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The transport mechanisms of organic cations and their zwitterionic derivatives across rat intestinal brush-border membrane. 1. Binding characteristics to the bio- and lipid-membranes

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The uptake mechanisms of organic cations such as tryptamine, tyramine, 5-benzyloxytryptamine (BOTA) and their zwitterionic derivatives (tyrosine, tryptophan, 5-benzyloxytryptophan (BOTP)) by rat intestinal brush-border membrane vesicles and liposome containing phosphatidylserine were studied and compared. As compared to their zwitterionic derivatives, uptake rates by rat intestinal brush-border membrane of these three cations were far superior. The binding of cationic compounds to the brush-border membrane was also higher than those of their zwitterionic derivatives. Furthermore, the binding behaviour of BOTA and tryptamine to phospholipid liposome clearly amplified with increasing amounts of phosphatidylserine. In contrast, the contents of phosphatidylserine, a negatively charged phospholipid, exhibited no effects on the binding of zwitterionic derivatives (tryptophan and BOTP). The double-reciprocal plot of tryptamine binding with BOTA to liposome showed competitive inhibition. These results suggest that the binding of organic cations to the membrane lipid has a relatively high specificity despite the absence of membrane protein such as a transport-carrier in the liposome, and that the binding of cationic compounds plays an important role in the uptake to the cell membrane systems.

Introduction

The membrane transport mechanisms of substrates are classified roughly into two systems, one is a carrier-mediated transport system associated to membrane protein and the other is the passive diffusion based on the physico-chemical interaction between the compound molecule and the membrane. It is well known that many endogenous compounds entered via these carrier-mediated transport systems into the intestinal [1–4] and renal [6–8] epithelial cells. In contrast, a lot of exogenous compounds, such as drugs, could transport across the plasma membrane by passive diffusion except for the analogue of various endogenous compounds [9–12]. However, despite numerous investigations [9–14], it remains unclear whether the differences among the structures of these analogues can be related to the participation in the carrier-mediated transport mechanism for the endogenous compounds. Furthermore, little information exists concerning detailed mechanisms of the passive diffusion focusing in the relationship between the diffusion process and the molecular structures of drugs.

Prior to this current investigation, we reported that all great absorbabilities of the hydrophilic drugs were not the results of carrier-mediated transport systems [15–19]. Recently, we found evidence that the uptake of organic cation, tryptamine and disopyramide, into rat intestinal brush-border membrane vesicles were affected markedly by the H^+, K^+-diffusion potential [20,21]. Moreover, the uptake of the cationic form of enoxacin was stimulated by the inside negative diffusion potential in rat intestinal brush-border membrane vesicles [22]. On the other hand, we confirmed that the inhibitory effect of several organic cations such as imipramine and chlorpromazine on tryptamine uptake by brush-border membrane vesicles was due to the binding to membrane, but not to intravesicular uptake [20]. Furthermore, there was a good correlation between the binding behaviour of spermine to the phospholipid liposome and that of brush-border membrane vesicles. Since the liposome membranes contain no proteins, and are capable of changing the composition

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of lipids, the binding experiment with liposome becomes a very useful method when the interaction between membrane lipids and drug is directly examined.

Therefore, the present investigation was designed to obtain further evidence concerning a common relationship of uptake mechanism among the organic cations into the intestinal brush-border membrane, and the binding characteristics of organic cations to bio- and phospholipid-membranes as compared to those of their zwitterionic derivatives.

Materials and Methods

Chemicals

Tyramine hydrochloride, tryptamine hydrochloride were purchased from Nacalai Tesque (Kyoto, Japan). L-tyrosine, L-tryptophan was obtained from Kyowa Hakko Kogyo (Tokyo, Japan). 5-Benzyloxytryptamine (BOTA), 5-benzyloxy-DL-tryptophan (BOTP), DL-α-phosphatidylcholine dipalmitoyl, L-α-phosphatidic acid (from egg yolk lecithin), L-α-phosphatidyl-L-serine (from bovine brain), L-α-phosphatidylethanolamine (from sheep brain) were from Sigma (St. Louis, MO, USA). L-[^{14}C]Tyrosine (13.1 GBq/mmol) was purchased from Amersham International (Buckinghamshire, UK). All other chemicals were of the highest grade available and used without further purification.

