol; β-adrenergic blocking agent; rat intestinal absorption; sodium cholate; bile salt; inhibitory effect; mechanism; micellar complex; micellar stability

In the previous paper, the intestinal absorption of various β-adrenergic blocking agents in rats has been demonstrated to be consistent with pH-partition theory by using the *in situ* ligated loop method.[1] The absorption of nadolol was found to be inhibited by trihydroxy bile salts, sodium cholate and its taurine and glycine conjugates, but not by dihydroxy bile salts, such as sodium chenodeoxycholate and sodium deoxycholate. However, sodium cholate did not inhibit the absorption of other β-blocking agents. Furthermore, the absorption of nadolol after oral administration was also inhibited by the presence of bile in the intestinal lumen.

The purpose of this paper was to elucidate the mechanism underlying the inhibition of intestinal absorption of nadolol by sodium cholate and its conjugates in rats.

Experimental

Materials——β-Adrenergic blocking agents (nadolol, atenolol, carteolol, pindolol and propranolol) and bile salts (sodium cholate and its taurine and glycin conjugates, sodium chenodeoxycholate and sodium deoxycholate) were prepared as described in the previous paper.[1] Sephadex G-15, G-25 and G-50 of fine grade were purchased from Pharmacia (Uppsala, Sweden). All other chemicals were of analytical-reagent grade.

Exsorption Experiments——Male Wistar rats, weighing about 200 g, were used after fasting overnight. The *in situ* single-pass perfusion technique in rat small intestine was performed according to the method of Kakemi *et al.*[2] The rat was anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The bile duct was ligated in all experiments. Nadolol (15 mg/kg) was intravenously administered from the tail vein, and the small intestine was perfused with pH 6.5 isotonic buffer at a rate of 1 ml/min. The perfusion solution was collected every 5 min, and the exsorption rate was calculated from the amount of drug in the solution. To investigate the effect of sodium cholate and its conjugates on membrane permeability, the small intestine was perfused first with pH 6.5 buffer for 30 min, and then with the buffer containing 20 mM bile salts for the next 30 min.

Pretreatment in Absorption Experiments——Male Wistar rats, weighing about 200 g, were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The intestine was exteriorized through a central mid-line incision, and the bile

duct was ligated. A loop of about 10 cm long was prepared from the upper part of the jejunum by ligature of both ends.[1] The solution of bile salts (0.02 mmol/0.5 ml) was injected into the loop. After 40 min, the loop was washed well with physiological saline to remove bile salts as completely as possible, and then the nadolol solution (0.01 mmol/0.2 ml) was injected into the loop as described in the previous paper.[1]

Uptake of Nadolol into the Intestinal Membrane——Male Wistar rats, weighing about 200 g, were anesthetized with ether. The isolated small intestine was everted by means of a glass rod. A loop of 5 cm long was prepared by ligature of both ends, and incubated at 37 °C in nadolol solution (4 ml, 0.1 mM in pH 6.5 isotonic buffer containing 0—40 mM bile salts). No solution was placed on the serosal side. After 15 min, the everted intestine was removed from the solution, and the uptake of nadolol into the intestinal membrane was calculated from the difference of drug concentrations between the initial and the final incubation media. Nadolol was not found on the serosal side after a 15-min incubation period.

Solubility of Nadolol——The solubility of nadolol was determined in a series of aqueous solutions containing various concentrations of sodium cholate (0—500 mM). An excess amount of nadolol was added to sodium cholate solution (3 ml) in a 10-ml glass-stoppered centrifuge tube. The tube was vigorously shaken at 37 °C for 4 d, and centrifuged. Thereafter, the supernatant liquid was filtered through a 0.45-μm filter (Millipore) to insure that no undissolved solid was present in the sample taken for analysis. The drug concentration and pH of the filtrate were measured.

Measurement of Micellar Interaction——Interactions between β-blocking agents and sodium cholate were determined by the molecular sieve technique of Ashworth and Heard[3] using Sephadex G-15. In the case of nadolol, Sephadex G-25 and G-50 were also used. The drug concentration was 0.1 mM, and sodium cholate concentration was in the range of 0—50 mM.

