Characteristics of the Gastrointestinal Absorption of Morphine in Rats[1)]

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The absorption characteristics of morphine were investigated by using rat gastrointestine. Absorption and transport experiments were carried out by the *in situ* loop and the *in vitro* everted sac methods, respectively. Brush border membrane vesicles (BBMVs) were used for uptake experiments. Morphine and its metabolites, morphine-3-glucuronide (M-3-G), and morphine-6-glucuronide (M-6-G), in biological samples were simultaneously determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection and electrochemical detection.

In the *in situ* loop method, morphine was well absorbed in the order of jejunal site > duodenal site > ileal site > middle intestinal site > rectal site, but it was poorly absorbed from the stomach. In each of the everted duodenal and jejunal sacs, 2,4-dinitrophenol, a metabolic inhibitor, inhibited the transport of morphine from the mucosal side to the serosal side. Further, $HgCl_2$ pretreatment reduced the absorption of morphine from the duodenal and the jejunal loops. The initial uptake of morphine by BBMVs was stimulated in the presence of an H^+ gradient (inner pH 7.5 and outer pH 5.0) and an overshoot phenomenon was observed. The initial uptake showed concentration dependence, *i.e.*, it was saturable.

Results obtained in this study indicate that carrier-mediated transport stimulated by the H^+ gradient is partly involved in the duodeno-jejunal absorption of morphine, although morphine is passively absorbed from other sites.

Keywords morphine; gastrointestinal absorption; *in situ* loop; *in vitro* everted sac; brush border membrane vesicle; carrier-mediated transport; high-performance liquid chromatography; rat

Introduction

Morphine has long been used for treatment of severe chronic pain in patients with advanced malignant disease. It is well known that morphine ($pK_a = 8.0$), which is a basic drug, is almost completely absorbed from the gastrointestinal tract,[2-4)] though it is almost wholly ionized at the physiological pH (pH 6.5) of the small intestine. However, the absorption mechanisms of morphine across the gastrointestinal membrane remain unexplored in detail, although the kinetics and metabolism have been widely studied in humans and some species of animals.[5-10)]

The present study was undertaken to investigate in detail the absorption characteristics of morphine by using rat *in situ* gastrointestinal loops, *in vitro* everted intestinal sacs and intestinal brush border membrane vesicles (BBMVs).

Experimental

Materials Morphine-3-glucuronide (M-3-G) and morphine-6-glucuronide (M-6-G) were prepared by the methods previously described.[11,12)] Morphine hydrochloride was obtained from a commercial source (Dainippon Pharmaceutical Co., Ltd.) with permission of the Government. Distilled water, methanol and acetonitrile were of high-performance liquid chromatography (HPLC) grade. All other chemicals were of analytical grade.

Animals Male Wistar rats weighing 180 to 230 g were used for this study.

Absorption Experiments Rats were anesthetized with sodium pentobarbital (40 mg/kg) after being fasted for 15 to 20 h prior to this experiment. In the loop experiments, rats under anesthesia scarcely survived at a dose much higher than 10 mg/kg of morphine hydrochloride. A dose of more than 0.5 mg/kg is required to determine the plasma concentrations of morphine and M-3-G. For these reasons, the dose range of 0.5 to 4 mg/kg was employed in this experiment.

Absorption from Gastrointestinal Loops: The gastrointestine was divided into six portions: stomach; duodenum (5 cm length below the pylorus); jejunum (20 cm length below Treitz's ligament); ileum (20 cm length above the ileo-cecal valve); middle intestine (about 20 cm length between the jejunum and the ileum); rectum (5 cm length above the anus). The bile duct was ligated in all experiments. The loop was washed gently with 20 ml of the warm saline (37 °C), then 0.25 ml of the saline containing morphine hydrochloride (0.4, 2.0, 3.2 mg/ml) was injected into the loop through the silicone tubing inserted into both ends. After 1 h, the contents were emptied into a 10 ml volumetric flask. The mucosal side of the loop was thoroughly rinsed with the saline to give a final volume of 10 ml. This fluid was subjected to assay. Blood samples (0.3 ml) were withdrawn at 15, 30, 45 and 60 min from the jugular vein after dosing, and the plasma was subjected to assay.