Preparation of membrane vesicles

Brush-border membrane vesicles were prepared from small intestine of rat (wistar, male; 170–230 g) by $CaCl_2$ precipitation [1] as described previously [17–19]. Membrane vesicles were suspended in the buffer used for the transport studies. The composition of each buffer was given in the legends of figures.

Uptake studies

The uptake study was performed by rapid filtration technique using a Millipore Filter (HAWP, 0.45 μm, 25 mm diameter) which was pre-treated with 0.3 96 polyethylenimine to avoid the nonspecific adsorption to the filter in the case of organic cations as described previously [18]. As a blank, membrane free incubation medium was handled in an identical manner.

Measurement of binding to liposome preparations by ultrafiltration

Liposomes derived from phospholipids (phosphatidylcholine, phosphatidic acid, and either phosphatidylserine or phosphatidylethanolamine) were prepared under nitrogen stream by sonication in a buffer solution (20 mM Mes-Tris (pH 5.5) containing 100 mM KCl and 100 mM D-mannitol) for 1 h at 4°C according to the method of Ustumi et al. [23]. The final phospholipid concentration was adjusted to 0.2 or 0.4 mg lipid/ml.

To examine the binding of tested compounds to liposome, a mixed solution of liposome and substrate was ultrafiltered through a Centriflow C3LGC appara-

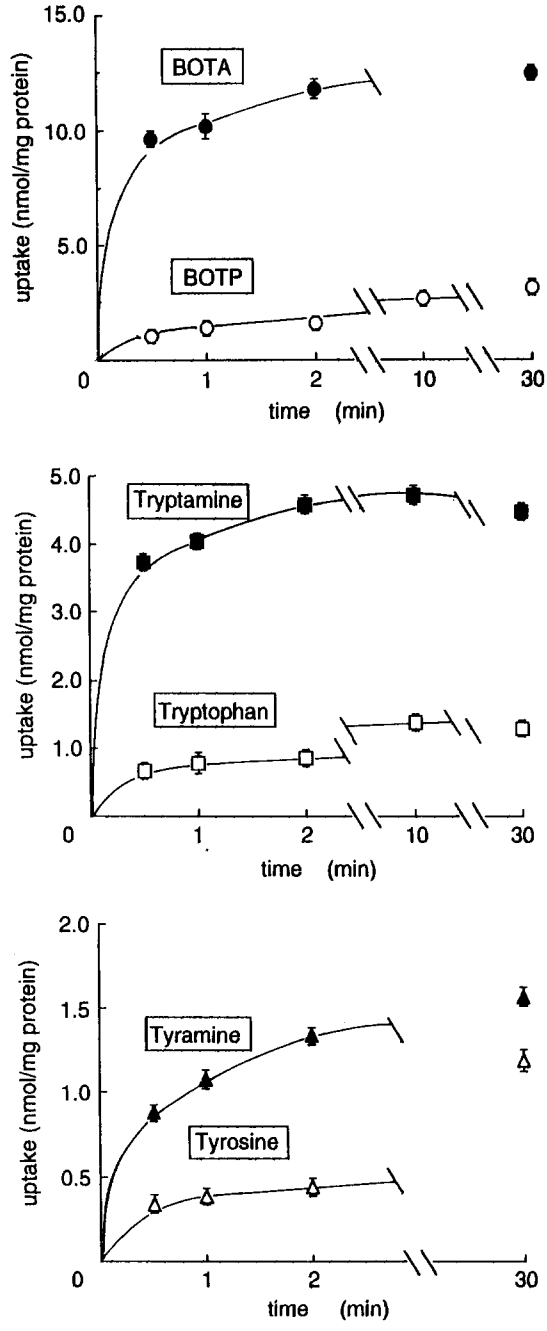

Fig. 1. Uptake of organic cations and their zwitterionic derivatives by rat intestinal brush-border membrane vesicles. The vesicles were preincubated in 100 mM KCl, 100 mM D-mannitol and 20 mM Mes-Tris buffer (pH 5.5). Uptake studies were performed in the same medium containing either organic cation or its zwitterionic derivative. Final substrate concentration was 1.0 mM (tyramine, ▲; tyrosine, △; tryptamine, ■; tryptophan, □) or 0.25 mM (BOTA, ●; BOTP, ○. Results represent the means with S.E. of 3–8 determinations.