Apparent Dissociation Constants of Micellar Complexes——The apparent dissociation constants of β-blocking agents–sodium cholate complexes were determined by the high-performance liquid chromatographic (HPLC) method developed by Uekama et al.[4] HPLC was carried out using a HP-1090 liquid chromatograph equipped with an autosampler, a photodiode-array detector and a HP-3392A integrator (Hewlett Packard, Waldbronn, F.R.G.). A stainless-steel column (40 × 4 mm i.d.) packed with Develosil ODS-5 (Nomura Chemical, Aichi, Japan) was used. The mobile phase was pH 6.5 or 7.4 isotonic buffer with various concentrations of sodium cholate (0—15 mM) at a flow-rate of 1 ml/min at 37 °C. The drugs were dissolved in isotonic buffer at a concentration of 10 mM. A 2-μl aliquot of the solution was injected into the column, and the retention time was measured.

Analytical Method——β-Adrenergic blocking agents were determined by HPLC. In the case of the exsorption study, nadolol in the perfusion solution was measured by gas chromatography (GC). HPLC and GC conditions were as described in the previous paper.[1]

Statistical Analysis——Absorption data were tested for statistically significant differences by means of Student's t test. A probability level of $p < 0.05$ was considered statistically significant.

Results and Discussion

The intestinal absorption of nadolol was inhibited by sodium cholate and its taurine and glycine conjugates, which are main components of bile salts.[1] Based on the report on the surfactant effects on drug absorption by Gibaldi and Feldman,[5] there seem to be two possible mechanisms by which sodium cholate could decrease nadolol absorption from the rat small intestine. First, sodium cholate may have a direct effect on the permeability characteristics of the intestinal mucosa, thereby decreasing the absorption of nadolol. Second, the complex formed with nadolol and sodium cholate may show a low absorption rate constant due to the loss of thermodynamic activity, resulting in the decrease of nadolol uptake into the intestinal mucosa.

Membrane Permeability to Nadolol

The effect of sodium cholate on the membrane permeability was investigated by using the drug exsorption technique. As shown in Fig. 1, the rate of exsorption to the small intestine of intravenously administered nadolol was not affected by the addition of 20 mM sodium cholate to the perfusate. Sodium taurocholate and sodium glycocholate (20 mM) also did not affect it. Next, the effect of a 40-min pretreatment with bile salts was examined (Table I). Pretreatment of the intestinal lumen with 0.02 mmol of sodium cholate, sodium taurocholate or sodium glycocholate did not alter the intestinal absorption of nadolol injected into the ligated loop. These results show that sodium cholate and its conjugates do not affect the permeability of
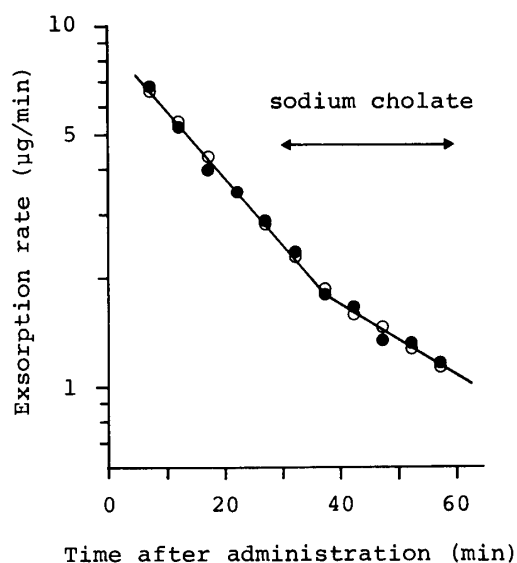
Fig. 1. Effect of Sodium Cholate on the Ex-
sorption Rate of Nadolol to Rat Small In-
testine after Intravenous Administration of 15
mg/kg of Nadolol

