Paper citation: Rochat, B., Baumann, P., and Audus, K.L. (1999) Transport mechanisms for the antidepressant citalopram in brain microvessel endothelium. Brain Res. 831, 229-236. PMID: 10412001

Keywords: Antidepressant, blood-brain barrier, bovine brain microvessel endothelial cells, carrier, enantiomers, P-glycoprotein, selective serotonin reuptake inhibitor, stereoselectivity, transport.

Abstract: Blood-brain barrier transport of the selective serotonin reuptake inhibitor and antidepressant, citalopram, was studied using monolayers of bovine brain microvessel endothelial cells (BMECs). This study provides for the first time, evidence of a transport mechanism for a selective serotonin reuptake inhibitor (SSRI). Carrier-mediated transport, efflux mechanisms, as well as inhibition of metabolizing enzymes of citalopram were investigated. Citalopram transport was saturable and temperature-dependent suggesting that passage of the drug across BMECs was mediated by a carrier mechanism. Since the apical to basolateral and basolateral to apical permeability coefficients were similar and cyclosporin A, a P-glycoprotein inhibitor, does not modify the transport of citalopram, it appeared that no active efflux systems were involved in this transport. Citalopram is only available as a racemic drug and its pharmacological effect resides mainly in the S-(+)-enantiomer. However, the passage of citalopram enantiomers across BMEC monolayers was not stereoselective. Finally, inhibition of the metabolizing enzymes of citalopram and monoamine oxidases did not modify the permeation of citalopram across BMECs. Collectively, our results suggested that citalopram crosses the blood-brain barrier via a non-stereoselective, bidirectional and symmetrical carrier-mediated mechanism without influences of active efflux mechanisms or monoamine oxidases.

Text of paper:

TRANSPORT MECHANISMS FOR THE ANTIDEPRESSANT CITALOPRAM IN BRAIN MICROVESSEL ENDOTHELIUM

Bertrand Rochat[1], Pierre Baumann[2] and Kenneth L. Audus[1,3]

[1]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047

[2]Unite de Biochimie et Psychopharmacologie Clinique, Departement Universitaire de Psychiatrie Adulte, CH-1008 Prilly, Switzerland.

[3]To whom correspondence should be addressed.

Text pages (including figures & tables): 28

Figures: 6

Tables: 0

Corresponding author:

Dr. Kenneth L. Audus

Department of Pharmaceutical Chemistry

University of Kansas

2095 Constant Avenue

Lawrence, KS 66047

phone: (785) 864-3609

fax: (785) 864-5736

e-mail address: audus@ukans.edu

URL: http://www.hbc.ukans.edu/phch/faculty/audus/audus.htm

Acknowledgements: The authors gratefully acknowledge the support of the Fonds national suisse de la recherche scientifique (BR) and Corning Costar for support of the Cellular Molecular Biopharmaceutics Handling Laboratory. We are grateful to Victoria Thiel, Tony Rice and Josh Snyder for skillful technical assistance for the isolation of endothelial cells and to Marlyse Amey for the stereoselective HPLC

analysis of the citalopram enantiomers. We appreciate the help of Professor Ronald T. Borchardt and Dr. Gian Camenisch for assistance with instrumentation.

Classification terms:

Theme D: Neurotransmitters, Modulators, Transporters, and Receptors

Topic: Uptake and transporters

Rochat, B., Baumann, P., and Audus, K.L. (1999) Transport mechanisms for the antidepressant citalopram in brain microvessel endothelium. Brain Res. 831, 229-236. PMID: 10412001. Publisher's official version: <http://dx.doi.org/10.1016/S0006-8993(99)01461-4> . Open Access version: http://kuscholarworks.ku.edu/dspace/.

1. Introduction

In general, few studies regarding the stereoselective levels of psychotropic drugs either in the brain or in the blood have been published. With the enantiomers of (E)-10-hydroxynortriptyline, evidence has strongly implicated a stereoselective, active transport of the molecule out of the cerebrospinal fluid [6]. In contrast, the difference between the brain levels of the enantiomers of fluoxetine may be the consequences of specific and stereoselective cerebral enzymatic activities [14].