Effect of $HgCl_2$ Pretreatment on Absorption: The gastrointestine was divided into four portions: duodenum; jejunum (5 cm length below Treitz's ligament); ileum (5 cm length above the ileo-cecal valve); rectum. After washing of the loop, Ringer's solution (pH 7.4) containing 2 mM $HgCl_2$ was injected into the loop through the silicone tubing inserted into both ends. After 2 min, the contents were emptied and the mucosal side of the loop was thoroughly rinsed with the isotonic phosphate buffer (pH 6.5). A 0.25 ml aliquot of the same buffer containing morphine hydrochloride (0.4 mg/ml) was injected into the loop. Subsequent procedures were carried out in the same manner as described above.

Transport and Accumulation by Everted Sacs Rats were killed by decapitation. The intestine was removed and immediately placed in ice-cold saline. The intestine was everted, and divided into four segments: duodenum; jejunum (5 cm length below Treitz's ligament); ileum (5 cm length above the ileo-cecal valve); rectum.

Effect of pH of Incubation Medium: A 0.25 ml aliquot of the drug-free isotonic phosphate buffer at various pHs was injected into each sac, and the sac was incubated in 20 ml of the same buffer containing 0.1 mM morphine hydrochloride at 37 °C under constant gassing with 5% carbon dioxide in oxygen. After 30 min, each sac was removed, washed thoroughly by shaking in about 50 ml of the ice-cold drug-free buffer and blotted with a filter paper. Each sac was emptied into a 10 ml volumetric flask, and the serosal side of the sac was thoroughly rinsed with the drug-free buffer to give a final volume of 10 ml. The fluid and the tissue were subjected to assay.

Effects of Metabolic Inhibitor and Temperature: 2,4-Dinitrophenol (DNP) and ouabain were used as metabolic inhibitors. As the DNP peak somewhat interferes with the morphine peak on HPLC chromatograms when the concentration of morphine hydrochloride in the incubation medium is 0.1 mM, 0.35 mM was employed as a concentration not subject to significant interference. Each sac containing 0.25 ml of the drug-free isotonic phosphate buffer (pH 6.5) was placed in 20 ml of the buffer containing 0.5 mM metabolic inhibitor and 0.35 mM morphine hydrochloride. Subsequent procedures were carried out in the same manner

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as described above. The effect of low temperature was examined at 25 °C, instead of 37 °C.

Preparation of Brush Border Membrane Vesicles (BBMVs) BBMVs were prepared by the method of Tsuji *et al.*[13] The duodeno-jejunal portion, about 30 cm below the pylorus, was used in this study. The membrane vesicles finally obtained were suspended in 10 mM Tris-HEPES buffer (pH 7.5) containing 270 mM mannitol to give a protein concentration of 1.2 ± 0.1 mg of protein per ml. The purity of the membrane vesicles was routinely evaluated in terms of the enrichment of alkaline phosphatase, an enzyme specific to the intestinal brush boder membrane. The specific activity of this enzyme was increased 12-fold in the final membrane suspension compared with the concentration found in the homogenate of the mucosa scrapings. The membrane vesicles were equilibrated for 1 h at 4 °C before use.

Uptake Experiments Uptake of morphine by the BBMVs was measured by the rapid filtration method described by Hopfer *et al.*[14] The specific conditions for each experiment are given in the figure legends. Considering the assay limit of morphine in BBMVs (0.2 mM), the suitable morphine concentration to evaluate the correlation between osmolarity and uptake (2 mM) and the suitable morphine concentration to evaluate the concentration-dependent uptake (0.25—4 mM), the concentration range of 0.25 to 4 mM was employed in this experiment. The uptake was started by adding 100 μl of the incubation medium (pH 5.0, 10 mM Tris-citrate buffer containing 270 mM mannitol; pH 7.5, 10 mM Tris-HEPES containing 270 mM mannitol) containing morphine to 20 μl of the membrane vesicle suspension. The medium osmolarity was adjusted with cellobiose. The transport reaction was carried out at 37 °C and was stopped at the predetermined time by adding 3 ml of the ice-cold stop solution containing 150 mM NaCl and 1 mM Tris-HCl (pH 7.5). The diluted samples were applied immediately on a Millipore filter (HAWP, 0.45 μm pore size) and washed rapidly twice with 4 ml of the ice-cold stop solution. Nonspecific adsorption on the filter was determined using the incubation medium instead of the membrane vesicle suspension. Although such nonspecific adsorption was so small as to be negligible, all uptake amounts were corrected by subtraction of this amount. Morphine trapped on the filter was extracted with 1 ml of 0.1 N HCl by shaking for 20 min and the resultant extract was directly analyzed by HPLC.