○, control; ●, with 20 mM sodium cholate. Sodium
cholate was perfused for 30 min after a 30-min per-
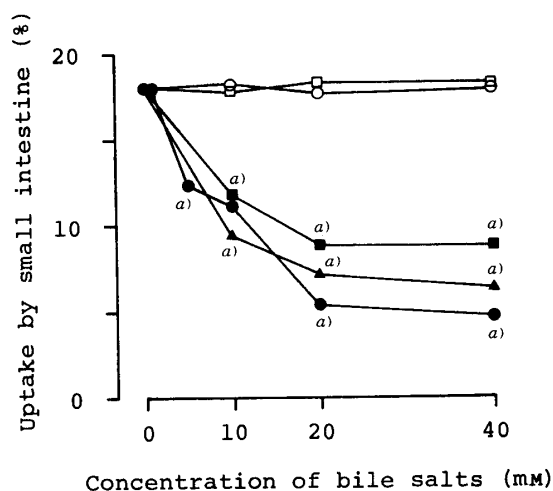fusion of pH 6.5 isotonic buffer. Each point represents
the mean value of 3 rats.

TABLE I. Effect of Pretreatment with Bile
Salts on Nadolol Absorption in Rats
with Jejunum Loop

Bile salts[a]	Absorption in 4 h (%)[b]
None	71.5 ± 1.7
Sodium cholate	70.3 ± 5.5
Sodium glycocholate	69.7 ± 4.0
Sodium taurocholate	68.2 ± 3.9

Nadolol (0.01 mmol) was administered into the ligated
loop. a) The intestinal lumen was pretreated with 0.02 mmol
bile salts for 40 min. b) Results are expressed as the
mean $\pm$ S.E. of 4—5 rats. No statistically significant difference
was observed.

Fig. 2. Effect of Bile Salts on the Uptake of
Nadolol by Rat Small Intestine

●, sodium cholate; ■, sodium glycocholate; ▲,
sodium taurocholate; ○, sodium chenodeoxycholate;
□, sodium deoxycholate. The concentration of
nadolol was 0.1 mM. Each point represents the mean
value of triplicate experiments. a) $p < 0.01$, compared
to the control.

the intestinal mucosa to nadolol.

Uptake of Nadolol into the Intestinal Membrane

The effect of bile salts on the uptake of nadolol into the intestinal membrane was studied.
The results are shown in Fig. 2. The uptake of nadolol was markedly reduced by sodium
cholate and its conjugates at concentrations over 5 mM, although the reduction was not
observed at a concentration of 1 mM sodium cholate. The inhibitory potencies were in the
order of sodium cholate, sodium taurocholate and sodium glycocholate, in good agreement
with the order of effects on nadolol absorption in the ligated jejunum loop.[1] The uptake of
nadolol was not affected by the addition of sodium chenodeoxycholate or sodium de-
oxycholate, which had no inhibitory effect on nadolol absorption.[1] Similar results were also
observed by using intestinal mucosa alone. These results suggest that the inhibition of nadolol
absorption occurs as a result of a reduction in the uptake of nadolol into the intestinal
membrane.

Interaction of Nadolol with Sodium Cholate

Bile salts are well known to be physiological surface-active agents, and appear to be important in the intestinal absorption of lipids, cholesterol and fat-soluble vitamins.[6] They also affect the absorption of drugs based on micellar complexation.[5] Therefore, we investigated the micellar interaction between nadolol and sodium cholate, since the effects of two conjugated trihydroxy bile salts, sodium taurocholate and sodium glycocholate, on the intestinal absorption of nadolol were similar to that of an unconjugated one, sodium cholate.[1]