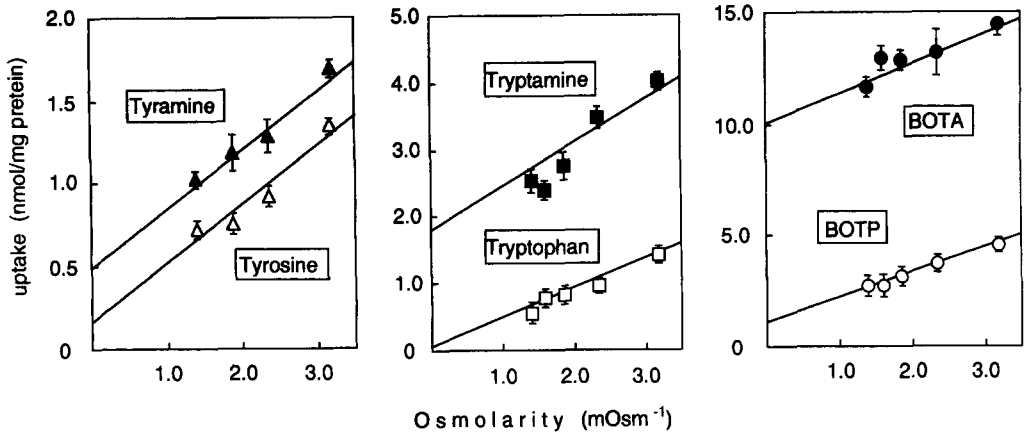

Fig. 2. Effect of the medium osmolarity on the uptake of organic cations and their zwitterionic derivatives by the brush-border membrane vesicles. Uptake of compounds were measured as a function of varying medium osmolarities (300–600 mosM). The symbols are the same as those in Fig. 1. Each point represents the mean with S.E. of 3–6 determinations.

tus (Millipore, Bedford, MA, USA) after equilibration of binding (1 h, room temperature).

Analytical methods

The tested compounds were determined by HPLC (Hitachi L-6000, Hitachi, Tokyo, Japan) equipped with an F-1000 Fluorometric monitor (Hitachi, Tokyo, Japan) at an excitation wavelength of 285 nm (for tryptamine and tryptophan) or 305 nm (for BOTA and BOTP), and an emission wavelength of 350 nm (for tryptamine and tryptophan) or 345 nm (for BOTA and BOTP). Separations were achieved on a reversed phase ODS column (Hitachi No. 3053, 5 μm, 4 mm i.d. × 250 mm) using the mobile phase consisting of acetonitrile/0.05 M phosphate buffer, pH 2.5 (20:80 for tryptamine and tryptophan; 40:60 for BOTA and BOTP) at a flow rate of 0.8 ml/min. To determine the tyramine concentration, samples were pre-labeled by fluorescamine, and then assayed by HPLC equipped with fluorometry at the wavelength of 380 nm (excitation) and 485 nm (emission). In this case, a reversed phase octyl column (Zorbax C8, 5 μm, 4.6 mm i.d. × 250 mm) was used for the separation of fluorescamine-labeled tyramine on a mobile phase of acetonitrile/0.05 M phosphate buffer, pH 6.5 (75:25) at a flow rate of 1.5 ml/min. The radioactivity of [^{14}C]tyrosine was measured by standard liquid scintillation technique. Protein was determined by the method of Lowry et al. [24].