Analysis of Tissue and Loop Samples Each intestinal tissue was weighed and homogenized using a BIOTRON (Kimura Co., Ltd.) with 3 ml of 0.1 N HCl. One milliliter of the homogenates or loop samples was added to a test-tube containing 2 ml of 0.5 M ammonium sulfate adjusted to pH 9.3 with ammonium hydroxide and 50 μl of nalorphine hydrochloride solution (2.4 μg/ml) as an internal standard. The mixture was shaken with 10 ml of chloroform–butanol (9 : 1) and centrifuged. Then 8 ml of the organic phase was transferred to a test-tube containing 2.5 ml of 0.1 N HCl. The tube was shaken and centrifuged, then 2 ml of the aqueous phase was transferred to a test-tube containing 2 ml of 0.5 M ammonium buffer and 5 ml of chloroform–butanol. This tube was shaken and centrifuged, and 4 ml of the organic phase was transferred to a test-tube and evaporated to dryness at 50 °C under vacuum. The residue was redissolved in the HPLC mobile phase (100 μl) and 20 μl of the solution was injected onto the HPLC column.

The overall extraction efficiencies of morphine and nalorphine by this method were 89.0 ± 0.5 and 96.8 ± 1.1%, respectively. The assay limit for morphine is 1 ng/ml.

Analysis of Plasma Samples The concentrations of morphine and its metabolites, M-3-G and M-6-G, in the plasma were determined simultaneously by the method of Svensson *et al.*[15,16] with some modifications. Briefly, 1 ml (0.1 ml) of plasma was added to a test-tube containing 3 ml (1 ml) of 0.5 M ammonium buffer (pH 9.3), and 50 μl of nalorphine hydrochloride solution (2.4 μg/ml). This mixture was applied to a Sep-Pak C_{18} cartridge (Waters Assoc.). The cartridge was washed with 20 ml of 5 mM ammonium buffer (pH 9.3), and 0.5 ml of distilled water. Morphine and its metabolites were eluted with 5 ml of 10% acetonitrile solution in 10 mM phosphate buffer adjusted to pH 2.1 with phosphoric acid. The eluate was washed with 5 ml of chloroform–butanol (9 : 1) by shaking. The aqueous phase was mixed with 2 ml of 0.5 M ammonium buffer, and treated on a second Sep-Pak C_{18} cartridge in the same way as on the first one. The eluate was evaporated to dryness at 50 °C under vacuum. The residue was redissolved in the HPLC mobile phase (100 μl) and 40 μl of the solution was injected onto the HPLC column.

HPLC Conditions A liquid chromatograph was assembled from the following components: M 6000 constant-flow pump (Waters Assoc.), U6K sample injector (Waters Assoc.), YMC A-312 ODS column 15 cm × 4 mm i.d. (Yamamura Chemical), UVIDEC 100-VI UV detector (210 nm, 0.005 AUFS; Jasco) and L-ECD 6A electrochemical detector (800 mV, 16 nA; Shimadzu). The mobile phase was a mixture of 0.1 M sodium dihydrogen phosphate buffer (containing 10 mM dodecyl sulfate and 30 nM ethylenediaminetetra acetic acid disodium salt (EDTA·2Na)), adjusted to pH 2.5 with phosphoric acid–acetonitrile–methanol (plasma samples, 68 : 27 : 5; other samples, 65 : 35 : 0). The flow rate was 2 ml/min for plasma samples and 1 ml/min for other samples.

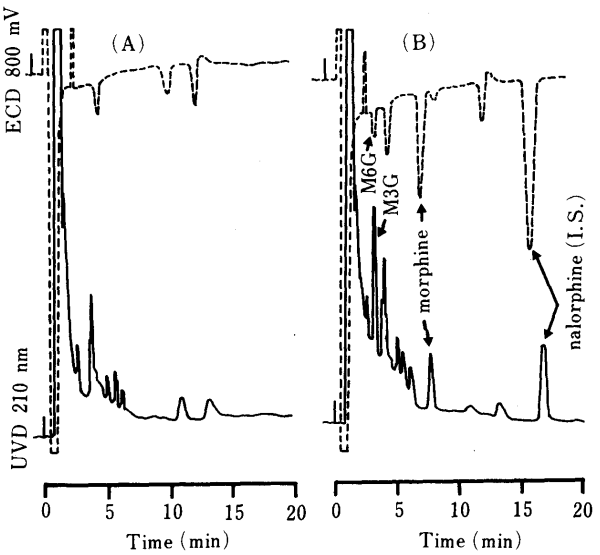

Fig. 1. Chromatograms of (A) Blank Plasma and (B) Plasma Spiked with Morphine, Morphine-3-glucuronide (M3G), Morphine-6-glucuronide (M6G), and Nalorphine Using UV and EC Detection

Figure 1 shows the chromatograms for the blank plasma and the plasma spiked with morphine, M-3-G, M-6-G and nalorphine.

