0090-9556/98/2611-1144–1148$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 26, No. 11
Printed in U.S.A.

TRANSPORT OF THE β-LACTAM ANTIBIOTIC BENZYLPENICILLIN AND THE DIPEPTIDE GLYCYLSARCOSINE BY BRAIN CAPILLARY ENDOTHELIAL CELLS *IN VITRO*

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(Received March 9, 1998; accepted June 24, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:

Peripherally administered β-lactam antibiotics, which are structural analogs of tripeptides, may cause neurotoxic reactions or induce seizures. Previous *in vivo* studies provided evidence for brain uptake of these antibiotics. In the present work, we studied the extent and mechanism of the uptake of benzylpenicillin and glycylsarcosine by brain microvessel endothelial cells *in vitro*, using freshly isolated and cultured porcine brain capillary endothelial cells. Characterization of the cell culture model demonstrated the functional expression of the system transporting the neutral amino acids leucine and phenylalanine. The initial rate of uptake of benzylpenicillin was >3-fold greater than the rate of uptake of the extracellular marker sucrose (ratio, 3.29 ± 0.37), whereas uptake of glycylsarcosine did not differ from that of sucrose. The differences in cellular uptake correlated with the octanol/buffer partition coefficients for glycylsarcosine and benzylpenicillin (1.16×10^{-3} for glycylsarcosine and 6.83×10^{-2} for benzylpenicillin). The concentration-dependent uptake of benzylpenicillin (1–2000 μM) was not saturable and was not sensitive to shifts in pH or temperature. The permeability-surface area product for the uptake of benzylpenicillin at pH 7.4 was determined from these experiments and was found to be 8.1×10^{-5} ml/sec/g of brain. This value was very close to the value determined in *in vivo* studies. Uptake of benzylpenicillin and glycylsarcosine was not reduced in the presence of 1 mM ceftibuten or 100 μM probenecid. The findings with cultured cell monolayers were confirmed using freshly isolated endothelial cells. These *in vitro* data are compatible with benzylpenicillin, but not glycylsarcosine, being able to penetrate endothelial cells. Uptake of benzylpenicillin by brain capillary endothelial cells occurs by a slow nonsaturable process, with no evidence for carrier-mediated transport.

Knowledge of drug disposition is an essential prerequisite for estimating drug effectiveness. It is of particular importance when drugs must cross epithelial or endothelial barriers to exert their actions at the desired target sites. Antibiotics must cross the blood-brain barrier to be suitable for use in the treatment of severe cerebral infections such as bacterial meningitis. However, reports on the blood-brain barrier permeation of β-lactam antibiotics and/or small peptides are controversial. On one hand, small peptides seem to cross the blood-brain barrier in only negligible amounts, which do not differ from those of extracellular markers (Himmelseher *et al.*, 1996; Vasquez *et al.*, 1992). On the other hand, β-lactam antibiotics (which are structural analogs of tripeptides) (Suzuki and Sugiyama, 1994) seem to penetrate the blood-brain barrier, because central neurotoxic reactions or the induction of seizures can be caused by peripherally administered β-lactam antibiotics (Grondahl and Langmoen, 1993; Schliamser *et al.*, 1991; Sunagawa and Nouda, 1996). Using an *in situ* brain perfusion technique, a probenecid-sensitive mechanism was suggested for the facilitated diffusion of some β-lactam antibiotics across the blood-brain barrier (Spector, 1987; Suzuki *et al.*, 1989). Other reports, using the carotid artery injection technique in rats, imply carrier-mediated uptake of antibiotics *via* the monocarboxylate carrier (Kang *et al.*, 1990).

Using *in vivo* techniques, such as those described above, the actual contribution of a certain cell type within the brain microvasculature to brain penetration of a given drug cannot be determined. The situation is complicated by the presence of two distinct barriers, *i.e.* the choroid plexus and the blood-brain barrier. For instance, it has been shown that a transport system for benzylpenicillin exists in the rat choroid plexus (Suzuki *et al.*, 1987b).