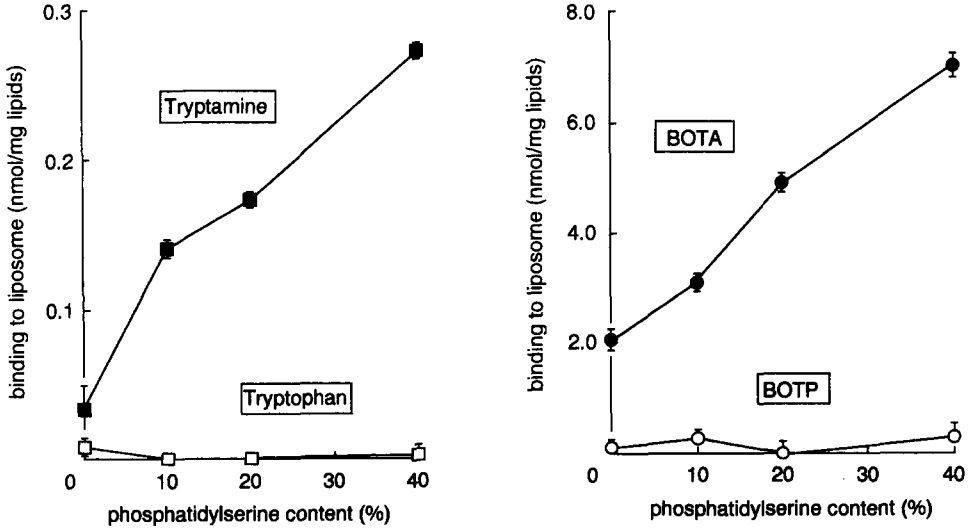

Fig. 3. The binding behaviour of organic cations and their zwitterionic derivatives to liposomes as a function of phosphatidylserine content. Initially each substrate concentration was 1 μM. Total phospholipids concentration was either 2 mg/ml for tryptamine and tryptophan, or 0.1 mg/ml for BOTA and BOTP in the experimental medium. Lipid composition of liposomes were 90–50% phosphatidylcholine, 0–40% phosphatidylserine, and 10% phosphatidic acid. Each point represents the mean with S.E. of three observations.

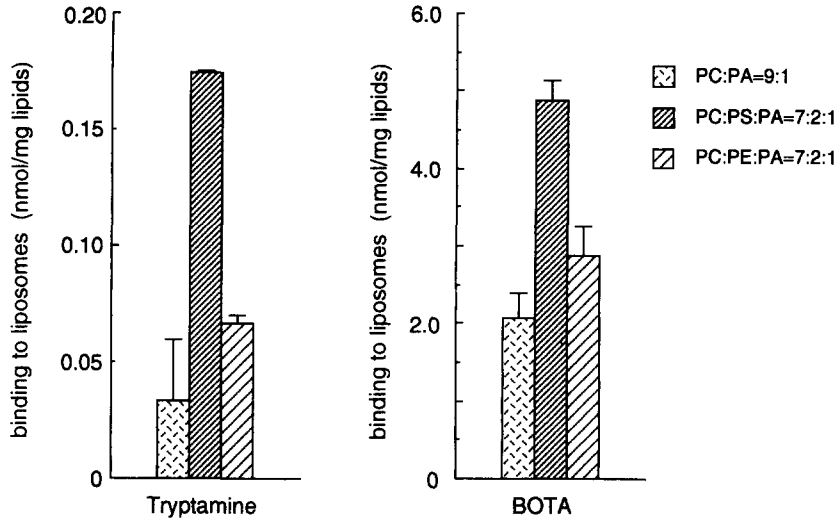

Fig. 4. Effect of phosphatidylserine on the binding of organic cations to the liposomes. Initially each substrate concentration was 1 μM. Total phospholipids concentration was either 2 mg/ml for tryptamine or 0.1 mg/ml for BOTA. Liposome was composed of phosphatidylcholine (PC), phosphatidic acid (PA) and either phosphatidylserine (PS) or phosphatidylethanolamine (PE). Columns represent the mean values with vertical bars showing S.E. of three observations.