Analysis of Alkaline Phosphatase and Protein Activity of alkaline phosphatase was measured by the method of Bessey *et al.*[17] Protein was measured by the method of Lowry *et al.*[18] with bovine serum albumin as a standard.

Measurement of pH of Gastrointestinal Fluid Each loop was filled with saline and after 1 h, the contents were thoroughly rinsed out with 2 to 3 ml of the saline. The pH of the contents was immediately measured with a pH meter (model TP 1000; Toko Chemical). The value was taken as representing the pH of the gastrointestinal fluid.

Results and Discussion

Absorption from Gastrointestinal Loops Absorption of morphine from rat intestine was estimated by subtracting the remaining amount from the initial amount. Figure 2 shows the apparent absorption of morphine from each site at doses ranging from 0.5 to 4 mg/kg. Morphine was well absorbed in the order of jejunum > duodenum > ileum > middle intestine > rectum, but was poorly absorbed from the stomach. It is noteworthy that morphine is very well absorbed from the duodenum in spite of the short length available for absorption compared with the jejunum and the ileum. The absorption of morphine from the duodenum also tended to decrease with increasing dose. However, it is difficult to demonstrate whether the absorption process is dose-dependent or not, because rats do not survive at a dose much higher than 10 mg/kg. On the other hand, the plasma concentrations of morphine and its metabolite, M-3-G, at a dose of 2.5 mg/kg are shown in Fig. 3. These results corresponded well to the results for the absorption from loops, indicating that morphine was transported across the mucosal membrane at each site. Similar results were obtained with other doses. In addition, it was found that morphine was extensively metabolized to M-3-G when absorbed from all sites. In the

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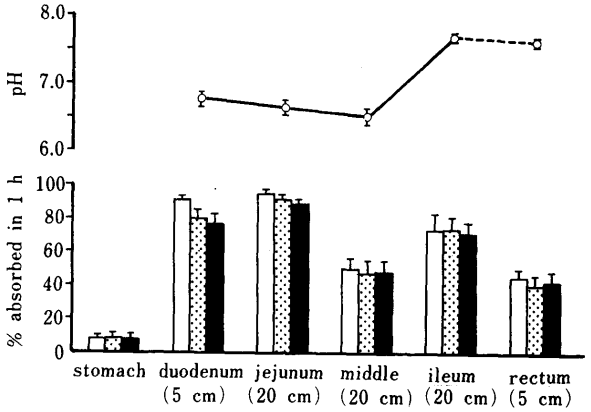

Fig. 2. Absorption of Morphine in Relation to Fluid pH in a Gastrointestinal Loop

Dose: □, 0.5 mg/kg; ▨, 2.5 mg/kg; ▩, 4.0 mg/kg. Each column or point represents the mean and S.E. of five animals.

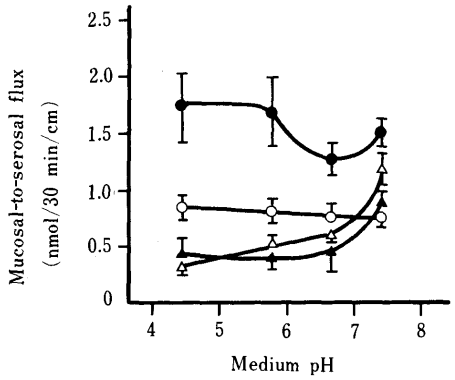

Fig. 4. Effect of pH of Incubation Medium on Mucosal-to-Serosal Flux of Morphine in Everted Rat Intestine

○, duodenum; ●, jejunum; △, ileum; ▲, rectum. Initial concentration of morphine in the mucosal fluid was 0.1 mM. Each point represents the mean and S.E. of three animals.