It was, therefore, the aim of the present study to determine the contribution of the brain capillary endothelial cells, which make up the blood-brain barrier, to the penetration of a β-lactam antibiotic (the tripeptide analog penicillin) and the metabolically stable dipeptide glycylsarcosine. The present study was carried out in an *in vitro* system, using freshly isolated or cultured porcine brain microvessel endothelial cells (Huwyler *et al.*, 1996). Because benzylpenicillin and glycylsarcosine are substrates of the dipeptide (oligopeptide) carrier expressed in small intestine (Dantzig and Bergin, 1988; Kramer *et al.*, 1992) and kidney (Boll and Daniel, 1995), special attention was paid to the possible contribution of this carrier system.

Materials and Methods

Chemicals and Reagents. [2-^{14}C]Glycyl[1-^{14}C]sarcosine (110 mCi/mmol) was from Movarek Biochemicals (Brea, CA). [*phenyl*-4-^{3}H]Benzylpenicillin (13.6 Ci/mmol), L-[4,5-^{3}H]leucine (67 Ci/mmol), L-[4-^{3}H]phenylalanine

This work was supported by the Swiss National Science Foundation (Grant 32–42179.94), a scholarship to M.T. from the Association of Chemical Industries (Basel, Switzerland), and the ASTRA Research Fund of the Department of Internal Medicine of the University Hospital (Basel, Switzerland).

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(15 Ci/mmol), [6,6′(N)-^{3}H]sucrose (16.6 Ci/mmol), and [U-^{14}C]sucrose (626 mCi/mmol) were from Amersham (Buckinghamshire, UK). Ceftibuten was a gift from Essex Chemie (Luzern, Switzerland). Probenecid was from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of the highest quality available.

Determination of PC[1] Values. Radioactively labeled benzylpenicillin and glycylsarcosine (0.3 μCi) were dissolved in 300 μl of 100 mM phosphate buffer, pH 7.4. The solution was added to the same volume of 1-octanol (Merck, Darmstadt, Germany), and this mixture was equilibrated overnight at room temperature before centrifugation for 30 min. The concentration of the tracer in each phase was determined by liquid scintillation counting.

Microvessel Endothelial Cell Isolation. Primary cultures of porcine brain capillary endothelial cells were prepared as described previously (Huwyler *et al.*, 1996). Briefly, cortical gray matter from six fresh porcine brains was minced and digested enzymatically using 0.5% dispase. Cerebral microvessels were obtained after centrifugation in 13% dextran and were subsequently incubated in buffer containing 1 mg/ml collagenase/dispase. The resulting cell suspension was supplemented with 10% horse serum and filtered through 150-μm nylon mesh, and brain capillary endothelial cells were separated on a continuous 50% Percoll gradient (Pharmacia, Uppsala, Sweden). Isolated endothelial cells were filtered through 35-μm nylon mesh before being seeded, at a density of 150,000 cells/cm^2, onto collagen/fibronectin (Boehringer-Mannheim)-coated, 24-well, cell culture plates. Cells were cultured under standard cell culture conditions [cell culture medium consisting of 45% minimal essential Eagle medium, 45% Ham's F-12 nutrient mixture, 100 μg/ml streptomycin, 100 μg/ml penicillin G, 100 μg/ml heparin, 13 mM $NaHCO_3$, and 20 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (all from Sigma), with 10% heat-inactivated horse serum (Gibco BRL, Basel, Switzerland)].