Figure 3 shows the solubility of nadolol in water at 37 °C at various concentrations of sodium cholate. The pH values of the saturated solution were constant at 10.9—11.0. The solubility of nadolol increased linearly with sodium cholate concentration, with a deflection point at 20 mM sodium cholate, after the critical micellar concentration (cmc) has been exceeded. The cmc value was approximately 3 mM at pH 10.9—11.0. The result indicates that nadolol forms two micellar complexes with sodium cholate, corresponding to concentrations of 3—20 mM and >20 mM. The two slopes of the linear portion of the solubilization curve, obtained after cmc, correspond to the ratios of micellar nadolol to micellar cholate. The molar ratios of nadolol to sodium cholate were 1 : 4 (3-20 mM sodium cholate) and 1 : 6 (>20 mM). Examination by the molecular sieve technique at pH 6.5 also showed two micellar complexes of nadolol and sodium cholate (Fig. 4). Namely, apparent micelle formation took place at 5 mM and 20 mM sodium cholate when Sephadex G-15 and Sephadex G-25 were used, respectively. In the case of Sephadex G-50, no apparent micelle formation was found. The results are in good agreement with the findings obtained by the solubility method. The apparent molecular weights of the two nadolol–sodium cholate micelles seemed to be in the ranges of 1500—5000 (3—20 mM sodium cholate) and 5000—30000 (>20 mM), indicating apparent aggregation numbers of 1—2 and 3—10, respectively. However, from the viewpoint of inhibitory effect on nadolol absorption, the formation of two micellar complexes may not be significant, because the uptake of nadolol into the intestinal membrane was significantly reduced by sodium cholate even at a concentration of 5 mM, as shown in Fig. 2. It is well known that bile salts form different micellar complexes themselves[7] or with other compounds such as p-xylene,[8] and lecithin[9] (so-called primary and secondary micelles).

The above two methods indicate that the cmc values of nadolol–sodium cholate micelle are similar (about 3 mM) at pH 6.5 and 11.0, which is consistent with the data on reduced uptake of nadolol into the intestinal membrane (Fig. 2). This concentration is sufficiently
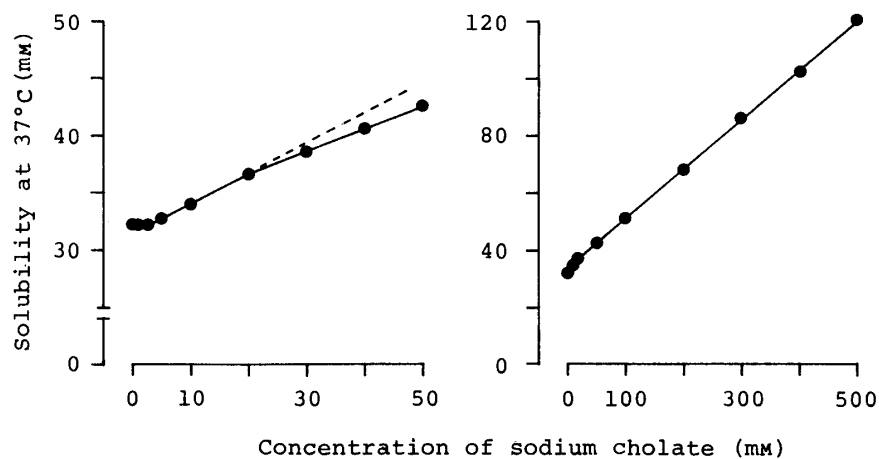

Concentration of sodium cholate (mM)

Fig. 3.   Effect of Sodium Cholate on the Solubility of Nadolol

Left, sodium cholate concentrations of 0—50 mM; right, concentrations of 0—500 mM. Each point represents the mean value of duplicate experiments.
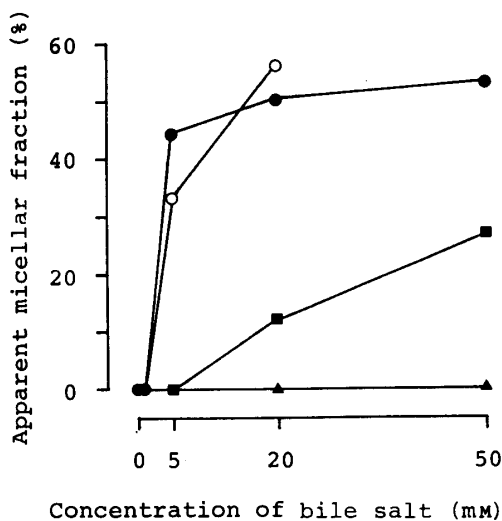
Fig. 4. Interaction of Nadolol with Bile Salts Examined by the Molecular Sieve Method