Results

Comparison of the uptake behaviour of organic cations and their zwitterionic analogues

Uptake of tyramine, tryptamine, BOTA and their zwitterionic derivatives as a function of time are depicted in Fig. 1. The uptake of each organic cation was superior to that of its zwitterionic derivative at not only the initial (0.5–2 min) but also the equilibrated (30 min) point. For the reasons, uptake values included the transport compartment into an intravesicular space and the binding compartment to the membrane surface following 30 min of incubation, uptake of each compound by the vesicles was measured under various medium osmolarities using D-cellobiose to estimate the binding values. The uptake of each compound was inversely proportional to medium osmolarity from 320 mosM to 715 mosM (Fig. 2). It was found that the binding of each organic cation to membrane surfaces estimated by the extrapolation to infinite extravesicular osmolarity (zero intravesicular space) was greater than that of its zwitterionic derivative (Fig. 2).

Effect of phosphatidylserine on the binding of organic cations to the liposome

The binding behaviour of organic cations and their zwitterionic derivatives to liposome are shown in Fig. 3. The binding of BOTA and tryptamine clearly amplified with increased amounts of phosphatidylserine, a negatively charged phospholipid. On the contrary, the binding of zwitterionic compounds, tryptophan and BOTP, were extremely small compared to that of organic cations, and were unaffected by any variation of phosphatidylserine content in the liposome. Furthermore, as illustrated in Fig. 4, there was no significant increase in binding of the organic cations to the liposome containing phosphatidylethanolamine, which has no negative charges. These results suggest that these cations interact electrostatically with the negative charge of phosphatidylserine on the lipid membrane surface.

Inhibition study between organic cations on the binding to liposome

Fig. 5 shows the inhibitory effect of BOTA, an organic cation, on the binding of tryptamine and BOTP to the liposome. The binding of tryptamine was concentration dependently inhibited by BOTA, while no comparable change was observed in the BOTP binding in the presence of BOTA.

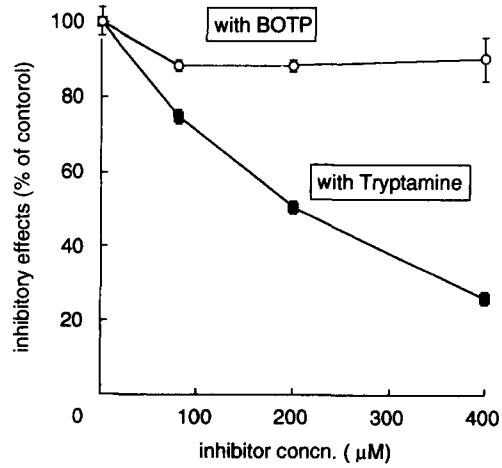

Fig. 5. Inhibitory effect of BOTA on bindings of tryptamine and BOTP to liposomes. Initial concentration of BOTA was 8 μM and liposome (2 mg/ml) composition was PC/PS/PA (7:2:1). Each point represents the mean with S.E. of three determinations.

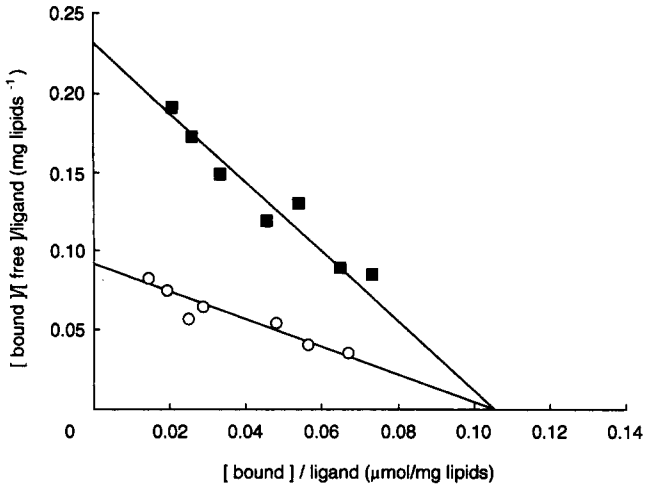

Fig. 6. Scatchard analysis of tryptamine binding to liposome (PC/PS/PA = 7:2:1). Binding of tryptamine was examined in the presence (○) or absence (■) of BOTA (200 μM). All values are means of three observations liposome concentration was 2 mg/ml. Intercept of line with the X-axes and slope are B_{max} (B_{max} = 107.0 nmol/lipid) and $-1/K_s$ (K_s = 0.4563 mM), respectively.

