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Intestinal Absorption of a β-Adrenergic Blocking Agent Nadolol. I. Comparison of Absorption Behavior of Nadolol with Those of Other β-Blocking Agents in Rats

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Intestinal absorption of nadolol, a new long-acting β-adrenergic blocking agent, was compared with those of six other β-blocking agents by the *in situ* ligated loop method in rats. It was found that nadolol was well absorbed from the duodenum, jejunum, ileum and colon, but not the stomach. The *in situ* intestinal absorption of β-blocking agents including nadolol was consistent with pH-partition theory. The absorption of nadolol was, however, strongly 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. An inhibitory effect on the absorption of nadolol was also found *in vivo* in rats with bile duct ligation. No inhibition of the absorption of other β-blocking agents by sodium cholate was observed. The results suggest a specific interaction between nadolol and trihydroxy bile salts, especially sodium cholate.

Keywords——nadolol; β-adrenergic blocking agent; rat intestinal absorption; pH-partition theory; sodium cholate; bile salt; inhibitory effect

Nadolol (*cis*-5-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-2,3-naphthalenediol, Fig. 1), a non-selective β-adrenergic blocking agent,[1] has been proved to be effective in the treatment of hypertension,[2] angina pectoris[3] and cardiac arrhythmias.[4] The drug is not metabolized[5,6] and has a long plasma elimination half-life of 12—20 h[5-7] in man. However, it has been demonstrated that the intestinal absorption of nadolol following oral administration is incomplete (20—30% of the dose in man[5,6] and 15—20% in rats[8]), in contrast to the cases of other β-blocking agents, such as propranolol and alprenolol, which are completely absorbed.[9]

The present study was designed to evaluate the intestinal absorption behavior of nadolol, compared with those of six other β-blocking agents, by using the *in situ* ligated loop method in rats.

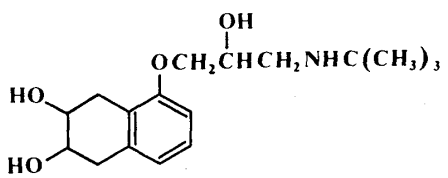

Fig. 1. Structure of Nadolol

Experimental

Materials——Nadolol was a gift from Squibb Institute (NJ, U.S.A.). Alprenolol, atenolol, carteolol, oxprenolol and propranolol were prepared as their hydrochlorides, and pindolol was prepared as the free base; the source materials were commercial products. Their purity was checked by gas chromatography (GC), high-performance liquid chromatography (HPLC) and elemental analysis.

Sodium cholate and its taurine and glycine conjugates, sodium deoxycholate, sodium chenodeoxycholate and sodium lithocholate were purchased from Nakarai Chemical Co. (Kyoto, Japan). They were used without further purification. All other chemicals were of analytical-reagent grade.

***In Situ* Experiments on Absorption from Rat Intestinal Loop**——Male Wistar rats weighing about 200 g were used after fasting overnight, with water *ad libitum*. Each rat was anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). The intestine was exteriorized through a central mid-line incision, and the bile duct was ligated. A loop of about 5 cm long was prepared from various parts of the intestine by ligature of both ends. The drug solution (0.2 ml) suspended in 0.5% tragacanth solution was injected into the loop with a syringe, and the loop was returned to the body. After a given time (usually 4 h after injection), the loop was removed. The contents were drained off with water followed by 0.01 N hydrochloric acid solution, and adjusted to 50 ml. The absorption of drugs was determined by subtracting the remaining amount from the amount administered in each loop.

***In Vivo* Experiments on Absorption in Bile Duct-Ligated Rats**——Male Wistar rats weighing about 200 g were used. The bile duct was ligated under anesthesia with sodium pentobarbital (40 mg/kg, i.p.). Next day, nadolol was orally administered at a dose of 20 mg/kg, and heparinized blood specimens were drawn by cardiac puncture at 1, 2, 4 and 6 h after dosing. Plasma levels of nadolol were measured and compared with those in sham-operated rats.

Apparent Partition Coefficients——*n*-Octanol and pH 7.4 isotonic buffer[10] were pre-equilibrated by shaking them together. The drug sample was dissolved in the buffer at a concentration of 0.1 mM, and a 5-ml aliquot of the drug solution was shaken with 5 ml of *n*-octanol for 2 h at 37 °C. After centrifugation, the amount of the aqueous layer was determined, and compared with that of the original aqueous solution before partition.