Morphological and Biochemical Characterization of Freshly Isolated Cells and 10-Day-Old Cell Cultures. At day 10 of growth, all cell monolayers used for transport experiments exhibited spindle-shaped cells (typical for primary cultures of capillary endothelial cells) (Audus and Borchardt, 1986). Contamination by cells of other shapes was rare, but cultures containing them were rejected. Cultured endothelial cells expressed enzymatic markers such as angiotensin-converting enzyme (EC 3.4.15.1.) (29.6 $\pm$ 4.4 nmol/mg/min) and alkaline phosphatase (EC 3.1.3.1) (549.8 $\pm$ 34.5 nmol/mg/min). In addition, the expression of the tight junctional marker protein ZO-1 by the cell cultures could be shown by immunostaining (Huwyler *et al.*, 1996). Tight junctions play an important role in maintaining the integrity of the blood-brain barrier; therefore, the expression of the zona occludens protein ZO-1 at confluency is evidence of the formation of a structurally intact and polarized monolayer. This has been demonstrated for several endothelial and epithelial cell layers *in vitro* and *in vivo, e.g.* Madin-Darby canine kidney and endothelial cells (Smith and Shine, 1992; Staddon *et al.*, 1995).

Uptake Assays. Uptake assays were performed at 20°C, using 10-day-old confluent monolayers of porcine brain capillary endothelial cells. Cells were grown in 24-well cell culture plates. The surface area was 2 cm^2/well. Cells were washed using transport buffer [142 mM NaCl, 3 mM KCl, 1.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.5 mM K_2HPO_4, 4 mM D-glucose, 10 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, pH 6.5 or, where indicated, pH 7.4]. The reaction was initiated by addition of 250 μl of transport buffer containing 0.3 μCi of ^{3}H- or ^{14}C-labeled tracer for the respective substrate, sufficient unlabeled substrate or inhibitor to bring the medium to the desired final concentration, and 0.3 μCi of the labeled extracellular marker sucrose. Incubations were terminated after 2 min ([^{3}H]benzylpenicillin, [^{3}H]leucine, or [^{3}H]phenylalanine) or 5 min ([^{14}C]glycylsarcosine) by rapid removal of the incubation medium by aspiration, followed by washing of the cells using ice-cold transport buffer. Incubations in the presence of inhibitors were performed with 1 mM ceftibuten or 0.1 mM probenecid. Cells were then detached from the wells by incubation with trypsin (0.25%) for 10 min and were transferred to scintillation vials. The amount of radiolabeled substrate taken up by the cells was determined by scintillation counting.

Uptake in freshly isolated brain capillary endothelial cells was assayed at 20°C. The viability of the cells was determined by trypan blue exclusion. Only cell populations with a viability of >95% were used for additional experiments. Total cell numbers were measured with a hemocytometer, and 2.0 $\times$ 10^6 cells were used in each incubation. The reaction was initiated by mixing 100 μl of cell suspension in transport buffer with 50 μl of transport buffer containing substrate (0.3 μCi of [^{3}H]benzylpenicillin or [^{14}C]glycylsarcosine), an extracellular marker (0.3 μCi of ^{14}C- or ^{3}H-labeled sucrose), and, when indicated, the inhibitor ceftibuten at a final concentration of 0.66 mM. The cells were incubated for 2 min ([^{3}H]benzylpenicillin) or 5 min ([^{14}C]glycylsarcosine) on a rotary shaker. One hundred microliters of the cell suspension were then transferred to microcentrifuge tubes containing 50 μl of 3 M KOH and 150 μl of silicone oil (1:1, v/v, mixture of silicone oil types Ar20 and Ar200; Wacker Chemie, München, Germany). The cells were immediately centrifuged in a tabletop microfuge (Hettich, Tuttlingen, Germany) capable of rapid acceleration. The centrifugation tubes were then transferred to liquid nitrogen. The amount of tracer taken up was quantified by cutting the frozen centrifugation tube just above the KOH/oil interface and placing the tip of the tube (with the cell pellet layer) in a scintillation vial containing 500 μl of Solutron tissue solubilizer (Kontron, Zürich, Switzerland). Samples were incubated overnight, sonicated in a water bath sonicator, and neutralized using 70 μl of glacial acetic acid. The amount of radiolabeled substrate in the pellet layer was determined by scintillation counting.