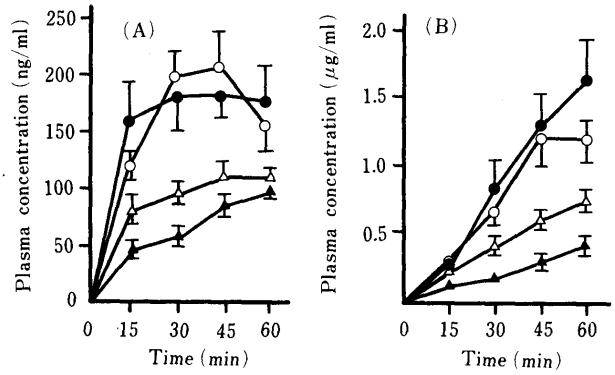

Fig. 3. Plasma Concentration–Time Curves of (A) Morphine and (B) Morphine-3-glucuronide after Intestinal Administration of Morphine to Rats

○, duodenum; ●, jejunum; △, ileum; ▲, rectum. Dose: 2.5 mg/kg. Each point represents the mean and S.E. of five animals.

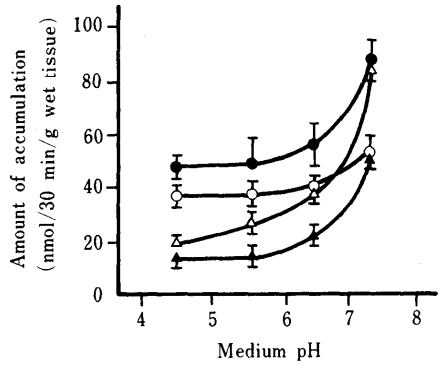

Fig. 5. Effect of pH of Incubation Medium on Accumulation of Morphine by Everted Rat Intestine

○, duodenum; ●, jejunum; △, ileum; ▲, rectum. Initial concentration of morphine in the mucosal fluid was 0.1 mM. Each point represents the mean and S.E. of three animals.

case of rats, M-6-G was not detected in the plasma, although it had been detected in human plasma.[19] When the absorption was considered on the basis of the pH-partition theory, it was predicted that morphine would probably be absorbed to a much greater extent from the lower small intestine having higher fluid pH than from the upper small intestine. However, morphine was well absorbed from the upper small intestine (duodeno–jejunal site) compared with the lower small intestine. This implies the involvement of an absorption mechanism not following the pH-partition theory in the absorption process of morphine at the upper small intestine, besides the simple diffusion mechanism. On the other hand, the absorption from below the duodeno–jejunal site can be explained on the basis of the pH-partition consideration.

Effects of Medium pH on Transport and Accumulation by Everted Sacs Figure 4 shows the effect of the medium pH on the transport of morphine from the mucosal side to the serosal side over a pH range of 4.46 to 7.24. The transport across the ileal and the rectal sacs increased with increasing medium pH and followed the pH-partition theory. On the other hand, the duodenum showed transport that was little influenced by the medium pH. The transport across the jejunum tended to increase in the acidic range (less than pH 5.5). These results imply that a specific transport process which can not be explained in terms of the pH-partition theory is probably involved in the transport of morphine at the upper small intestine.

On the other hand, the accumulation of morphine in each tissue increased with increasing medium pH (pH-dependent accumulation; Fig. 5). These results did not correspond to those for the transport. In separate experiments, although the magnitude of the mucosal-to-serosal flux in each tissue was three- to five-fold greater than that of the serosal-to-mucosal flux, there was no difference in the apparent accumulation in the tissue, irrespective of the direction of the flux (data not shown). Therefore, the pH-dependent binding of morphine to the tissue may be partly involved in the apparent accumulation.

Effects of Metabolic Inhibitors and Temperature on Transport by Everted Sacs Figure 6 shows the effects of metabolic inhibitors, DNP and ouabain, and lower temperature (25 °C) on the transport of morphine from the mucosal side to the serosal side. The transport of morphine across the duodenal and the jejunal sacs was significantly inhibited by DNP and lower temperature. However, no inhibition was found with ouabain. Therefore, it seems that Na^+–K^+ adenosine triphosphatase (ATPase) is probably not involved in the transport process of morphine. On the other hand, the transport of morphine across the ileal sac

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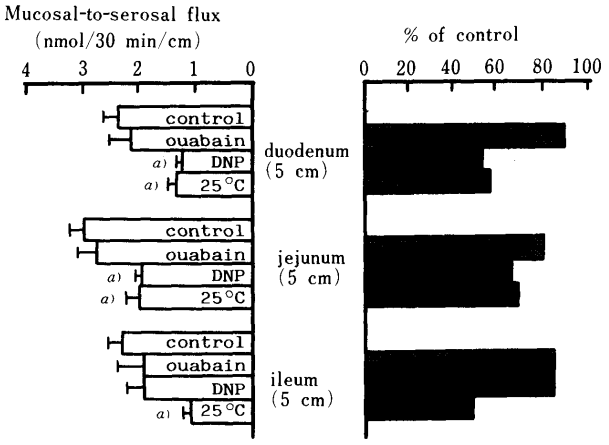

