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# Intestinal Absorption of a β-Adrenergic Blocking Agent Nadolol. III.[1] Nuclear Magnetic Resonance Spectroscopic Study on Nadolol–Sodium Cholate Micellar Complex and Intestinal Absorption of Nadolol Derivatives in Rats

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The stable micellar complex formation between nadolol and sodium cholate was studied. The proton nuclear magnetic resonance spectroscopic study showed that the signals of protons in the hydrophobic site of cholic acid, *i.e.*, the methine signals of C-7 and C-12, and the methyl signals of C-18 and C-19, exhibited rather larger shifts than those of the other protons. The longitudinal relaxation times of the aromatic ring protons (C-4, C-6, C-7 and C-8) of nadolol were shortened by about 10% in the presence of sodium cholate, indicating that the aromatic ring protons were more immobilized upon complex formation. These results suggest that the hydrophobic protons of sodium cholate and nadolol interact with each other, and complex formation with the hydrophobic protons inside and the hydrophilic protons outside may occur in aqueous solution. The intestinal absorption of four nadolol derivatives was examined by the *in situ* ligated loop method in rat jejunum in the presence of sodium cholate. The absorption of the *trans*-2,3-diol isomer was inhibited as effectively as that of nadolol. The absorption of the 2- and 3-monohydroxy derivatives and *cis*-2,3-dimethoxy derivative was inhibited by sodium cholate at 1 h after dosing, but no inhibition was observed at 4 h. This absorption behavior of nadolol derivatives was well correlated with the apparent dissociation constants of their micellar complexes with sodium cholate. It is concluded that the stability of the complexes of β-adrenergic blocking agents with sodium cholate depends upon the steric features of both hydrophobic and hydrophilic parts of the drug molecules. The hydrophilic *cis*-2,3-diol moiety of nadolol plays an important role in stabilizing the micellar complex with sodium cholate.

**Keywords**——nadolol; sodium cholate; rat intestinal absorption; inhibitory effect; micellar stability; hydrophobic–hydrophilic interaction; micelle $^1$H-NMR; relaxation time

The intestinal absorption of the β-adrenergic blocking agent nadolol has been demonstrated by the *in situ* ligated loop method in rats to be inhibited by trihydroxy bile salts, sodium cholate and its taurine and glycine conjugates, but not by dihydroxy bile salts.[2] The specific inhibitory effect on nadolol absorption was interpreted in terms of the loss of thermodynamic activity of nadolol due to stable micelle formation with sodium cholate, resulting in the decrease of the uptake of nadolol into the intestinal membrane.[1] Other β-blocking agents also formed micellar complexes with sodium cholate, but their complexes were ten times less stable than that of nadolol. Thus, the stability of the micellar complex was considered to be an important factor affecting the inhibition of the intestinal absorption of nadolol, and seemed to depend not only on the chemical nature of bile salts but also on that of the drug.

There are many reports on the chemical nature of micellar complexes with bile salt itself[3–6] or bile salt–lecithin–cholesterol system,[7–9] but little information is available on the nature of bile salt–drug micellar complexes, except for the solubilizing effect.[10] In the case of

nadolol–sodium cholate, it has been shown that there are two kinds of micellar complexes depending on the sodium cholate concentration.[1] The primary micelle (3—20 mM sodium cholate) showed a molar ratio (nadolol : sodium cholate) of 1 : 4, an apparent molecular weight of 1500—5000 and an apparent aggregation number of 1—2, and the corresponding values for the secondary micelle (>20 mM sodium cholate) were 1 : 6, 5000—30000 and 3—10. However, it is not clear why nadolol forms a more stable micellar complex than other β-blocking agents.

The purpose of this study was to examine the stable micellar complex formation between nadolol and sodium cholate. First, a $^1$H-nuclear magnetic resonance (NMR) spectroscopic study was performed, and then the *in situ* intestinal absorption of nadolol derivatives was examined in the presence of sodium cholate.

## Experimental

**Materials**——Nadolol (*cis*-5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-2,3-naphthalenediol, I) was a gift from the Squibb Institute (NJ, U.S.A.). The following nadolol derivatives were prepared by the method of Condon *et al.*[11]: *trans*-2,3-dihydroxy isomer of nadolol (II), 2-monohydroxy derivative (III), 3-monohydroxy derivative (IV) and *cis*-2,3-dimethoxynadolol (V). Sodium cholate was purchased from Nakarai Chemical Co. (Kyoto, Japan). All other chemicals were of analytical-reagent grade.

