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Report

Carrier-Mediated Transport of Baclofen Across Monolayers of Bovine Brain Endothelial Cells in Primary Culture

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The mechanism of transport of baclofen, a centrally acting muscle relaxant, across the blood–brain barrier (BBB) was studied using an *in vitro* model. The model consisted of a monolayer of bovine brain endothelial cells grown in primary culture on a porous regenerated cellulose membrane. The transport of baclofen across the monolayer expressed time and concentration dependency and was saturable. Transport data were corrected for diffusion and fitted to the Michaelis–Menten V_{max} model: K_m = 58.5 μ*M*, V_{max} = 0.23 nmol/min. The results validate the use of the *in vitro* BBB model as described and support the hypothesis that baclofen penetrates the BBB by means of a carrier-mediated transport system.

KEY WORDS: blood–brain barrier; baclofen; endothelial cells; bovine brain; amino acid carrier.

INTRODUCTION

Baclofen [4-amino-3-(*p*-chlorophenyl)-butyric acid; Lioresal], which is commonly used as a muscle relaxing agent (1), has its primary site of action in the central nervous system. Since baclofen is active after peripheral administration, it is assumed that the drug readily crosses the blood–brain barrier (BBB)[4] (2). It is unlikely that the mechanism of transport of baclofen is based solely upon passive diffusion because of its unfavorable physicochemical properties (i.e., low lipophilicity) (3–5).

The large neutral amino acid (LNAA) carrier at the BBB is responsible for transport of circulating amino acids, including centrally acting drugs that are amino acids, to the central nervous system (6). Previous studies have shown that baclofen inhibits the carrier-mediated transport of leucine across the BBB (7). Therefore, it is not unlikely that BBB transport of baclofen is due mainly to LNAA carrier-mediated uptake. The aim of our study was to verify this hypothesis experimentally by using an *in vitro* BBB model consisting of primary monolayer cultures from bovine brain microvessel endothelial cells (8). In this *in vitro* model system, specific properties associated with the BBB *in vivo* remain intact (8,9). In contrast to isolated brain microvessel preparations, this model offers the possibility of studying transcellular transport rather than cellular uptake (10–12). Thus, the investigation of transendothelial transport of baclofen demonstrates a specific application of the *in vitro* BBB model.

MATERIALS AND METHODS

Chemicals

Baclofen and [^{14}C] baclofen (sp act, 210 kBq/mg; 5.67 μCi/mg; Fig. 1) were a gift from Ciba-Geigy, Ardsley, N.Y. All other chemicals were of analytical grade; double-distilled water was used throughout experiments.

Isolation and Culture of Microvessel Endothelial Cells

Cells were isolated from cerebral gray matter of bovine brains by means of subsequent enzyme digestions and gradient centrifugation as previously detailed by Audus and Borchardt (8). Isolated cells were suspended in culture medium and seeded on collagen/fibronectin-coated regenerated cellulose disks (MW cutoff, 160,000; Sartorious, Goettinggen, FRG) placed in 100-mm plastic culture dishes (Corning Glass Works, Corning, N.Y.). Regenerated cellulose disks with complete monolayers (9–12 days in culture) were removed aseptically from the culture dishes and used for transport studies. Characterization of the obtained primary cultures has previously been presented (3,7,8).

Transendothelial Transport Studies

Transendothelial transport of baclofen was studied by placing a regenerated cellulose disk covered with cells in Side-Bi-Side diffusion cells (Crown Glass Co., Somerville, N.J.). Collagen-coated disks served as controls. The system was thermostated at 37 or 4°C by means of a thermal jacket and a circulating water bath. The donor chamber was filled with 3.0 ml assay buffer (122 m*M* NaCl, 25 m*M* $NaHCO_3$, 3 m*M* KCl, 1.4 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 0.4 m*M* K_2HPO_4,

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[4] Abbreviations used: BBB, blood–brain barrier; LNAA, large neutral amino acid.