Analytical Method——The drug contents of the intestinal loop and the partitioned samples were measured by HPLC, by direct injection of sample solution into a Waters model ALC/GPC 204 liquid chromatograph equipped with a dual-delivery pump (model 6000A), an automatic sampler (model 710B), an ultraviolet absorption detector (model 440; 254 or 280 nm) and a recorder with an integrator (model 730) (Waters Assoc., MA, U.S.A.). A stainless-steel column (30 cm × 4 mm i.d.) packed with μ Bondapak C_{18} (10 μm; Waters Assoc.) was used with a mobile phase of methanol–1/15 M potassium dihydrogen phosphate (1 : 2—3 : 2) at a flow rate of 1 ml/min.

Plasma levels of nadolol after oral administration or after injection into the loop (20 mg/kg) were determined by GC equipped with a nitrogen-selective detector as described previously.[11] The procedure for sample preparation was briefly as follows. The internal standard solution and 5 N sodium hydroxide solution were added to a plasma sample (0.5 ml). The mixture was shaken with ethyl ether (5 ml), and centrifuged. The organic layer (4 ml) was transferred into another tube and evaporated to dryness under a gentle stream of air at 40 °C. The residue was dissolved in *n*-butylboronic acid solution (1 mg/ml of ethyl acetate containing 5% of dimethyl sulfoxide), and an aliquot of the solution was injected onto a column packed with 3% SP-400 on Gas Chrom Q (80—100 mesh). The cyclohexylidene derivative of nadolol was used as an internal standard.

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

Intestinal Absorption of Nadolol

Figure 2 shows the time course of the intestinal absorption of nadolol after injection into ligated loops of rat duodenum and jejunum. When nadolol was administered at a dose of 0.01 mmol (3.1 mg) per loop, the percentage absorption of nadolol reached a plateau at 4 h after dosing in the duodenum and at 2 h in the jejunum. The absorption of nadolol at 4 h after dosing was 64.9 and 71.5% of the dose in the duodenum and the jejunum, respectively. The intestinal absorption of nadolol was dose-independent: 68—72% of the dose was absorbed in 4 h after the injection of 0.1—10 mg (about 0.5—50 mg/kg) into the jejunum loop. A small and constant amount of nadolol (3—5% of the dose) was found in the tissue of the loop after dosing of 0.1—10 mg, indicating that the percentage absorption may be reasonably determined as the difference between remaining amount and amount administered in each loop. The absorption of nadolol during 4 h after injection into ligated loops of various parts of rat intestine was examined (Fig. 3). Nadolol was well absorbed from the duodenum, jejunum, and ileum, 65—79% of the dose being absorbed from lumen during the 4 h period, and 43% from the colon. However, the absorption from the stomach was extremely small (1.4%).

Plasma levels of nadolol after injection into the ligated jejunum loop were compared to those after oral administration at a dose of 20 mg/kg. As shown in Fig. 4, plasma levels of nadolol were maximal at 2 h after dosing, with a level of 0.46 μg/ml. The percentage

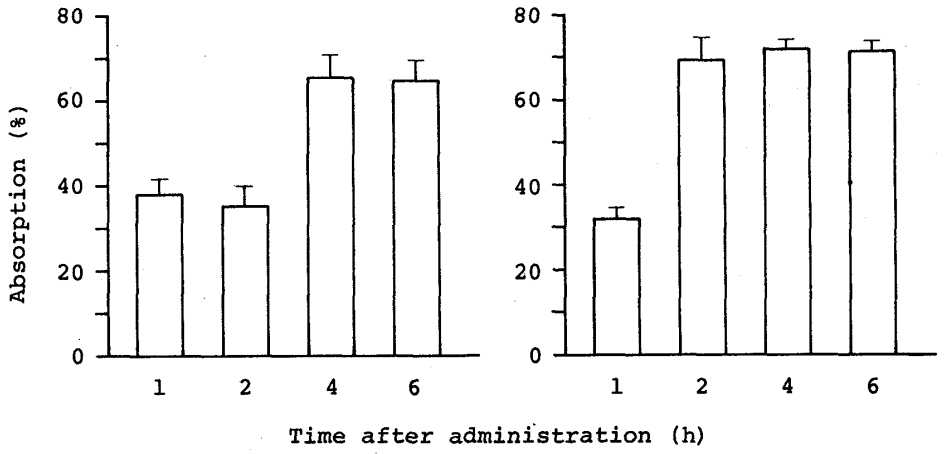

Fig. 2. Time Course of the Intestinal Absorption of Nadolol in Rats with Duodenum (Left) and Jejunum (Right) Loops

The dose of nadolol was 0.01 mmol/rat. Results are expressed as the mean ± S.E. of 5 rats.