Moreover, a certain saturability was observed in the tryptamine binding to the liposome at a concentration of more than ca. 200 μM when tryptamine concentration increased from 10 to 860 μM. Scatchard analysis of tryptamine binding with/without BOTA resulted in a competitive inhibition (Fig. 6), and revealed that a single population of binding sites was observed in phospholipid membrane (B_{max} = 107.0 nmol/mg lipid, K_s = 0.456 mM).

Discussion

In the previous paper [19], we demonstrated the uptake behaviour of the cationic form of enoxacin, which is a zwitterionic antimicrobial agent (pK_{a1} = 6.2, pK_{a2} = 8.8), play an important role in the intestinal transport process of this drug. Furthermore, we confirmed that the enoxacin uptake by brush-border membrane vesicles was stimulated by an electrical transmembrane potential (interior negative) [19].

In regard to the binding characteristics of various organic cations to brush-border membrane, it has been clarified that the interaction of quaternary ammonium compounds to the membrane was a first step in their transport mechanism driven by physiological membrane potential [25], and that the binding of polyamines such as spermine, spermidine, and putrescine to the outside and inside of brush-border membrane vesicles could be a contributory factor in the high accumulation of these polyamines in the intestine [26,27]. Moreover, several investigators [28–30] reported that the anionic phospholipids were the binding sites in the renal brush-border membrane for the polycationic aminoglycoside such as gentamicin, and that the binding was due to a charge interaction between the anionic phospholipid and the aminoglycoside. Our experimental results in the present study were in close agreement with those of gentamicin [28–30] and polyamines [26,27]. Thus, we conclude that there was a close relationship between the binding to membrane (static interaction) and the transport characteristics (dynamic interaction) in transport processes of these cations into the membrane.

Electrostatic interaction finding between the cationic compound and the phospholipid (Fig. 3) were in agreement with the results reported by Lullmann and Wehling [31] and Kubo et al. [32]. The binding of BOTA and tryptamine to the liposome increased proportionally with the content of phosphatidylserine, a component of the biological membrane (Fig. 4). In contrast, the interaction between phosphatidylserine and their zwitterionic derivatives was not observed. These results suggest that the anionic charged residue of zwitterionic derivatives can prevent cationic charge from interacting with the membrane surface. On the other hand, the binding of organic cations to the membrane has a relatively high specificity due to that there was a competitive manner revealed in the inhibition study. This finding was observed in the liposome containing no membrane protein, such as a transport carrier. Thus, it can be assumed that the specificity of transport and/or binding was caused by not only carrier proteins but also charge density and relative location of membrane lipid. There can exist a similar manner in binding of cationic compounds between native cell membrane and phospholipid liposome due to that the mutual inhibition between organic cations on binding to liposome was similar to the result obtained from the native intestinal brush-border membrane vesicles.

In conclusion, in this study, higher binding of each cationic compound tested to rat intestinal brush-border membrane vesicles was exhibited than those of their zwitterionic derivatives. The binding of these cations was also observed in liposome-binding studies, and binding increased proportionally with the content of phosphatidylserine in the liposome. A competitive inhibition between the organic cations indicates the structure-specificity of binding to phosphatidylserine.

References

1 Kessler, M., Acuto, O., Strelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154.
2 Simon, M.J. (1989) Biochim. Biophys. Acta 979, 132–138.
3 Wilson, D., Barry, J.A. and Ramaswamy, K. (1989) Biochim. Biophys. Acta 986, 123–129.
4 Miyamoto, Y., Thompson, Y.G., Howard, E.F., Ganapathy, V. and Leibach, F. (1991) J. Biol. Chem. 266, 4742–4745.
5 Gutierrez, M.M., Brett, C.M., Ott, R.J., Hui, A. C. and Giacomini, K.M. (1992) Biochim. Biophys. Acta 1105, 1–9.