**$^1$H-NMR Spectroscopy**——Three samples were prepared for NMR measurement: nadolol (10 mM), sodium cholate (10 mM) and a mixture of nadolol (10 mM) and sodium cholate (10 mM). They were dissolved in deuterium oxide, and pD was adjusted to 7.0 with isotonic buffer. NMR spectra were recorded at 23 °C on a Bruker WH-270 (Karlsruhe, F.R.G.) equipped with an ASPECT-2000 minicomputer. Sodium 4,4-dimethyl-4-silapentane-1-sulfonate was used as an internal reference for chemical shifts. The longitudinal relaxation time ($T_1$) was measured by the inversion recovery method ($\pi$-$\tau$-$\pi/2$).

***In Situ* Experiments on Absorption from Rat Jejunum Loop**——Male Wistar rats weighing about 200 g were used after fasting overnight. The procedure for the jejunum loop preparation was described in the previous paper.[2] The drug (0.01 mmol/0.2 ml) was injected into the loop (5 cm long) with or without an equimolar amount of sodium cholate. At 1 and 4 h after the injection, the contents in the loop were drained off with water followed by 0.01 N hydrochloric acid solution, and the volume was adjusted to 50 ml. The absorption of drugs was determined as the difference between amount remaining and amount administered in each loop.

**Micellar Interaction of Nadolol Derivative–Sodium Cholate**——Interactions between nadolol derivatives and sodium cholate were measured by the molecular sieve technique of Ashworth and Heard,[12] and the procedures were described in the previous paper.[1] The apparent dissociation constant of nadolol derivative–sodium cholate micellar complex was determined by the high-performance liquid chromatographic (HPLC) method.[1]

**Analytical Method**——Nadolol derivatives were determined by HPLC under the conditions described in the previous paper.[2] Briefly, a reversed-phase column was used (μBondapak C18, 10 μm, 30 cm × 4 mm i.d., Waters), with a mobile phase of methanol–1/15 M potassium dihydrogenphosphate (2 : 3—1 : 1) at a flow rate of 1 ml/min. Detection was by ultraviolet absorbance measurement at 280 nm.

**Statistical Analysis**——Absorption data were compared for statistical significance by using Student's *t* test. A probability level of $p<0.05$ was considered statistically significant.

## Results and Discussion

### NMR Study on Nadolol–Sodium Cholate Micellar Complex

The difference spectrum between nadolol–sodium cholate (both 10 mM) solution and sodium cholate (10 mM) solution is shown in Fig. 1. The methine signals of C-7 and C-12 of cholic acid and the methyl signals of C-18 and C-19, positioned in the hydrophobic site of cholic acid, exhibited rather larger shifts than those of the other protons of cholic acid. If there were no interactions between nadolol and sodium cholate, signals from cholic acid should be cancelled out, and only the signals from nadolol would be observed. However, not only the signals from nadolol, but also the shifted and broadened signals of cholic acid were seen in the difference spectrum. This is direct evidence of the interaction of sodium cholate with nadolol in aqueous solution. The $T_1$ measurements allowed us to identify the protons of nadolol