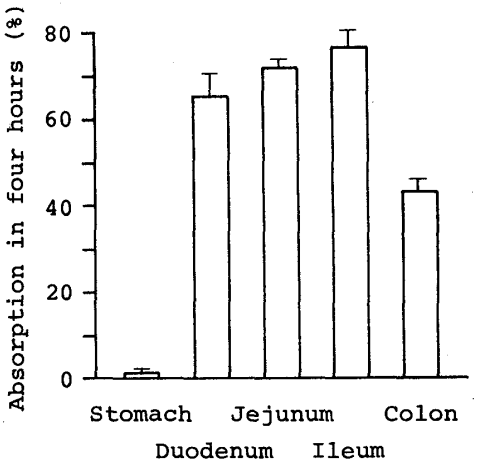

Fig. 3. Intestinal Absorption of Nadolol in Rats with the Intestinal Loops

Loop preparations of the stomach, duodenum, jejunum, ileum and colon were used. The dose of nadolol was 0.01 mmol/rat. Results are expressed as the mean ± S.E. of 5 rats.

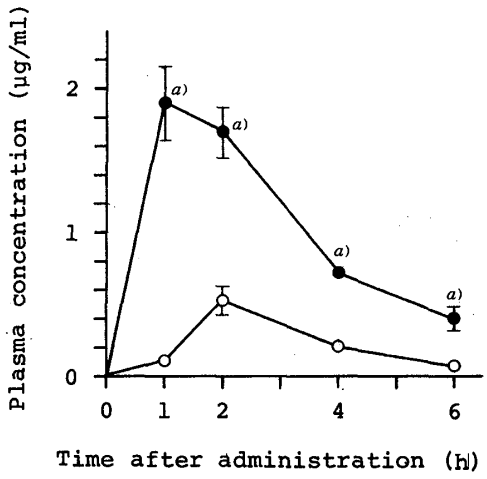

Fig. 4. Plasma Levels of Nadolol in Rats after Oral Administration (○) or after Injection into the Jejunum Loop (●)

The dose of nadolol was 20 mg/kg. Each point represents the mean ± S.E. of 5 rats. *a*) $p<0.01$, compared to oral administration.

absorption of nadolol after oral dosing, calculated from the area under the curve divided by that after intravenous dosing, was 18.0% of the administered dose. A similar result was reported by Dreyfuss *et al.*,[8] who obtained an oral absorption of 15—20% in rats. On the other hand, mean peak plasma level of nadolol after administration (20 mg/kg) into the jejunum loop was 1.9 μg/ml, four times higher than that after oral administration, which was in good agreement with the ratio of the percentage absorption (71.5%) from the jejunum to that (18.0%) after oral administration.

Absorption of β-Adrenergic Blocking Agents

The intestinal absorptions of seven β-blocking agents including nadolol were compared after injection into ligated loops of rat jejunum. Table I shows the percentage absorptions from the ligated loops, together with the partition coefficients. As is evident from the table, nadolol and the other β-blocking agents seem to be absorbed *in situ* to extents that correspond to their partition coefficients: the percentage absorption increased progressively with increas-

TABLE I. Apparent Partition Coefficient and *in Situ* Intestinal Absorption of β-Adrenergic Blocking Agents in Rats

Drug	Partition coefficient[a]	Absorption (%)[b]	
		1 h	4 h
Atenolol	0.030	24.1±4.2	70.3±2.0
Nadolol	0.035	31.5±2.0	71.5±1.7
Carteolol	0.21	83.1±3.1	97.2±0.7
Pindolol	0.69	91.8±2.2	97.9±1.3
Oxprenolol	2.04	94.5±1.3	>99
Alprenolol	13.1	93.5±1.3	>99
Propranolol	34.9	95.8±0.9	>99

Drugs (0.01 mmol) were administered into the ligated jejunum loop. *a*) *n*-Octanol/pH 7.4 isotonic buffer. *b*) Results are expressed as the mean±S.E. of 4—5 rats.