Metabolic Stability. The radiochemical purity of tracers, as determined by the supplier using HPLC analysis, was 98.9% for [^{14}C]glycylsarcosine and 97.5% for [^{3}H]benzylpenicillin. The metabolic stability of [^{3}H]benzylpenicillin was examined by TLC (Spector, 1986; Suzuki *et al.*, 1987a). Samples were spotted on a silica gel plate (precoated silica gel 60; Merck, Darmstadt, Germany) and then developed in a solvent system of methanol/isopropanol (7:3, v/v). The locations of unlabeled benzylpenicillin and its possible metabolites were determined under UV light. An autoradiograph of the silica gel plate was used to locate isotope-labeled benzylpenicillin and possible radiolabeled metabolites. During a 2-min incubation of the substrate with cells, there was negligible formation of metabolites.

Data Analysis. To represent data and to obtain estimates of kinetic parameters, a nonlinear regression program was used (Microcal Origin, version 3.5; Origin, Microcal Software, Inc., Northampton, MA). For statistical comparison, data of groups were compared by analysis of variance. The level of significance was $p = 0.05$. If this analysis revealed significant differences, pairwise comparisons within groups were performed with two-sided unpaired *t* tests. The *p* values were adjusted by Bonferroni's correction for multiple comparisons.

Results

Structural and Functional Characterization of the *In Vitro* Cell Culture System. It was our goal to study the contribution of brain microvessel endothelial cells to brain uptake of β-lactam antibiotics, with benzylpenicillin and glycylsarcosine as model compounds, and to correlate the *in vitro* data with previous *in vivo* findings. Primary cultures of porcine brain capillary endothelial cells were used as an *in vitro* system. This system was first characterized to establish its suitability.

Microscopic inspection of the cultured cells clearly demonstrated a homogeneous cell population. Biochemical and immunochemical characterization demonstrated the preservation of important characteristics in isolated and cultured brain endothelial cells, such as endothelial marker enzymes and the zona occludens-associated protein ZO-1 (see *Materials and Methods*), thus confirming our own recent data (Huwyler *et al.*, 1996, 1997). In this report, validation of the model was extended to functional assays, to demonstrate the intactness of a carrier system (the L-system of neutral amino acid transport). Initial rates of uptake of leucine and phenylalanine as functions of their concentrations in the incubation mixture were determined. The overall uptake of leucine consisted of two components, *i.e.* a linear term representing passive diffusion and a second term representing a specific transport system. The latter was saturable and showed Michaelis-Menten-type kinetics, with a K_M of 19 μM and

[1] Abbreviations used are: PC, octanol/buffer partition coefficient; PS, permeability-surface area.

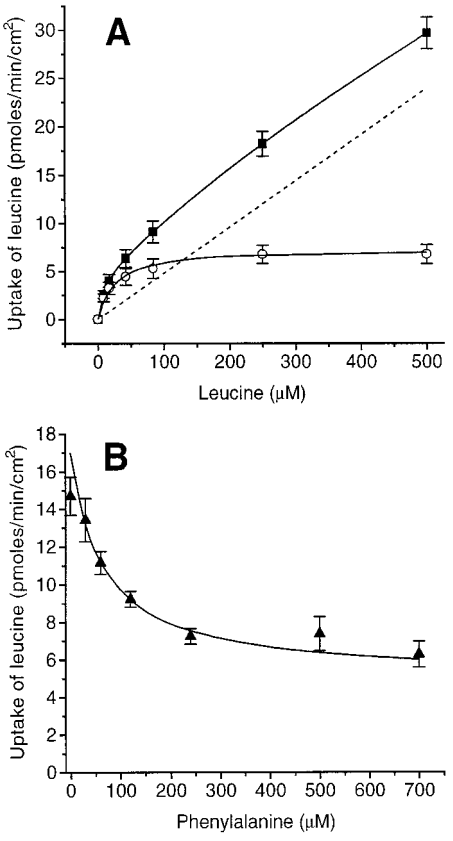

FIG. 1. *Functional characterization of cultured brain microvessel endothelial cells.*