Citalopram (CIT), Figure 1, is a selective serotonin reuptake inhibitor (SSRI) and antidepressant, which has recently been introduced as a racemic mixture [26]. The drug is well-absorbed with an absolute oral bioavailability of 80%. The pharmacological effect of CIT resides mainly in the S-(+)-enantiomer [18]. In humans, CIT is stereoselectively biotransformed mainly by N-demethylation to demethylcitalopram by cytochrome P450 (CYP) 2C19, 3A4 and 2D6 [27] but also by deamination to a propionic acid derivative (CIT-PROP) by monoamine oxidases (MAO) and aldehyde oxidase [29]. The serum half-life of CIT is approximately 35 hours [35]. Knowledge of the biotransformation and elimination of CIT and other SSRIs has improved drug efficacy or safety and, in some cases, individualized drug treatment [8,12].

Although SSRI metabolism has been extensively studied *in vivo* and *in vitro* [5], no data are available for the transport mechanisms into the brain. Indeed, the exchange of drugs between systemic circulation and the central nervous system is severely restricted by the so-called blood-brain barrier (BBB) [2]. The microvessels of the BBB consist of a single continuous layer of cerebral endothelial cells effectively sealed together by tight intercellular junctions. Cells surrounding the brain endothelium

(i.e. neurons, astrocytes and pericytes), as well as the basal lamina, also contribute to the stability and the barrier characteristics of the brain microvessel cells (BMECs) [19]. The presence of the efflux pump P-glycoprotein (Pgp) at the apical membrane of brain endothelial cells, compounds the picture of the BBB [36]. Pgp decreases the permeability across the BBB by effluxing many drugs and peptides back into the blood [2]. Moreover, brain endothelial cells contain a substantial volume of mitochondria, indicating that the BBB could contribute to signfficant MAO biotransformation of xenobiotics [16,25].

The purpose of this study was to evaluate CIT transport across the BBB using a simple, well characterized *in vitro* model of the brain endothelium: BMEC monolayers [1]. Specifically, carrier mechanisms, as well as Pgp and metabolism, were investigated as possible factors regulating the distribution of CIT across the blood-brain barrier.

2. Materials and Methods

Chemicals. Citalopram hydrobromide was obtained from Lundbeck (Copenhagen, Denmark). Serotonin, dopamine, L-tryptophan, cyclosporin A and rhodamine 123 were purchased from Sigma Chemical Company (St. Louis, MO). All other compounds used were of highest quality commercially available.

High performance liquid chromatography analysis (HPLC). Non-stereoselective HPLC analysis of CIT racemate was performed on a C18 analytical column coupled with a Shimadzu system (Shimadzu, Inc., Tokyo, Japan) consisting of LC-6A pump, a SIL-10A autoinjector and a SCL-10A controller. The injection volume was 200 μl. The

mobile phase consisted of 35% acetonitrile, 65 % triethylamine buffer 1% (vol/vol) adjusted with acetic acid to pH = 6. 1. The flow rate was 1.2 ml/min.

Stereoselective analysis of CIT racemate was performed on a CHIROBIOTIC V analytical column (150 mm x 4.6 mm). The mobile phase was 99.885% methanol with 0.055% triethylamine and 0.060% acetic acid. The flow rate was 1.0 ml/min. The fluorescence detector (Perkin-Elmer LC 240) was coupled after the columns and set at 240 nm and 296 nm for excitation and emission wavelengths, respectively. Samples were directly injected into the column and quantitation was obtained after comparison of peak areas with a calibration curves. Calibration curves were linear between 25 nM and 30 μM. Samples were kept at 4°C for less than one month prior to HPLC analysis. In a previous study [28], the enantiomers of CIT showed high stability regarding temperature and in the solvents used above.

Cell culture. BMECs were isolated from gray matter of cerebral cortices by enzymatic digestion and subsequent centrifugation, and seeded into primary culture as detailed previously [1]. The 100 mm tissue cultures dishes (Coming Costar Corporation, Cambridge, MA) were pretreated with rat tail collagen and bovine fibronectin (Sigma Chemical Co., St. Louis, MO). Culture media consisted of a minimum of 45% essential media, 45% F-12 Ham nutrient mix (Gibco, Life Technologies, Grand Island, IL), 10 mM HEPES, pH 7.4, 13 mM sodium bicarbonate, 10% plasma-derived equine serum, 100 μg/ml heparin, 100 μg/ml streptomycin, 100 μg/ml penicillin G, 50 μg/ml polymixin B and 2.5 μg/ml amphotericin B (Sigma Chemical Co., St. Louis, MO). Isolated BMECs were cultured at 37°C with 95% humidity and 5% CO_2 until confluent monolayers were formed (10 to 14 days). Cell surfaces facing the culture media, and those sealing the culture dishes, mimic apical and basolateral membranes of brain

microvessel cells, respectively [1]. The purity of the isolated BMECs was monitored by periodic evaluation of the enrichment of enzyme markers, γ-glutamyl transpeptidase and alkaline phosphatase, and the endothelial origin confirmed by positive staining for Factor VIII antigen [1,3].