TABLE II. Effect of Intestinal Treatment on Nadolol Absorption in Rats

Treatment		Absorption in 4 h (%)[a]	
		Jejunum	Colon
None		71.5±1.7	43.0±2.4
Washout		69.0±2.7	45.4±3.5
Antibiotics[b]	Carbenicillin	74.7±2.7	40.4±4.4
	Gentamicin	68.2±5.3	44.4±3.0
Bile[c]		37.3±1.1[d]	

Nadolol (0.01 mmol) was administered into the ligated loop. The total injection volume was 0.2 ml in all experiments. *a*) Results are expressed as the mean±S.E. of 5 rats. *b*) Carbenicillin (100 mg/kg) or gentamicin (20 mg/kg) was orally administered twice a day for 5 d. *c*) Nadolol dissolved in rat bile was injected into the jejunum loop. Bile was collected from separate rats during a 1 h period by cannulation of the common bile duct. *d*) $p<0.01$, compared with the value without treatment.

ing partition coefficient.

β-Blocking agents were completely absorbed (more than 90%) after oral administration, except for nadolol and atenolol which showed the absorptions of 18% and 48—56%,[12] respectively. Oral absorption of nadolol was about one-third that of atenolol, although the *in situ* absorption of nadolol was better than that of atenolol.

Inhibition of Nadolol Absorption

The effect of intestinal components such as food, flora and bile on nadolol absorption in rats is shown in Table II. No inhibition was observed on the absorption of nadolol from the jejunum and the colon after the washout of intestinal contents. The oral dosing of antibiotics, carbenicillin and gentamicin, also did not affect the absorption of nadolol. When nadolol dissolved in bile was administered into the jejunum loop, the absorption was significantly decreased to 37% of the dose ($p<0.01$). In addition, the absorption of nadolol progressively decreased with increasing bile volume, and seemed to be saturable over the ratio of 0.5 (Fig. 5). Plasma levels of nadolol in bile duct-ligated rats are presented in Fig. 6. Plasma levels of unchanged nadolol in bile duct-ligated rats after oral administration (20 mg/kg) of nadolol were significantly higher than those in sham-operated rats.

Figure 7 shows the effect of bile salts on nadolol absorption after injection into the jejunum loop. The intestinal absorption of nadolol was strongly inhibited by trihydroxy bile salts (sodium cholate and its taurine and glycine conjugates), but not by dihydroxy bile salts

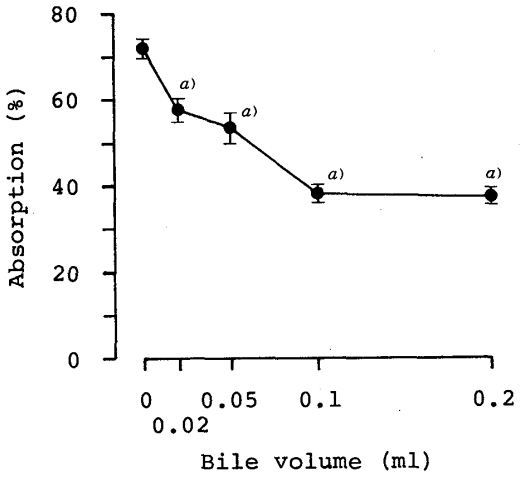

Fig. 5. Effect of Bile on Nadolol Absorption at 4 h after Injection into the Rat Jejunum Loop

The dose of nadolol was 0.01 mmol/rat, and the total injection volume was 0.2 ml in all experiments. Each point represents the mean ± S.E. of 5 rats. *a*) $p<0.01$, compared to the control (without bile).

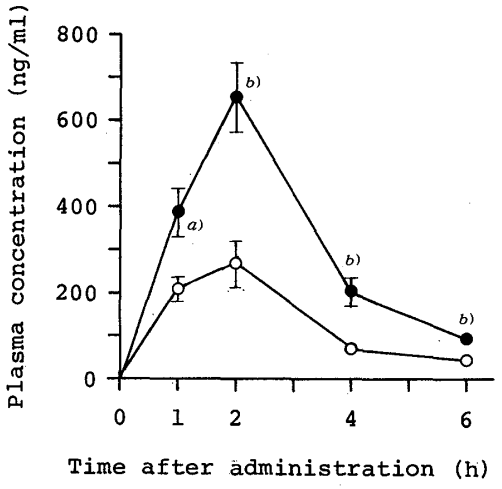

Fig. 6. Plasma Levels of Nadolol in Rats with Bile Duct Ligation after Oral Administration of 20 mg/kg of Nadolol

●, bile duct-ligated rats; ○, sham-operated rats. Each point represents the mean ± S.E. for at least 4 rats. *a*) $p<0.05$, *b*) $p<0.01$, compared to sham-operated rats.