A, Carrier-mediated, and therefore saturable, uptake of leucine by endothelial cells (○), shown as a function of increasing substrate concentration. This curve represents the difference between the overall uptake of leucine (■) and the calculated simple diffusion term (*dashed line*). *B*, Competitive inhibition of leucine uptake by cultured brain endothelial cells in the presence of different concentrations of phenylalanine. Data points are means ± SE ($N = 8$).

a V_{max} of 6.9 pmol/min/cm^2. The parameters for uptake of phenylalanine were determined in a separate set of experiments; the K_M was 40 μM and the V_{max} was 12 pmol/min/cm^2. When the uptake of leucine was measured in the presence of increasing concentrations of phenylalanine, which shares the leucine carrier in other cell types (Oldendorf, 1971b), transport was apparently competitively inhibited, with a K_i of 13 μM (fig. 1*B*). Here again, only specific transport could be inhibited, resulting in a reduction of leucine uptake by a maximum of 70%; the remainder represents passive diffusion. In these experiments, sucrose was used as an extracellular marker. Cell-associated sucrose was found to be minimal, and <0.07% of the applied dose of sucrose was typically recovered. This value did not change with incubation time (determined for incubation times ranging from 1 to 30 min).

Uptake of Benzylpenicillin and Glycylsarcosine by Cultured Brain Microvessel Endothelial Cells. The initial rates of uptake of the peptide analog benzylpenicillin and the dipeptide glycylsarcosine were measured with confluent monolayers of cultured porcine brain capillary endothelial cells. The cells were incubated with the respective drug on the luminal side, which corresponds to the blood-directed cell surface *in vivo*. The initial rates of uptake were directly compared with the uptake of the extracellular marker sucrose. The benzylpenicillin/sucrose uptake ratio was 3.29 ± 0.37 ($N = 10$, mean ± SE, pH 6.5). The glycylsarcosine/sucrose uptake ratio was 0.74 ± 0.03 ($N = 10$, pH 6.5). Thus, cellular uptake of benzylpenicillin was >3-fold

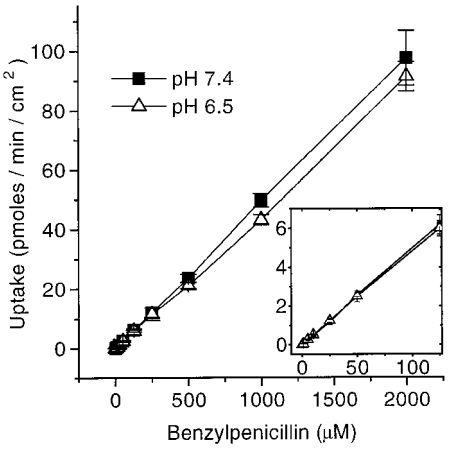

FIG. 2. *Concentration-dependent uptake of benzylpenicillin by brain microvessel endothelial cells at pH 6.5 and pH 7.4.*

Inset, magnification of the first section of the graph. Data points represent means ± SE ($N = 4$).

greater than that of sucrose (statistically significant difference by Student's t test, $p < 0.001$), whereas that of glycylsarcosine did not differ from the corresponding sucrose value ($p = 0.39$). These differences in cellular uptake correlated with differences in the lipophilicity of these compounds. The PC of benzylpenicillin was >1-log unit greater than that of sucrose (log PC of [^{14}C]sucrose = −2.971 ± 0.092, $N = 3$; log PC of [^{3}H]benzylpenicillin = −1.165 ± 0.004, $N = 5$; $p < 0.001$). The PC of glycylsarcosine (log PC of [^{14}C]glycylsarcosine = −2.935 ± 0.008, $N = 5$) equals that of sucrose ($p = 0.62$).