Transport studies. BMECs were seeded onto collagen- and fibronectin-coated, translucent, polycarbonate membranes (pore size 0.4 μm) placed in 100 mm tisssue culture dishes. Monolayer confluency was verified by inspection of the areas around the polycarbonate membranes using an inverted microscope. The basolateral side of the cells was defined as the side facing the collagen matrix. Once confluency was obtained, the membranes were carefully inserted in a horizontal Side-bi-Side™ diffusion apparatus (Crown Glass, Inc., Somerville, NJ) for transendothelial permeability studies. The area of the diffusion membrane was 0.636 cm^2. The donor and receiver chambers were filled with 3.0 ml of culture media and the temperature was maintained with an external circulating water bath at either 37°C or 4°C. The contents of each chamber were continuously stirred with Teflon coated magnetic stirred bars at the speed of 600 r/min driven by an external drive console (Crown Glass, Inc., Somerville, NJ). The apical to basolateral transport of CIT was studied with a 5 μM drug pulse added to the donor chamber. In some experiments, cyclosporin A (1 μM), serotonin (250 μM), dopamine (250 μM), tryptophan (250 μM), pargyline (100 μM), selegiline (50 nM) or clorgyline (50 nM) were added in both chambers at 50 min. In these cases, permeability coefficients were calculated for CIT permeation before and after the treatment. For cyclosporin A, pargyline, selegifine and clorgyline, a pre-incubation was performed for a specific time before the collection of the subsequent samples. A 200 μl sample was taken from the

receiver chambers at various times. Each sample volume was replaced with equal volume of fresh medium. CIT transport at 37°C with no additional treatment present was always performed as control.

Calculation and statistics. Permeability coefficients were calculated using the following equation: $P = F/(A*C_{donor})$; where F, the flux, is the slope of the drug appearing in the receiver chamber per time; A, the area of the membrane (0.636 cm^2) and C_{donor}, the initial concentration in the donor chamber [1].

Kinetic data (CIT flux versus CIT concentration) were analyzed by means of a nonlinear least square curve fitting program (Statistics for Windows, StatSoft, Inc.) and treated according to a monophasic model of Michaelis-Menten equation in order to determine K_m and J_{max} values (Flux-max) of the CIT. All experiments were carried out in at least three replications and expressed as means ± standard deviation.

3. Results

Concentration-dependence of CIT transport. The time-dependent permeation of CIT across BMEC monolayers was determined using concentrations ranging from 1 μM to 1000 μM. The flux of CIT across BMEC monolayers for 30 min was linear over the concentration range examined at 37°C (data not shown). Figure 2 illustrates the concentration dependence of the apical to basolateral transport of CIT across BMEC monolayers at 37°C. The flux (Figure 2A) and the apparent permeability coefficients (Figure 2B) for CIT passage across BMEC monlayers was a saturable process at concentrations higher than 100 μM. The apparent permeability

coefficient for CIT passage across the BMEC monolayers was significantly greater than for sucrose (data not shown), a marker for paracellular leakage of the monolayers. Collectively, these results argued for a carrier-mediated mechanism for CIT transport across BMECs. Michaelis-Menten parameters, K_m and J_{max} values, were determined to be 487 μM and 7.4 nmol/min, respectively.

Temperature dependence of CIT transport. The effect of temperature on the apical to basolateral permeation of CIT (5 μM) across the BMECs is presented in Figure 3A. After following the flux of CIT for 50 min, control diffusion systems were maintained at 37°C, while others were cooled to 4°C with ice. Flux values were recorded for an additional 40 min in both diffusion systems. The apparent permeability coefficients for CIT at 4°C and 37°C are represented in Figure 3B. The permeability coefficients decreased significantly ($P < 0.05$) from $22.5 \pm 4.9 \times 10^{-5}$ cm/s at 37°C to $0.5 \pm 0.2 \times 10^{-5}$ cm/s at 4°C. The sensitivity of the apparent permeability coefficients for CIT to temperature was also suggestive of a transport process rather than a saturation of a binding process since the latter likely would be much less affected by low temperature.