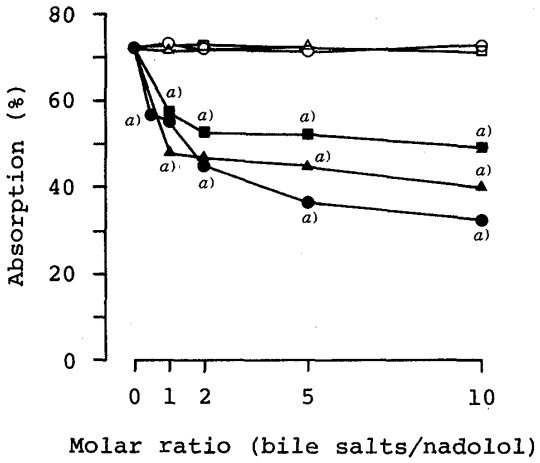

Fig. 7. Effect of Bile Salts on Nadolol Absorption at 4 h after Injection into the Rat Jejunum Loop

●, sodium cholate; ■, sodium glycocholate; ▲, sodium taurocholate; ○, sodium chenodeoxycholate; □, sodium deoxycholate; △, sodium lithocholate. The dose of nadolol was 0.01 mmol/rat, and that of bile salts was 0.005—0.1 mmol/rat. Each point represents the mean value for at least 4 rats. *a*) $p<0.01$, compared to the control (without bile salts).

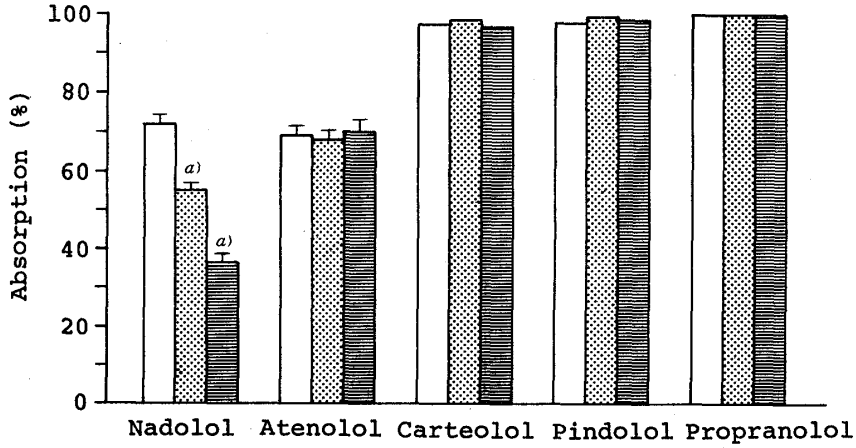

Fig. 8. Effect of Sodium Cholate on the Absorption of β-Adrenergic Blocking Agents at 4 h after Injection into the Rat Jejunum Loop

□, without sodium cholate; ▨, with equimolar amounts of sodium cholate; ▤, with 5-fold molar quantities of sodium cholate. The dose of drugs was 0.01 mmol/rat. Results are expressed as the mean ± S.E. of 5 rats. *a*) $p<0.01$, compared to the control (without sodium cholate).

(sodium deoxycholate and sodium chenodeoxycholate) or by a monohydroxy bile salt (sodium lithocholate). The percentage absorption decreased with increasing amount of sodium cholate and its conjugates, and these curves were similar to that presented in Fig. 5. The extent of inhibition by bile salts was in the order of sodium cholate, sodium taurocholate and sodium glycocholate. On the other hand, no inhibition by sodium cholate was observed on the absorption of other β-blocking agents such as atenolol, carteolol, pindolol and propranolol (Fig. 8). It was therefore found that the inhibition of the intestinal absorption by sodium cholate and its conjugates was specific for nadolol among β-blocking agents.

The effect of synthetic surfactants on nadolol absorption after injection (0.01 mmol) into the jejunum loop was also studied. The absorption of nadolol was not inhibited by sodium lauryl sulfate (0.01 mmol) or polysorbate 80 (1%); the absorptions were 72.3% and 68.0%, respectively.