To determine whether there was carrier-mediated transport, initial rates of cellular uptake of benzylpenicillin were determined at pH 7.4 and pH 6.5, over a wide range of concentrations (1–2000 μM) (fig. 2). Uptake was linear at pH 7.4, as well as at pH 6.5, over the entire range of concentrations and could be approximated by linear regression (coefficients of correlation under the two pH conditions were >0.999), thus giving no indication for carrier-mediated transport. The PS product for the uptake of benzylpenicillin at pH 7.4 could be calculated from the data shown in fig. 2. The slope of the line represents the PS product and equals 48.9 (pmol/min/cm^2)/mM. This corresponds to a PS product of 8.1×10^{-5} ml/sec/g of brain (assuming a value of 100 cm^2/g of brain) for the surface area of the blood-brain barrier *in vivo* (Pardridge *et al.*, 1990).

Uptake experiments with benzylpenicillin and glycylsarcosine were also performed at different temperatures and in presence of inhibitors of the dipeptide carrier, to clarify the potential involvement of this carrier system (table 1). The cephalosporin antibiotic ceftibuten is a substrate of the dipeptide carrier (Matsumoto *et al.*, 1995; Sugawara *et al.*, 1994) and was therefore used as a potential competitive inhibitor of benzylpenicillin and glycylsarcosine uptake. Probenecid has been reported to interact with *in vivo* brain uptake of benzylpenicillin (Suzuki *et al.*, 1989). In addition, it was suggested that a probenecid-sensitive drug efflux system might exist at the blood-brain barrier (Dykstra *et al.*, 1993; Wong *et al.*, 1992). In the inhibition experiments, the concentrations of ceftibuten and probenecid were 1 and 0.1 mM, respectively. These concentrations correspond to those of previously performed studies with ceftibuten (Matsumoto *et al.*, 1995) or probenecid (Suzuki *et al.*, 1987a). Our results indicate that uptake of benzylpenicillin and uptake of glycylsarcosine at low temperatures and in the presence of inhibitors of the dipeptide carrier were not statistically significantly different from controls determined at pH 6.5.

Uptake of Benzylpenicillin and Glycylsarcosine by Freshly Iso-

TABLE 1

Uptake of benzylpenicillin and glycylsarcosine under different incubation conditions

Tracer	Condition	Tracer/Sucrose Ratio	*N*	*p* Value[a]
		% of control		
[^{3}H]Benzylpenicillin	pH 6.5	100[b]	4	
	pH 7.4	88.5 ± 4.5	4	NS
	Ceftibuten (1 mM), pH 6.5	166.7 ± 27.8	10	NS
	Probenecid (100 μM), pH 6.5	88.3 ± 7.6	4	NS
	4°C, pH 6.5	88.1 ± 2.7	4	NS
	4°C, pH 7.4	94.6 ± 5.2	4	NS
[^{14}C]Glycylsarcosine	pH 6.5	100[b]	4	
	pH 7.4	95.9 ± 2.8	4	NS
	Ceftibuten (1 mM), pH 6.5	87.3 ± 5.3	6	NS
	Probenecid (100 μM), pH 6.5	95.7 ± 1.3	4	NS
	4°C, pH 6.5	103.2 ± 1.3	4	NS
	4°C, pH 7.4	102.1 ± 1.9	4	NS

Values are means ± SE of *N* experiments.
[a] By two-tailed Student's *t* test. NS, statistically not significant ($p > 0.05$). The *p* values were adjusted by Bonferroni's correction for multiple comparisons.
[b] Control value, which was defined as 100%.