Fig. 6. Effects of Metabolic Inhibitors and Temperature on Morphine Transport across Everted Rat Intestine Sacs at pH 6.5

Initial concentration of morphine in the mucosal fluid was 0.35 mM. All the experiments were carried out at 37 °C except for the experiment at 25 °C. Significant difference from the control: *a*) $p<0.05$. Each column represents the mean and S.E. of three animals.

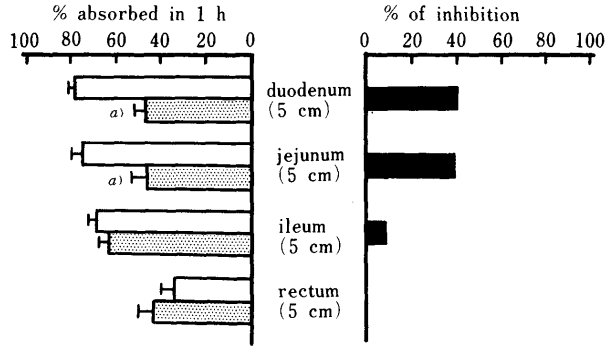

Fig. 7. Effect of $HgCl_2$ Pretreatment on Absorption of Morphine in Rat Intestinal Loop

□, control; ▨, $HgCl_2$ pretreatment. Dose: 0.5 mg/kg. The intestine was pretreated with 2 mM $HgCl_2$ for 2 min. Significant difference from control: *a*) $p<0.01$. Each column represents the mean and S.E. of six animals.

was not inhibited by DNP or ouabain, although it was significantly inhibited at lower temperature. These results indicate that the transport process of morphine at the upper small intestine requires metabolic energy, implying the involvement of an active transport mechanism.

Effect of Pretreatment with $HgCl_2$ on Absorption When the mucosal surface was modified by short-term pretreatment with 2 mM $HgCl_2$, the absorption of morphine from the duodenal and the jejunal loops was significantly reduced (Fig. 7). A contribution of protein and/or sulfhydryl groups within the mucosal membrane to the absorption process of morphine is suggested. On a different day, the effect of this pretreatment on the absorption of morphine from the duodenal loop was evaluated by measuring the plasma concentrations of morphine and M-3-G. Although morphine and M-3-G showed somewhat delayed absorption patterns compared with the results in Fig. 3, the effect of the pretreatment was reflected in the plasma concentrations (Fig. 8).

It is well known that morphine is extensively metabolized to the glucuronide in the small intestine.[20,21] Hartiala *et al.*[22] have also reported that the activity of glucuronyl transferase in rat small intestine varied seasonally and the

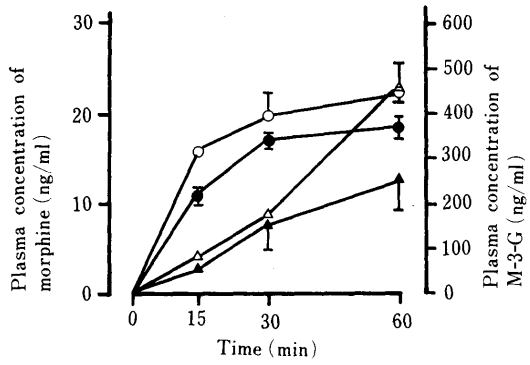

Fig. 8. Plasma Concentration–Time Curves of Morphine and Morphine-3-glucuronide (M-3-G) after Duodenal Administration of Morphine to $HgCl_2$-Pretreated Rats

Control: ○, morphine; △, M-3-G. $HgCl_2$ pretreatment: ●, morphine; ▲, M-3-G. Dose: 0.5 mg/kg. The intestine was pretreated with 2 mM $HgCl_2$ for 2 min. Each point represents the mean and S.E. of three animals.

activity was higher in the winter than in the spring. In our case, only the experiment shown in Fig. 8 was carried out in the winter. In this experiment, the molar ratio between the areas under the plasma concentration–time curve for M-3-G and morphine was in the range of 6.7 to 8.4, whereas in the results shown in Fig. 3, the corresponding value was in the range of 2.0 to 4.2. Thus, we consider that the difference in the plasma concentration profiles may be due to the seasonal difference in the activity of glucuronyl transferase and/or the absorbability.