Apparent micellar fraction (%) of sodium cholate was measured on Sephadex G-15 (●), G-25 (■) and G-50 (▲); that of sodium chenodeoxycholate was measured on Sephadex G-15 (○). The nadolol concentration was 0.1 mM. Each point represents the mean value of triplicate experiments.
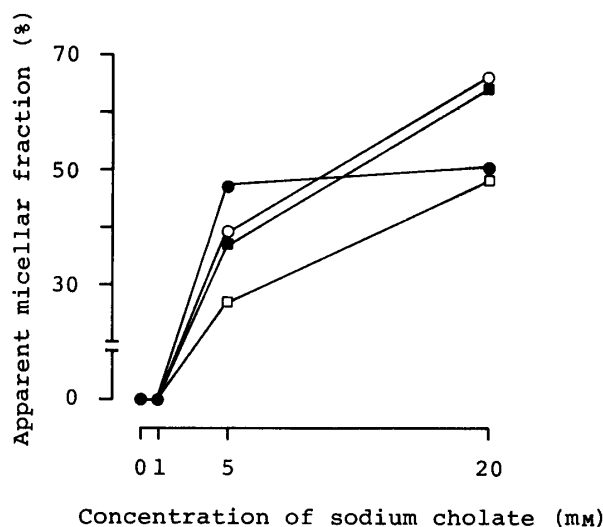
Fig. 5. Interaction of β-Adrenergic Blocking Agents with Sodium Cholate Examined by the Molecular Sieve Method (Sephadex G-15)

●, atenolol; ■, carteolol; ○, pindolol; □, propranolol. The drug concentration was 0.1 mM. Each point represents the mean value of triplicate experiments.

TABLE II. Apparent Dissociation Constants of β-Adrenergic Blocking Agent-Bile Salt Micellar Complexes

Drug	Bile salt	Dissociation constant (mol)	
		pH 6.5	pH 7.4
Nadolol	Sodium cholate	2.3×10^{-3}	1.0×10^{-3}
	Sodium chenodeoxycholate	38.5×10^{-3}	10.3×10^{-3}
Atenolol	Sodium cholate	26.3×10^{-3}	9.8×10^{-3}
Carteolol	Sodium cholate	55.6×10^{-3}	13.4×10^{-3}
Pindolol	Sodium cholate	29.4×10^{-3}	12.5×10^{-3}
Propranolol	Sodium cholate	31.3×10^{-3}	—

lower than that in the proximal intestine, which is considered to be of the order of 40 mM.[5,6] This suggests that the inhibition of nadolol absorption occurs as a result of micelle formation, even if the biliary secretion of sodium cholate is less than one-tenth of normal. The biliary secretion of sodium cholate in hepato-biliary diseases may increase or decrease 2—3 fold depending on the alteration of metabolism and pool size of bile salts.[6] It is therefore presumed that the intestinal absorption of nadolol remains inhibited even in patients with hepato-biliary diseases.

Micelle formation of lipolytic products by bile salts is important for normal fat digestion and absorption,[6] but the micellar complex is considered not to be absorbed intact,[10] presumably due to its relatively high molecular weight and reduced lipophilicity. Nadolol was found to form a micellar complex with sodium chenodeoxycholate (Fig. 4). As described in the previous paper,[1] the intestinal absorption of nadolol was not inhibited by sodium chenodeoxycholate. Moreover, other β-adrenergic blocking agents, such as atenolol, carteolol, pindolol and propranolol, also formed micellar complexes with sodium cholate (Fig. 5).