6 Skopicki, H.A., Zikos, F.D., Flouret, G. and Peterson, D.R. (1989) Am. J. Physiol. 257, C971-C975.
7 Jorgensen, K.E. and Sheikh, M.I. (1987) Biochem. J. 248, 533–538.
8 Tiruppathi, C., Ganapathy, D. and Leibach, F.H. (1987) Pediatr. Res. 22, 641–646.
9 Katura, T., Maegawa, H., Tomita, Y., Takano, M., Inui, K.-I. and Hori, R. (1991) Am. J. Physiol. 261, F774–F778.
10 Schron, C.M., Washington, C. Jr. and Blitzer, B.L. (1985) J. Clin. Invest. 76, 2030–2033.
11 Sugawara, M., Toda, T., Iseki, K., Miyazaki, K., Shiroto, H., Kondo, Y. and Uchino, J.-I. (1992) J. Pharm. Pharmacol. 44, 968–972.
12 Friedman, D.I. and Amidon, G.L. (1989) J. Pharm. Sci. 78, 995–998.
13 Tsuji, A., Simanjuntak, M.T., Tamai, I. and Terasaki, T. (1990) J. Pharm. Sci. 79, 1123–1124.
14 Tsuji, A., Tamai, I., Nakanishi, M. and Amidon, G.L. (1990) Pharm. Res. 7, 308–309.
15 Iseki, K., Mori, K.-I., Miyazaki, K. and Arita, T. (1987) Biochem. Pharmacol. 36, 1837–1842.
16 Iseki, K., Mori, K.-I., Miyazaki, K. and Arita T. (1987) Biochem. Pharmacol. 36, 1843–1837.
17 Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K. and Arita, T. (1988) J. Pharm. Pharmacol. 40, 701–705.
18 Iseki, K., Sugawara, M., Saitoh, H., Miyazaki, K. and Arita, T. (1989) J. Pharm. Pharmacol. 41, 628–632.
19 Sugawara, M., Saitoh, H., Iseki, K., Miyazaki, K. and Arita, T. (1990) J. Pharm. Pharmacol. 42, 314–318.
20 Sugawara, M., Sasaki, M., Iseki, K. and Miyazaki, K. (1992) Biochim. Biophys. Acta 1111, 145–150.
21 Takahashi, Y., Itoh, T., Kobayashi, M., Sugawara, M., Saitoh, H., Iseki, K., Miyazaki, K., Miyazaki, S., Takada, M. and Kawashima, Y. (1993) J. Pharm. Pharmacol., in press.
22 Iseki, K., Hirano, T., Fukushi, Y., Kitamura, Y., Miyazaki, S., Takada, M., Sugawara, M., Saitoh, H. and Miyazaki, K. (1992) J. Pharm. Pharmacol. 44, 722–726.
23 Utsumi, T., Aizono, Y. and Funatsu, G. (1987) FEBS Lett. 216, 99–103.
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 234, 466–468.
25 Saitoh, H., Kawai, S., Iseki, K., Miyazaki, K. and Arita, T. (1988) J. Pharm. Pharmacol. 40, 776–780.
26 Iseki, K., Kobayashi, M. and Miyazaki, K. (1991) Biochim. Biophys. Acta 1068,105–110.
27 Kobayashi, M., Iseki, K. and Miyazaki, K. (1992) Biochim. Biophys. Acta 1105, 177–183.
28 Hori, R., Okuda, M., Ohishi, Y., Yasuhara, M. and Takano, K. (1992) J. Pharmacol. Exp. Ther. 255, 867–874.
29 Kishore, B.K., Kallay, Z., Lambright, P., Laurent, G. and Tulkens, P.M. (1990) J. Pharmacol. Exp. Ther. 261, 867–874.
30 Sastrasinh, M., Knauss, T.C., Weinberg, J.M. and Humes, H.D. (1982) J. Pharmacol. Exp. Ther. 222, 350–358.
31 Lullman, H. and Wehling, M. (1979) Biochim. Pharmacol. 28, 3409–3415.
32 Kubo, M., Gardner, M.F. and Hostetler, K.Y. (1980) Biochem. Pharmacol. 35, 3761–3765.