It has been reported that a carrier-mediated mechanism participated in the accumulation of narcotic bases including morphine by the kidney[23,24] and choroid plexus.[24,25] On the other hand, Jackson concluded that a carrier-mediated mechanism did not participate in the transport of narcotic bases including morphine across the small intestine *in vitro*.[26] However, the small intestine used in his experiments corresponds to the middle intestine used in our experiments. Our results support the suggestion that the carrier-mediated transport does not operate in the middle intestine. There has been no report which suggests the participation of carrier-mediated transport in the upper small intestinal absorption of narcotic bases. However, from the results obtained in Figs. 6, 7 and 8, it seems resonable to suggest that a carrier-mediated transport is partly involved in the absorption process of morphine from the upper small intestine.

Effect of Medium Osmolarity on Uptake by BBMVs Tamai *et al.*[27] and Tsuji *et al.*[28] reported that the uptake rate of cefixime, a cephalosporin antibiotic, by an everted jejunal sac significantly increased in the acidic range (less than pH 5) compared with the neutral range, and the maximum uptake by BBMVs was obtained in the vicinity of pH 5.0, the rate being about 6 times higher than that at pH 7.5. In this study, the transport of morphine across the everted jejunal sac was found to increase in the acidic range (less than pH 5.5), although morphine is almost completely ionized in this pH region. Thus, although the pH value of the maximum transport across the sac does not necessarily coincide with that of the maximum uptake rate by BBMVs, it suggests that the maximum uptake rate by BBMVs may be in the range of pH 4 to 5.5. Therefore, subsequent studies were carried out in an incubation medium buffered

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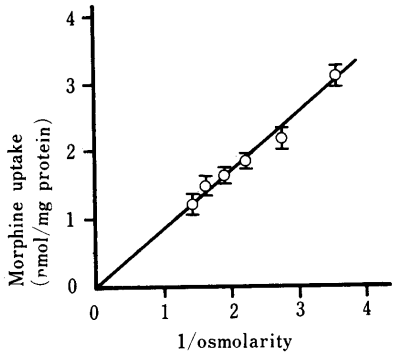

Fig. 9. Effect of Medium Osmolarity on Morphine Uptake by Rat Intestinal Brush-Border Membrane Vesicles

Membrane vesicles were preloaded with 10 mM Tris-HEPES buffer (pH 7.5) containing 270 mM mannitol. Uptake of 2 mM morphine was measured at 37 °C for 10 min by incubating the membrane vesicles in 10 mM Tris-citrate buffer (pH 5.0) containing 270 mM mannitol and various concentrations of cellobiose (0—250 mM), shown as inverse osmolarity. Each point represents the mean and S.E. of five experiments.

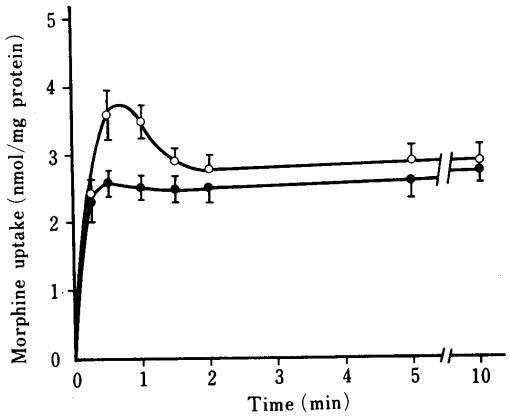

Fig. 10. Time Course of Morphine Uptake by Rat Intestinal Brush-Border Membrane Vesicles

Membrane vesicles were preloaded with 10 mM Tris-HEPES buffer (pH 7.5) containing 270 mM mannitol and 10 mM Tris-citrate buffer (pH 5.0) containing 270 mM mannitol. Uptake of 2 mM morphine was measured at 37 °C by incubating the membrane vesicles in 10 mM Tris-citrate buffer (pH 5.0) containing 270 mM mannitol. ○, inner pH 7.5/outer pH 5.0; ●, inner pH 5.0/outer pH 5.0. Each point represents the mean and S.E. of twelve experiments.

with Tris-citrate to pH 5.0.

To distinguish between binding of morphine to BBMVs and its transport into the intravesicular space, the steady-state uptake was measured at 10 min by increasing the osmolarity of the outer medium with D-cellobiose. As shown in Fig. 9, the steady-state uptake of morphine decreased significantly with increasing medium osmolarity, indicating that morphine is taken up into an osmotically sensitive vesicular space. The value obtained from the extrapolation of osmolarity to infinity suggests that the amount of binding to the membrane is negligible.