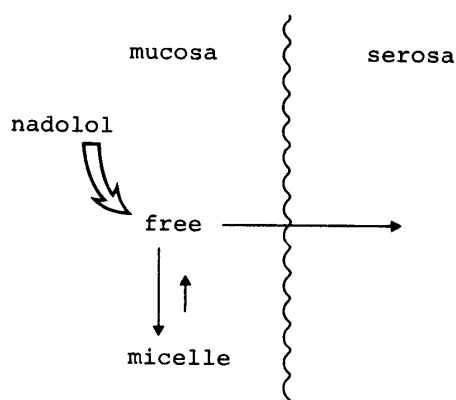mucosa   serosa

nadolol

free ⟶

↓ ↑

micelle

Fig. 6.   Schematic Representation of the Absorption Behavior of Nadolol in the Intestinal Lumen

No inhibition of the absorption of these drugs was observed.[1] The percentages of micellar complex formation of nadolol and other β-blocking agents at 20 mM sodium cholate were similar (in the range of 50—65%). On the basis of these results, the micellar complex must dissociate during the process of drug absorption, and the specific inhibition of nadolol absorption by sodium cholate can not be explained simply by micelle formation itself. The stability of primary micelles of nadolol and other β-blocking agents was therefore studied. The results are presented in Table II. The apparent dissociation constants of nadolol–sodium cholate micelle at pH 6.5 and 7.4 were 2.3×10^{-3} and 1.0×10^{-3} mol, respectively, being one-tenth or much smaller than those of other β-blocking agents–sodium cholate micelles at both pHs. These results suggest that nadolol–sodium cholate micelle is more stable than the other micelles.

In conclusion, the inhibitory effect of sodium cholate and its conjugates on nadolol absorption can be interpreted in terms of loss of thermodynamic activity of nadolol due to stable micelle formation, resulting in the decrease of drug uptake into the intestinal membrane. The following mechanism underlying the inhibition of intestinal absorption of nadolol in the intestinal lumen can be proposed, as illustrated in Fig. 6. Generally, a β-adrenergic blocking agent after oral administration forms a micellar complex with sodium cholate and its conjugates, and the free drug and micellar complex attain equilibrium in the intestinal lumen. Under these conditions, nadolol is hardly dissociated from the micelles, even though free nadolol is absorbed, this results in the specific inhibition of nadolol absorption. However, the other β-blocking agents are easily dissociated from the micelles due to the lower stability of the complexes, and thus are absorbed. Kimura et al. reported similar inhibitory effects on the absorption of imipramine and quinine as a result of stable micellar complex formation with sodium taurodeoxycholate or sodium taurocholate.[11,12]

The above proposed behavior of nadolol in the intestinal lumen led to the pharmacokinetic model shown in Chart 1. In the model, all the rate processes (k_{ij}) are assumed to be first-order. Plasma levels of nadolol (C) in compartment 2 at time t after oral administration are given by the following equations;

$$C = (D/V)k_{02}[(k_{10}+k_{15}-\alpha)\exp(-\alpha(t-t_0))/(\beta-\alpha)/(k_{24}-\alpha)$$

$$+(k_{10}+k_{15}-\beta)\exp(-\beta(t-t_0))/(\alpha-\beta)/(k_{24}-\beta)$$

$$+(k_{10}+k_{15}-k_{24})\exp(-k_{24}(t-t_0))/(\alpha-k_{24})/(\beta-k_{24})] \tag{1}$$

$$\alpha+\beta = k_{01}+k_{02}+k_{03}+k_{10}+k_{15} \tag{2}$$

$$\alpha\beta = k_{01}k_{15}+(k_{02}+k_{03})(k_{10}+k_{15}) \tag{3}$$

where D is the dose, V the volume of distribution and t_0 the lag time. V (5.3 l/kg) and k_{24} (0.829 h^{-1}) were calculated from the plasma levels after intravenous administration.[1] Plasma