Discussion

In the present study, nadolol was found to be well absorbed in rats from the duodenum, jejunum, ileum and colon, but not the stomach, after injection into the ligated loop (Fig. 3). This absorption (71.5% from the jejunum) was slightly less than those of lipophilic β-adrenergic blocking agents (carteolol, pindolol, oxprenolol, alprenolol and propranolol), which showed absorptions of more than 90% of the dose. From the results in Table I, the *in situ* intestinal absorption of β-blocking agents seemed to increase with increasing partition coefficient, indicating that β-blocking agents may be absorbed from the intestinal lumen according to the pH-partition theory. This behavior is similar to that found by Schoenwald and Huang for β-blocking agents in the rabbit eye.[13]

The intestinal absorption of nadolol was markedly decreased when nadolol dissolved in bile was injected into the jejunum loop, while it was not changed after oral antibiotics or after washout of the intestinal lumen (Table II). In addition, the absorption of nadolol progressively decreased with increasing bile volume (Fig. 5). Furthermore, the inhibitory effect on the absorption of nadolol was also observed after oral administration: peak plasma levels of nadolol after oral administration in bile duct-ligated rats were three times higher than that in sham-operated rats (Fig. 6). These results strongly suggest that the intestinal absorption of nadolol is inhibited by a component of bile, but not by food or intestinal flora.

The intestinal absorption of nadolol was strongly inhibited by trihydroxy bile salts, sodium cholate and its taurine and glycine conjugates, but not by di- and monohydroxy bile salts such as sodium deoxycholate, sodium chenodeoxycholate and sodium lithocholate (Fig. 7). However, the absorption of other β-blocking agents was not affected by sodium cholate (Fig. 8). The absorption of nadolol was also not reduced by synthetic surfactants such as sodium lauryl sulfate and polysorbate 80, which have frequently been used in studies on drug absorption. In addition, the solubility of nadolol was increased in the presence of bile or sodium cholate; a suspension of nadolol for injection into the loop was clarified by the addition of bile or sodium cholate. These results strongly suggest a specific interaction between nadolol and trihydroxy bile salts, especially sodium cholate.

Bile salts, which are physiological surface-active agents, have been shown to be important in the intestinal absorption of lipids, cholesterol and fat-soluble vitamins.[14] It is well known that the intestinal absorption of many drugs is enhanced by bile salts.[15,16] For example, the absorptions of poorly water-soluble drugs such as griseofulvin[17] and ethynylestradiol-6,7-^{3}H-3-cyclopentyl ether[18] were enhanced as a result of an increase of solubility or dissolution rate, and those of salicylate,[19] phenol red[20] and sulfaguanidine[21,22] were enhanced owing to an increase in membrane permeability. On the other hand, very limited information is available on the reduction of drug absorption by bile salts. Although

Kimura *et al.*[22,23] reported a decreasing effect of bile salts on the absorption of sulfadimethoxime, imipramine and quinine in rats by using the *in situ* recirculation technique, the decreasing effect of bile salts on the absorption of these drugs after oral administration is not clear. Accordingly, the findings that the absorption of nadolol is inhibited by bile salts both *in situ* and *in vivo* may give a new insight into the role of bile salts in drug absorption.

In the series of β-adrenergic blocking agents examined, nadolol and atenolol can be regarded as hydrophilic drugs from the pharmacokinetic and pharmacodynamic points of view.[9] Nadolol was better absorbed than atenolol from the jejunum loop in rats. This result is markedly different from that obtained after oral administration. The percentage absorption of oral atenolol was 48—56% of the dose in rats[12] and 42—62% in man,[24] and that of nadolol was 18% in rats and 20—30% in man,[5,6] *i.e.*, one-half or less as compared with atenolol. This means that the oral absorption of nadolol in man is similar to that in rats. Moreover, sodium cholate inhibited the absorption of nadolol, but not that of atenolol (Fig. 8). It can therefore be presumed that the oral absorption of nadolol in man is inhibited in the same manner as in rats, since the bile acids composition in man is qualitatively similar to that in rats.[14]

Although lipophilic β-adrenergic blocking agents, such as propranolol and alprenolol, are completely absorbed in man, the percentages of the oral therapeutic dose reaching the systemic circulation are only about 25%[25] and 10%,[26] respectively, due to the first-pass effect. While the intestinal absorption of nadolol is incomplete (20—30%),[5,6] the amount of drug taken into the systemic circulation is in the same range, because nadolol is not metabolized.[5,6] Thus, nadolol is considered to be effective against hypertension and coronary heart disease with equivalent potency to the existing β-blocking agents.

The results of experiments on the mechanism of inhibition of nadolol absorption by sodium cholate and its conjugates will be presented in the following paper.

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