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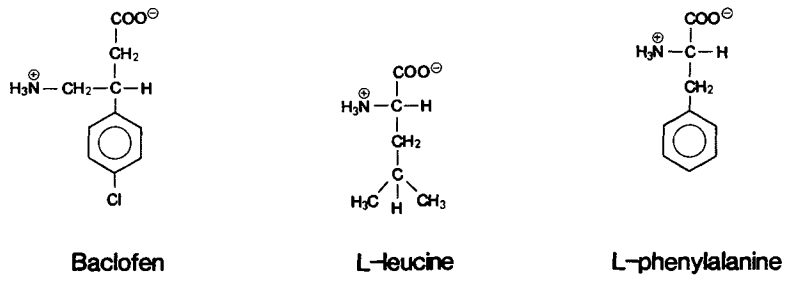

Fig. 1. Molecular structures of baclofen, leucine, and phenylalanine.

10 m*M* HEPES, pH 7.4, 10 m*M* glucose) (7) containing different concentrations of unlabeled baclofen and was pulsed with 100 μl [^{14}C]baclofen (0.5 μCi) at the beginning of the experiment.

The acceptor chamber was filled with 3.0 ml of blank assay buffer. Both chambers were stirred by Teflon stirring bars at a constant speed (600 rpm). At 30 sec after pulsing with radiolabeled baclofen, a 100 μl sample was drawn from the donor chamber in order to determine the precise amount of tracer added. The donor chamber volume was readjusted to 3.0 ml with assay buffer containing baclofen. Aliquots, 200 μl, were drawn from the acceptor chamber at various times after the start of the experiment. After each sampling, the acceptor chamber volume was readjusted up to 3.0 ml with assay buffer. Scintillation cocktail (3a70, Research Products Int. Corp., Mt. Prospect, Ill.) was added to each sample, and samples were thoroughly mixed and then counted in a scintillation counter (Beckman Model 6800, Fullerton, Calif.) for 5 min. Raw counts were transformed to dpm by means of a standard quench curve. Counts from acceptor side samples were corrected for the amount of radioactivity removed by previous sampling. The transport rate was determined from the slope of the linear regression on the amount of baclofen transported versus time. V_{max} and K_m were calculated using a nonlinear alogrithm with a simplex optimization routine (13).

RESULTS

Figure 2 illustrates the flux of baclofen across collagen-coated regenerated cellulose membranes, in the presence and absence of endothelial cells at 37°C. When thermostated at 4°C, comparable results were obtained, but transport rate values were substantially lower. All time-dependent transport curves showed a good linearity; values for correlation coefficients ranged between 0.989 and 0.999.

Figure 3 shows the relationship between baclofen concentration and transendothelial transport rate at 37 and 4°C. At 4°C a linear relationship exists between baclofen concentration and transcellular transport rate. At this temperature, transport did not show concentration dependency or apparent saturability, thus implying that the main transport mechanism is passive diffusion. At 37°C, a concentration dependency and saturation were observed, hence transport is likely to be governed by a carrier mechanism in addition to passive diffusion. The terminal part of the 37°C curve is parallel to the 4°C line as can be seen in Fig. 3, allowing transport values at 37°C to be corrected for diffusion (4°C data). Using "nondiffusional" transport values, a good fit was obtained to the Michaelis–Menten model (V_{max} = 0.23 nmol/min and K_m = 58.5 μ*M*). The computed curve is depicted in Fig. 3.