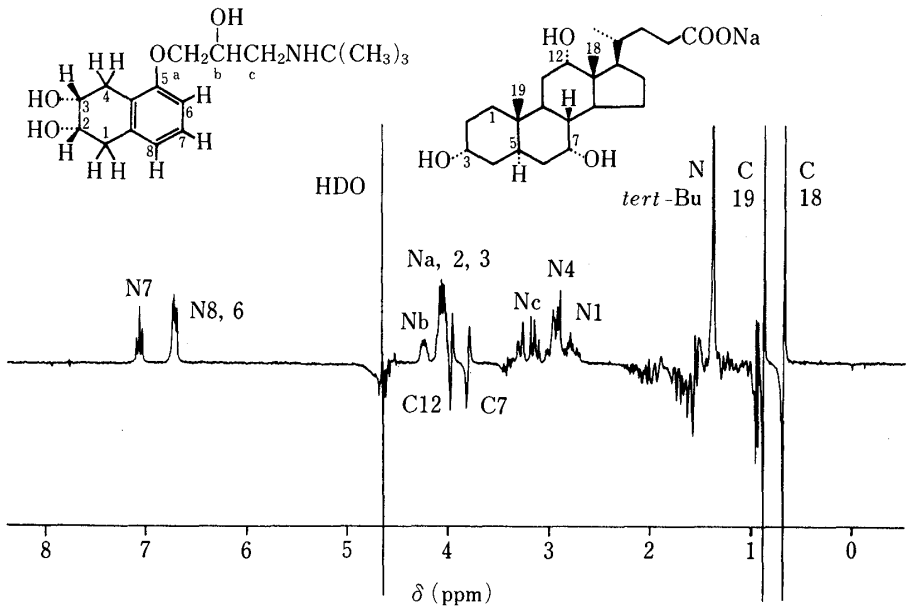



Fig. 1. $^1$H-NMR Difference Spectrum between Nadolol–Sodium Cholate and Sodium Cholate

Sample: nadolol (10 mM) and sodium cholate (10 mM), pD 7.0.
Reference: sodium cholate (10 mM), pD 7.0.
Symbols, N and C, represent the proton signals from nadolol and sodium cholate, respectively.

TABLE I. Longitudinal Relaxation Time of Proton Signals of Nadolol

| | Longitudinal relaxation time (s)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | C-1 | C-4 | C-6 | C-7 | C-8 | C-b | C-c | C-c′ | *tert*-Butyl |
| $T_1$[b] | 0.44 | 0.41 | 1.31 | 1.34 | 0.97 | 1.21 | 0.36 | 0.41 | 0.56 |
| $T_1'$[c] | 0.45 | 0.37 | 1.16 | 1.23 | 0.89 | 1.12 | 0.37 | 0.41 | 0.56 |
| $T_1'/T_1$ | 1.02 | 0.90 | 0.89 | 0.92 | 0.92 | 0.93 | 1.03 | 1.00 | 1.00 |

*a*) Values were measured at 23 °C by the inversion recovery method. The carbon numbering is shown in Fig. 1. *b*) Nadolol (10 mM), pD 7.0. *c*) Nadolol (10 mM) and sodium cholate (10 mM), pD 7.0.

participating in the complex formation (Table I). In the presence of sodium cholate, $T_1$'s of the C-4, C-6, C-7 and C-8 protons of nadolol were shortened by about 10% from those in the absence of sodium cholate, while the $T_1$ values of protons of the side chain (C-c, C-c′ and *tert*-butyl protons) showed little change. These results indicate that the aromatic ring protons of nadolol are more immobilized upon complex formation, and the mobilities of protons of the side chain are not altered. Accordingly, the hydrophobic portions of sodium cholate and nadolol interact with each other. In the aqueous medium, complex formation with the hydrophobic portion inside and the hydrophilic portion outside seemed to be preferred. This type of complex formation has been proposed for bile salt itself[3] or for bile salt–lecithin micellar complex.[7]

The results of the NMR spectroscopic study are consistent with previous work[1] showing the micellar complex formation of other β-adrenergic blocking agents with sodium cholate. Other β-blocking agents which also have a hydrophobic portion (the aromatic ring) and a hydrophilic portion (the side chain) should be able to form similar complexes. In practice, the NMR study of the propranolol–sodium cholate micellar complex gave results similar to those obtained with nadolol: namely, the signals of C-7, C-12, C-18 and C-19 of cholic acid

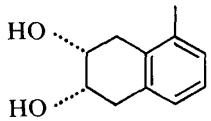
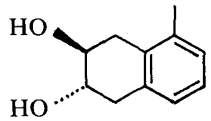
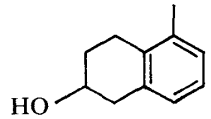
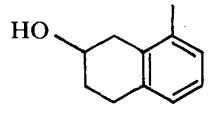
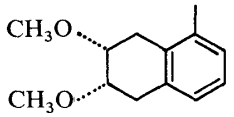
exhibited large shifts, and $T_1$'s of the aromatic ring protons of propranolol were shortened. However, the nadolol micellar complex was at least ten times more stable than the micellar complexes of other β-blocking agents.[1] Therefore, the stability of complexes of β-blocking agents with sodium cholate may depend on the steric features of both hydrophobic and hydrophilic parts of the drug molecules. The hydrophilic *cis*-2,3-diol in the 1,2,3,4-tetrahydronaphthalene ring of nadolol seems to play an important role in stabilizing the micellar complex with sodium cholate, while other β-blocking agents do not have such hydrophilic groups other than the side chain.