Polarity of CIT transport. In separate experiments, CIT (5 μM) or rhodamine 123 (10 μM) were added either in the chambers facing the cell membranes or in those facing the basolateral surface of the BMEC monolayers. Apparent permeability coefficients for CIT and rhodamine 123 were calculated for both directions of transport (Figure 4). The permeability coefficients for CIT were similar in apical to basolateral or in basolateral to apical directions and were $16.3 \pm 2.9 \times 10^{-5}$ cm/s and $15.0 \pm 2.0 \times 10^{-5}$ cm/s, respectively. In contrast, rhodamine 123, a well characterized Pgp substrate, exhibited a permeability coefficient about 4 times lower in the apical to basolateral transport in comparison to the basolateral to apical transport (i.e., 3.6 ±

2.4×10^{-5} cm/s and $14.3 \pm 3.8 \times 10^{-5}$ cm/s, respectively). In other experiments, CIT flux across BMEC monolayers was monitored for up to 50 min at which time cyclosporin A (1 μM), a Pgp inhibitor, was added to both diffusion chambers for a 30 min incubation period. Following this incubation period, the apparent permeability coefficients for both CIT and rhodamine 123 were re-determined over an additional assay period of 40 min (Figure 4). In the presence of cyclosporin A, the apical to basolateral permeability coefficient increased for rhodamine 123 over two-fold to $8.1 \pm 2.9 \times 10^{-5}$ cm/s. In contrast, in the presence the apparent permeability coefficient for the apical to basolateral flux of CIT, $15.9 \pm 2.4 \times 10^{-5}$ cm/s, was not statistically different from the untreated control. These results strongly suggested that CIT was not a substrate for Pgp or controlled by other unidirectional efflux systems in the BMECs.

Stereoselectivity of CIT transport. The stereoselective quantitation of CIT enantiomers was performed after CIT transport (5 μM and 50 μM) across BMECs for 30 min. In the receiver chambers, S(+)-CIT levels represented 50.6 % ± 2.9% of the total S-(+)-CIT and R-(-)-CIT enantiomers and R-(-)-CIT levels represented 49.4% ± 2.9%. Therefore, a stereoselective transport of the enantiomers of CIT was not observed for BMEC transport.

Dopamine, serotonin, and L-tryptophan effects on CIT transport. The effect of 250 μM of dopamine or serotonin or L-tryptophan on the passage of CIT (5 μM) across BMECs was examined. These compounds were pulsed into the donor chamber after the flux of CIT had been sampled for 50 min. Following the pulse of the potential competitors, samples were continually collected out to 100 min. No significant effects on the passage of CIT across the monolayers were observed for any of these compounds (data not shown).

Effect of MAO inhibitors on citalopram transport. As previously reported [31], inhibition of MAO activity in BMECs was obtained with a 30 min preincubation of pargyline (100 μM),

selegiline (50 nM) or clorgyline (50 nM) as non-selective, selective MAO type B and MAO type A inhibitors, respectively. No significant modification of the transport of CIT (5 μM) was observed in the presence of MAO inhibitors (data not shown). In addition, the CIT metabolite produced by MAO, CIT-PROP, was not detected in either receiver chambers or donor chambers under the conditions of these experiments.

4. Discussion

In BMECs, the polarity of transport mimics the blood to brain and brain to blood passage for some substances [1]. In the present study, we provided evidence that citalopram was transported across brain microvessel endothelium via a saturable mechanism in the apical-to-basolateral direction. Consistent with carrier-mediated mechanisms versus passive diffusion, the passage of CIT across the cells was extremely sensitive to low temperature. However, it also appeared that the process was not selective for S-(+)-CIT, the pharmacologically active enantiomer, suggesting that the transport process was non-stereoselective.

The absence of stereoselectivity is associated with carrier mechanisms of broad specificity such as the unidirectional Pgp efflux mechanism [30] and asymmetry in transport is reflective of such unidirectional, polyspecific efflux or uptake mechanisms [1]. In this study, the transport of CIT and the representative PgP substrate, rhodamine 123 [13], across BMECs was assayed in both directions. Apical to basolateral and basolateral to apical transport of CIT were similar and unaltered in the presence of a known Pgp inhibitor, cyclosporin A [13]. By contrast, the apical to basolateral transport of our positive control, rhodamine 123, was strongly impaired by the efflux pump, Pgp and modified on exposure to cyclosporin A. The lack of asymmetric transport and the absence of a sensitivity to cyclosporin A strongly suggested that CIT is not a substrate of Pgp or known alternative asymmetric active efflux systems like

the multidrug resistant associated protein (MRP), a recently described efflux mechanism in brain endothelium [24]. The relatively poor lipophilicity of CIT [4], a feature of most Pgp and MRP substrates [33], would further suggest that the drug would not necessarily fit the physicochemical profile of a substrate for these typical efflux mechanisms. Therefore, nonstereoselective, active, and unidirectional efflux mechanisms do not seem to be involved in regulating CIT distribution across brain endothelium. Bidirectional, saturable transport is a characteristic of non-energy requiring facilitated diffusion mechanisms at the blood-brain barrier [2] and appears likely to mediate CIT distribution across BMEC monolayers.