lated Brain Capillary Endothelial Cells. The uptake of benzylpenicillin and glycylsarcosine was measured in freshly isolated brain capillary endothelial cells (table 2) to exclude the possibility of a regulatory effect of the culture conditions on the expression of carrier proteins by brain capillary endothelial cells. These experiments confirmed the results obtained with confluent monolayers, in that the ratio between the uptake of benzylpenicillin and the uptake of sucrose was 4.41 ± 0.47 ($N = 5$, pH 6.5, $p < 0.001$). The ratio between the uptake of glycylsarcosine and the uptake of sucrose was 0.89 ± 0.01 ($N = 5$, $p = 0.78$). Furthermore, the uptake of benzylpenicillin and glycylsarcosine in the presence of ceftibuten was not statistically significantly different from control values ($p > 0.1$).

Discussion

β-Lactam antibiotics are used for the treatment of central nervous system infections, although their therapeutic use is often hampered by the low blood-brain barrier permeability of many common antibiotics. There is still controversy regarding how the transfer of β-lactam antibiotics is mediated. Central nervous system side effects have been clearly observed for some of these drugs (Schliamser *et al.*, 1991), and there is evidence from *in vivo* studies showing that β-lactam antibiotics may penetrate the brain (Matsushita *et al.*, 1991; Spector, 1987). However, *in vitro* data describing the mechanism of transport in more detail are lacking. Therefore, the uptake of benzylpenicillin and the dipeptide glycylsarcosine was characterized using an *in vitro* model of the blood-brain barrier that consists of cultured or freshly isolated porcine brain capillary endothelial cells. Furthermore, we correlated our *in vitro* findings with previous *in vivo* studies on brain uptake of β-lactam antibiotics, to obtain information on the predictive ability of our cell culture model.

The β-lactam antibiotic benzylpenicillin is a derivative and structural analog of a tripeptide and shares the uptake system for di- and tripeptides in the intestine and in the kidney (Ganapathy *et al.*, 1995). Glycylsarcosine, an hydrolysis-resistant dipeptide, was used as a second substrate. This compound was used previously to characterize the peptide carriers PEPT 1 and PEPT 2 in intestine and kidney (Ganapathy *et al.*, 1995; Thwaites *et al.*, 1993; Tomita *et al.*, 1995). Glycylsarcosine is hydrophilic and has a low PC. Its uptake was marginal and even lower than the uptake of the extracellular marker sucrose. In contrast, the uptake of benzylpenicillin, which has a considerably higher PC than sucrose, was >3-fold greater than the endothelial uptake of sucrose. This *in vitro* finding parallels *in vivo* observations where, using a single-pass brain-uptake technique, penicillin was shown to penetrate from the blood into rat brain, although the rate of transport was low (Oldendorf, 1971a).

From the concentration-dependent uptake experiment with benzylpenicillin, the PS product was determined to be 8.1×10^{-5} ml/sec/g of brain. This *in vitro* value is very close to the *in vivo* PS product of 9×10^{-5} ml/sec/g of brain that was determined using a brain perfusion technique, at a substrate concentration where transport was most efficient (Suzuki *et al.*, 1989). Thus, our results, which were obtained using a cell culture system, are in very good agreement with *in vivo* data.

Experiments were carried out to determine whether the uptake of benzylpenicillin or glycylsarcosine might be mediated by a carrier system. Special attention was thus given to a possible involvement of the dipeptide carrier system. Concentration-dependent uptake of benzylpenicillin by brain endothelial cells showed no saturation and was not temperature sensitive, indicating that endothelial uptake of benzylpenicillin represents simple diffusion. In addition, the uptake of benzylpenicillin was not stimulated by acidification of the incubation medium to pH 6.5, which is in contrast to the pH-sensitive stimulation of transport by the intestinal and renal peptide carriers (Ganapathy *et al.*, 1995; Thwaites *et al.*, 1993). Also, the uptake of glycylsarcosine

TABLE 2

Uptake of benzylpenicillin and glycylsarcosine by freshly isolated brain capillary endothelial cells