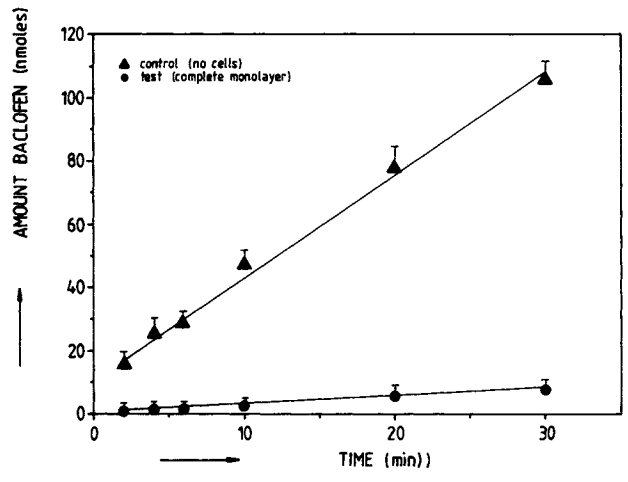

Fig. 2. Flux of baclofen vs time across either monolayers grown on regenerated cellulose membranes (test) or collagen-coated regenerated cellulose membranes alone (control). Each data point represents the mean ± SD from three different monolayers.

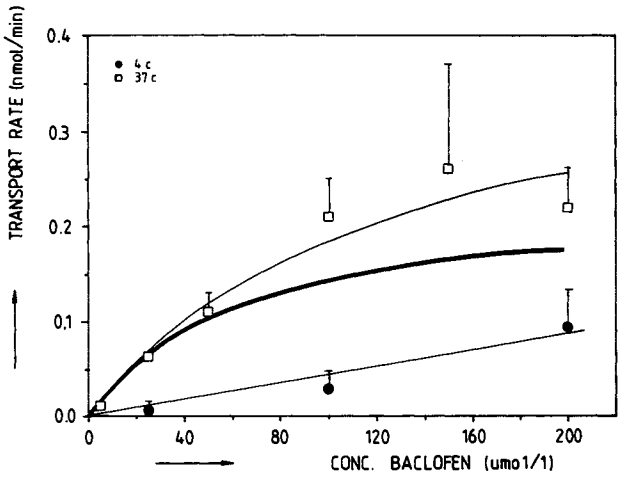

Fig. 3. Concentration dependency of baclofen transport at 37°C (□) and 4°C (●) across monolayers grown on regenerated cellulose membranes. The baclofen transport curve (heavy solid line) was generated from diffusion-corrected data using a Michaelis–Menten V_{max} model (see Ref. 13). Each data point represents the mean ± SD from three different monolayers.

DISCUSSION

Primary cultures of bovine brain microvessel endothelial cells have been used to obtain *in vitro* information on the mechanism by which baclofen crosses the BBB. It is demonstrated by this study that the *in vitro* BBB model system can be useful in obtaining qualitative and quantitative information on BBB transport mechanisms as has also been demonstrated previously (7,8).

The results of our experiments demonstrate that the centrally acting muscle relaxant, baclofen, is transported across the BBB mainly by a mechanism other than passive diffusion. Transport characteristics showed concentration dependency and saturation, thus indicating carrier-mediated transport. When combined with results presented previously, demonstrating baclofen's ability to inhibit leucine transport in the same model system, it appears likely that baclofen is transported by the LNAA carrier. Furthermore, there exists evidence that baclofen is transported from rat jejunum by a mechanism with similar characteristics (14). These findings also suggest that at the cellular level baclofen is transported by a uniform system (i.e., an amino acid carrier) throughout the body.

Baclofen exhibits a higher apparent affinity than leucine for the amino acid carrier in this *in vitro* model; V_{max} values are of the same order of magnitude (7). Among various amino acids, leucine does not have the highest apparent affinity for the LNAA carrier (15,16). Aromatic amino acids such as phenylalanine have apparent affinities up to three times higher than that of leucine (Fig. 1) (16). That baclofen, also an aromatic amino acid, has a higher apparent affinity for the LNAA carrier is consistent with previous findings (15,16).

To conclude, we have shown that transendothelial transport of baclofen is time, temperature, and concentration dependent. To validate further the hypothesis that baclofen transport is LNAA carrier mediated, additional experiments on competition for and inhibition of baclofen transport by other LNAA are required.

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