**Intestinal Absorption of Nadolol Derivatives**

To confirm the conclusions based on the NMR spectroscopic study, the intestinal absorption behavior of the following derivatives of nadolol was studied and compared with that of nadolol (I): *trans*-2,3-diol derivative (II), 2-monohydroxy derivative (III), 3-monohydroxy derivative (IV) and *cis*-2,3-dimethoxynadolol (V). Their structures are shown in Table II. These derivatives were selected to examine which functional groups contribute predominantly to the stability of nadolol–sodium cholate micellar complex: the diol as a whole or a part of the diol (the monohydroxy group and the lone pair of the oxygen atom). The molecular sieve technique using Sephadex G-15 showed that all nadolol derivatives formed micellar complexes with sodium cholate, and the percentage of the micellar complex formation of these derivatives (50—60%) was similar to that of nadolol and other β-blocking agents.[1]

The intestinal absorption of nadolol derivatives at 1 and 4 h after injection into a ligated loop of rat jejunum was determined in the presence or absence of equimolar amounts of sodium cholate (Table III). At 1 h after the injection into the loop, the absorption of all derivatives was inhibited by sodium cholate. However, at 4 h after the injection, the inhibition

TABLE II. Structures and Partition Coefficients of Nadolol Derivatives

| Drug | OH<br>R–$OCH_2CHCH_2NHC(CH_3)_3$ | Partition[a] coefficient |
|---|---|---|
| I (nadolol) |  | 0.035 |
| II |  | 0.063 |
| III |  | 0.64 |
| IV |  | 0.24 |
| V |  | 0.70 |

a) *n*-Octanol/pH 7.4 isotonic buffer.

TABLE III. *In Situ* Intestinal Absorption of Nadolol and Its Derivatives in Rat Jejunum Loop and Apparent Micellar Dissociation Constants with Sodium Cholate at pH 6.5

| Drug[a] | Sodium cholate | Absorption (% of dose)[b] | | Dissociation constant (mol) |
|---|---|---|---|---|
| | | 1 h | 4 h | |
| I (nadolol) | − | 31.5±2.0 | 71.5±1.7 | |
| | + | 23.6±1.3[c] | 55.2±1.2[d] | $2.3\times10^{-3}$ |
| II | − | 45.5±4.2 | 92.2±1.7 | |
| | + | 29.2±3.6[c] | 49.5±4.7[d] | $2.1\times10^{-3}$ |
| III | − | 90.1±2.2 | 99.4±0.4 | |
| | + | 66.4±1.7[d] | 98.2±0.8 | $4.7\times10^{-3}$ |
| IV | − | 81.5±2.9 | 93.6±0.7 | |
| | + | 60.2±4.2[c] | 94.5±1.2 | $5.6\times10^{-3}$ |
| V | − | 96.4±1.6 | 99.2±0.5 | |
| | + | 75.3±2.7[d] | 98.6±1.1 | $4.8\times10^{-3}$ |

*a*) Each drug (0.01 mmol/rat) was injected into the jejunum loop with (+) or without (−) sodium cholate (0.01 mmol). *b*) Results are expressed as the mean±S.E. of 4—5 rats. *c*) $p<0.01$, *d*) $p<0.001$, compared with the value without sodium cholate.

was maintained only for nadolol and its *trans*-isomer. No inhibition was observed on the absorption of compounds III, IV and V. These results show that the intestinal absorption behavior of β-adrenergic blocking agents in the presence of sodium cholate can be classified in three groups: first, drugs such as nadolol and its *trans*-isomer, the absorption of which is inhibited by sodium cholate; second, drugs such as compounds III, IV and V, the absorption of which is inhibited only for a short time; third, drugs such as atenolol, carteolol, pindolol and propranolol, the absorption of which is not inhibited.[2] These phenomena were well correlated with the apparent dissociation constant of the drug–sodium cholate micellar complex, as shown in Table III or in the previous paper.[1] The first group has a relatively smaller dissociation constant of $2\times10^{-3}$ mol at pH 6.5. The second group has a moderate constant of about $5\times10^{-3}$ mol, and the third group a large constant of more than $20\times10^{-3}$ mol. It may therefore be concluded that the stable micellar complex formation between nadolol and sodium cholate is due to the 2,3-diol moiety in the 1,2,3,4-tetrahydronaphthalene ring of nadolol.