Tracer	Condition	Tracer/Sucrose Ratio	*N*	*p* Value[a]
		% of control		
[^{3}H]Benzylpenicillin	pH 6.5	100[b]	5	
	Ceftibuten (0.66 μM), pH 6.5	139.6 ± 5.4	5	NS
[^{14}C]Glycylsarcosine	pH 6.5	100[b]	5	
	Ceftibuten (0.66 μM), pH 6.5	97.9 ± 1.6	5	NS

Values are means ± SE of *N* experiments.
[a] By two-tailed Student's *t* test. NS, statistically not significant ($p > 0.05$, adjusted by Bonferroni's correction for multiple comparisons).
[b] Control value, which was defined as 100%.

at pH 7.4 showed no statistically significant difference from the uptake at pH 6.5. To assess whether uptake of benzylpenicillin and glycylsarcosine could be inhibited by another substrate of the dipeptide carrier, we used the cephalosporin antibiotic ceftibuten (Matsumoto *et al.*, 1995; Saito *et al.*, 1995). Ceftibuten caused no statistically significant reduction of uptake. However, there may be differences between swine and other species in the transport of benzylpenicillin and ceftibuten.

Although *in vitro* systems have repeatedly been shown to accurately predict the blood-brain barrier permeability found *in vivo* (Audus and Borchardt, 1986; Dehouck *et al.*, 1992; this study), a possible underestimation of carrier-mediated transport using *in vitro* systems may be attributed to the loss of expression of carrier proteins in endothelial cell culture (Pardridge *et al.*, 1990). Biochemical and functional characterization of the cell culture model used suggested, however, that typical attributes of capillary endothelial cells were maintained. Carrier systems such as the amino acid transporter and P-glycoprotein (Huwyler *et al.*, 1996) were functionally active. To exclude the possibility of the loss of carrier activity during the culture period, uptake of benzylpenicillin and glycylsarcosine was also determined using freshly isolated brain capillary endothelial cells. The rates of uptake of benzylpenicillin and glycylsarcosine were very similar to the values obtained using endothelial cells in culture. Here again, incubations with and without the inhibitor ceftibuten were not significantly different.

Endothelial uptake of benzylpenicillin and glycylsarcosine was not affected by the presence of the organic anion probenecid. Therefore, it is unlikely that carrier-dependent uptake was masked by an active drug efflux system in brain endothelial cells, as was recently suggested for zidovudine (3′-azido-3′-deoxythymidine) (Dykstra *et al.*, 1993; Wong *et al.*, 1992). It is important to note that our *in vitro* model offers the advantage of direct access to the brain capillary endothelial cells, thus avoiding interference with other structures of the brain. This could be important in the present case, because penicillins and some cephalosporins are known to be accumulated in the choroid plexus by a probenecid-sensitive active transport system (Spector, 1987). Subsequent animal studies demonstrated probenecid inhibition of penicillin efflux from the cerebrospinal fluid (Suzuki *et al.*, 1987a). Thus, the slow entry of penicillins and cephalosporins in the brain is counteracted by a vigorous transport system in the choroid plexus, which transfers these agents from the cerebrospinal fluid into the blood. It is tempting to speculate that this mechanism could impede brain penetration of other antibiotics, such as cephalexin (Sakane *et al.*, 1991).

In summary, the present study provides a very good correlation between *in vitro* results obtained with cultured capillary endothelial cells and *in vivo* data obtained previously. The cell culture model can be used to study transport processes separate from other structures in the brain. The data show that benzylpenicillin, but not glycylsarcosine, is able to penetrate endothelial cells by simple diffusion. There was no indication for carrier-mediated transport of benzylpenicillin by porcine brain capillary endothelial cells.

Acknowledgments. We thank U. Behrens for excellent technical assistance and Dr. J. Baumann for critical revision of the manuscript.

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