levels of nadolol after oral administration $(20\,mg/kg)^{1)}$ were analyzed on the basis of Eqs. 1—3 by means of the non-linear least-squares method $(MULTI).^{13)}$ The values of rate constants and lag time were estimated to be $k_{01} = 97.5\,h^{-1}$, $k_{02} = 12.2\,h^{-1}$, $k_{03} = 8.16\,h^{-1}$, $k_{10} = 1.88\,h^{-1}$, $k_{15} = 1.02\,h^{-1}$ and $t_0 = 0.9\,h$. The theoretical curve obtained by using these k values was in good agreement with the experimental data (Fig. 7). Moreover, the amount of nadolol absorbed after oral administration was estimated to be 22% of the dose, which is similar to that (18%) determined from the area under the plasma concentration curve divided by that after intravenous dosing.[1] The unabsorbed amounts excreted as free nadolol and micellar nadolol were calculated to be 15% and 63%, respectively. If nadolol dissociated easily from the micellar complex, as in the case of other β-blocking agents, increased plasma levels would be expected. In fact, peak plasma levels of nadolol can be estimated to be 2.6 times higher than the experimental values (broken line in Fig. 7), if the dissociation rate constant of micellar nadolol (k_{10}) is increased ten-fold. The result shows that the micellar complex of nadolol–sodium cholate is stable, and that nadolol is hardly dissociated from the micellar complex. Therefore, the proposed model (Fig. 6) may well interpret the inhibited absorption behavior of nadolol in the intestinal lumen.
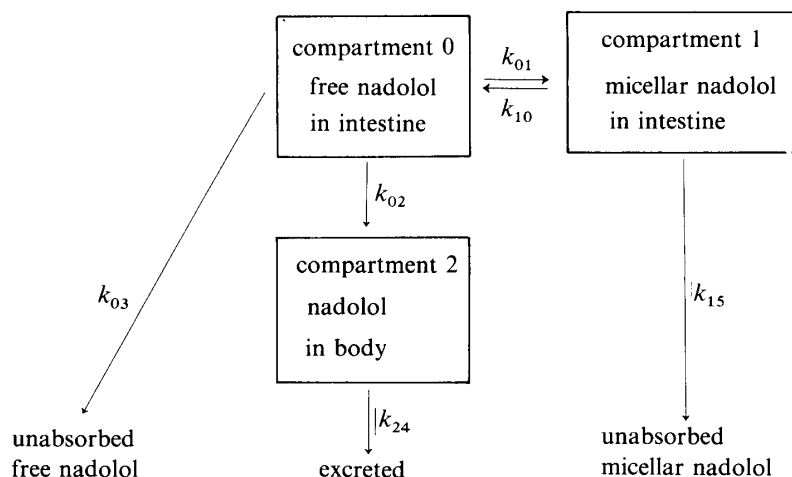

Chart 1. Pharmacokinetic Model for the Absorption Behavior of Nadolol in the Intestinal Lumen
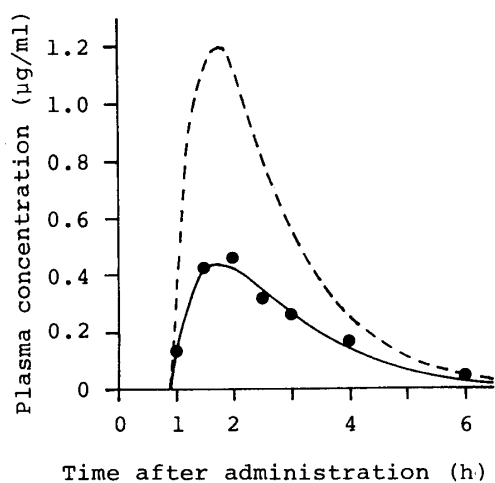

Fig. 7. Observed and Predicted Plasma Levels of Nadolol in Rats after Oral Administration of 20 mg/kg of Nadolol

Each point represents the mean value of 4—5 rats. The solid line is the theoretical curve calculated with the parameter values described in the text according to the model shown in Chart 1. The broken line represents the curve which would be obtained by increasing the dissociation rate constant of micellar nadolol (k_{10}) ten times.

From the observed intestinal absorption behavior of the β-adrenergic blocking agents examined, it is concluded that the stability of the micellar complex may be an important factor for the absorption-decreasing effect of bile salts, as in the case of nadolol. The micellar

stability seems to be associated with not only the chemical nature of bile salts but also that of the drug.

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