Time Course of Uptake by BBMVs The time course of morphine uptake by BBMVs at a concentration of 2 mM is shown in Fig. 10. As seen in Fig. 10, the initial uptake in the presence of an H^+ gradient (inner pH 7.5 and outer pH 5.0) was stimulated against the concentration gradient, and an overshoot phenomenon was observed, implying uphill trans port. On the other hand, in the absence of an H^+ gradient (inner pH 5.0 and outer pH 5.0), the initial uptake was depressed and the overshoot phenomenon faded, although

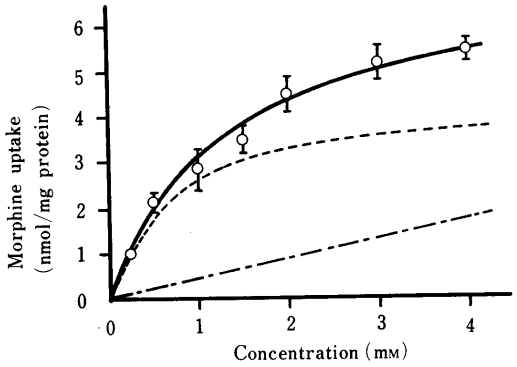

Fig. 11. Concentration Dependence of Morphine Uptake by Rat Intestinal Brush-Border Membrane Vesicles

Membrane vesicles were preloaded with 10 mM Tris-HEPES buffer (pH 7.5) containing 270 mM mannitol. Uptake of morphine at various concentrations (0.25—4 mM) was measured at 37 °C for 30 s by incubating the membrane vesicles in 10 mM Tris-citrate buffer (pH 5.0). —○—, total uptake; ----, carrier-mediated uptake; —·—, passive diffusion. Each point represents the mean and S.E. of four experiments.

the uptake at the steady state was not altered. The effect of Na^+ on morphine uptake was also examined when the pH of the medium containing 270 mM mannitol as a control or 100 mM mannitol and 100 mM sodium chloride was 5.0. The presence of Na^+ did not influence morphine uptake in the presence of the H^+ gradient (control, 3.60 ± 0.41; Na^+, 3.37 ± 0.26 nmol/30 s/mg protein). There was also no appreciable enhancement of morphine uptake in the absence of the H^+ gradient (inner pH 5.0/outer pH 5.0: control, 2.80 ± 0.39; Na^+, 2.36 ± 0.18 nmol/30 s/mg protein). These results indicate that the H^+ gradient plays an important role in the uphill transport of morphine by BBMVs.

Concentration Dependence of Uptake by BBMVs The effect of the concentration of morphine in the medium on its uptake at 30 s by BBMVs was studied over a range of 0.25 to 4 mM. As shown in Fig. 11, the concentration-dependent uptake suggests that the uptake consisted of two components, a saturable process and a nonsaturable one. The kinetic parameters were estimated from the following equation: $V = V_{max} \cdot C/(K_m + C) + K_d \cdot C$ where V and C represent the apparent uptake rate and the initial concentration of morphine, respectively. V_{max} is the apparent Michaelis constant and K_d is the first-order rate constant. V_{max} was 4.60 nmol/30 s/mg protein, K_m was 0.76 mM and K_d was 0.43 nmol/30 s/mg protein per mM. Thus, these results suggest the involvement of a carrier-mediated transport in the uptake of morphine by BBMVs.

Amino-β-lactams such as amoxicillin, cyclacillin, cephalexin, cephradine and cefadroxil, which have very low lipid solubility and a zwitter ionic structure, have been shown to be absorbed through dipeptide carrier systems in the intestinal brush border membrane.[29-32] Recent studies provided evidence that the uptake of dipeptides by the intestinal BBMVs is entirely independent of the presence of Na^+ but is dependent on an H^+ gradient.[33-36] Results obtained in this study suggest that the carrier-mediated transport stimulated by the H^+ gradient is partly involved in the duodeno-jejunal absorption process of morphine, although morphine does not bear any structural resemblance to the compounds described above. However, it is not clear whether morphine is absorbed through dipeptide carrier systems in the intestinal brush border membrane, because

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the effect of membrane potential and the inhibitory effect of dipeptides on morphine uptake have not yet been examined in detail. Further investigation will be required to elucidate this.

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