It was reported that the absorption of imipramine in rats was inhibited by taurocholate due to stable micellar complex formation, based on *in situ* recirculation studies.[13] To compare the absorption behavior of imipramine with that of β-adrenergic blocking agents in the presence of sodium cholate, the intestinal absorption of imipramine was studied in rat jejunum by the same *in situ* ligated loop method. The absorption behavior of imipramine corresponded to that of the second group described for β-blocking agents. Namely, at 1 h after injection into the loop, the absorption was inhibited by sodium cholate (from 85.7% to 20.8% of the dose), but at 4 h no inhibition was observed (97.2% and 96.8%). Moreover, the apparent dissociation constant of imipramine–sodium cholate micellar complex was $5.2\times10^{-3}$ mol, which is similar to that of the drugs in the second group. Consequently, the inhibitory effect of sodium cholate on the absorption of drugs seems to depend upon the stability of the drug–sodium cholate micellar complex.

It is well known that many drugs form complexes in the intestinal lumen with other drugs, bile salts, metallic ions and components in the pharmaceutical dosage form, such as binders, suspending agents and surfactants,[14] and in some cases the intestinal absorption is retarded. Typical examples are nadolol with sodium cholate and tetracyclines with metallic ions ($Fe^{2+}$, $Mg^{2+}$ and $Ca^{2+}$).[15] In these cases, the free drug is hardly dissociated from the

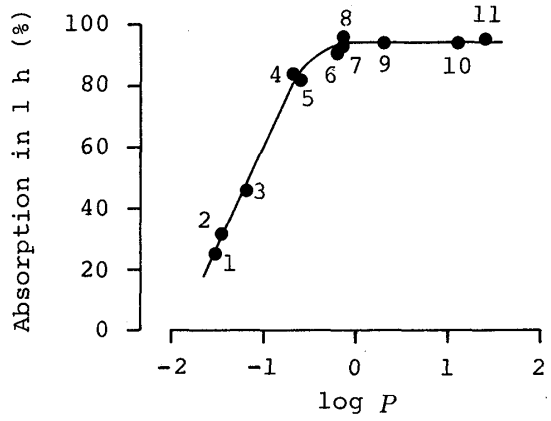



Fig. 2. Correlation between the Percentage Absorption at 1 h after Injection into the Rat Jejunum Loop and log $P$ for $\beta$-Adrenergic Blocking Agents

1, atenolol; 2, nadolol; 3, II; 4, carteolol; 5, IV; 6, III; 7, pindolol; 8, V; 9, oxprenolol; 10, alprenolol; 11, propranolol.

Dose of drugs was 0.01 mmol/rat. Each point represents the mean value of 4—5 rats.

complex, leading to the reduced intestinal absorption. On the other hand, in the case of other $\beta$-adrenergic blocking agents, such as atenolol, carteolol, pindolol and propranolol, the free drug is easily dissociated from the complex, since it is less stable. Therefore, it is concluded that the reduction of the intestinal absorption may not be due to the complex formation, but due to the stability of the complex formed.

In our previous paper on intestinal absorption behavior, we found that the seven $\beta$-adrenergic blocking agents including nadolol seemed to be absorbed from the rat jejunum loop proportionally to their partition coefficients ($P$).[2] The degrees of ionization of these agents in the intestinal lumen were essentially the same, because of the relatively narrow range of $pK_a$ (9.5—9.7).[16] The intestinal absorption of seven agents was compared with that of four nadolol derivatives. Figure 2 shows a plot of the percentage absorption 1 h after injection into the ligated loop of rat jejunum against log $P$. The relatively hydrophilic drugs ($P<0.3$, atenolol, nadolol, II, carteolol and IV) showed a linear increase in the absorption with log $P$. A plateau region was observed for the relatively lipophilic drugs ($P>0.6$, III, pindolol, V, oxprenolol, alprenolol and propranolol). Therefore, it was further confirmed that the intestinal absorption of $\beta$-blocking agents is consistent with the pH-partition theory. Similar behavior was reported by Schoenwald and Huang[16] for $\beta$-blocking agents in the rabbit eye.

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