Although binding studies have shown that the tricyclic antidepressant imipramine and the SSRI, paroxetine, have low and high binding sites on isolated porcine microvessels [10,11], these binding sites remain to be fully characterized and could be either ATP dependent transporters or ATP-independent carriers. The imipramine binding site on BMECs is believed to label the serotonin transporter [11]. Citalopram has been shown to competitively inhibit serotonin uptake and transport at submicromolar concentrations and is therefore, considered a very effective and specific serotonin uptake inhibitor [7,17,35]. However, we were not able to establish significant decreases in the transport of CIT across BMECs in the presence of either serotonin or L-tryptophan. Moreover, CIT did not inhibit dopamine transport. Sufficiently high expression of carriers on BMEC membranes, high maximum uptake rate (J_{max}) and/or participation of various carriers may explain the apparent lack of inhibitory effects of potential endogenous substrates on CIT transport at these concentrations. The low affinity and absence of significant competition with other potential substrates at the concentrations here would also be consistent with the observations for transport properties of the high capacity, polyspecific cationic carriers that are present in BMECs and other tissues [21]. Some authors have suggested that a panel of stably transfected cell lines expressing specific transporters could be an appropriate tool for this purpose [30] to identify more potent and specific inhibitors for such mechanisms. Using this approach,

Breidert et al. [9], was recently able to demonstrate in such a constructed cell line stably expressing OCT1r that transported several substrates in a saturable fashion, including norepinephrine, dopamine, and serotonin. However, the transport of the norepinephrine, dopamine, and serotonin by the OCT1r expressing cells was resistant to specific inhibitors such as desipramine, cocaine, and CIT, respectively. Our results were consistent with this observation and could be suggestive of the involvement of a polyspecific carrier in CIT transport. The absence of effects of potential inhibitors of CIT transport underlines the difficulties in identifying and kinetically characterizing the involvement of polyspecific carriers involved in the uptake and transport of drugs by cells.

Brain microvessel endothelium contains a very low level of cytochrome P-450 and, in contrast, contains high quantities of MAO type A or type B, metabolizing-isozymes found in mitochondrial membranes [25]. It appears that the levels of MAO type A in BMECs approach those of the gray matter but in contrast, MAO type B activity is a few times lower compared to activities of gray matter [3]. Some authors have suggested that microvessels should be considered as a potential site of inactivation of some neurotransmitters or drugs [3,20,25]. For instance, the transport of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a dopaminergic neurotoxin, across primary cultures of BMECs seems to be restricted by oxidation mediated by MAO [32]. In the presence of pargyline, seleguine, or clorglyine, the passage of MPTP increased significantly and the appearance of its oxidative metabolites was decreased. A combination of SSRI and MAO inhibitors have been shown to produce more adverse effects than SSRI alone [22]. Considering that CIT is biotransformed by both MAO type A and type B [29], it was interesting to evaluate the inhibitory effect of MAO activities on CIT transport across BMECS. MAO inhibition obtained with pargyline, selegiline, or clorgyline, did not modify the apparent permeability coefficients for CIT. Our results suggested that CIT metabolizing

enzymes would not be involved in the passage of CIT across the BBB. Moreover, the high permeability coefficient of CIT, in contrast to Pgp substrates, was consistent with the probable insignificant role of MAO for CIT deliverance at the BBB. Indeed, some authors have suggested that reducing the rate of the transport of cyclosporin A across Caco-2 cells increases its biotransformation rate [15]. Similar to CIT, pargyline has been observed to not modify dopamine uptake in perfused brains of guinea pigs [23]. It was suggested that BBB uptake of dopamine was independent of MAO type B, a dopamine metabolizing enzyme. Taking into consideration these data, interindividual differences of cerebral levels of the CIT enantiomers and other SSRIs (e.g., fluoxetine) might result from its stereoselective metabolism in the liver and in the brain [14,34], rather than due to stereoselective transport or biotransformation localized at the BBB.

To conclude, this study provides for the first time, evidence of transport mechanisms of a SSRI antidepressant across the BBB using monolayers of BMECS. CIT seems to cross the blood-brain barrier via a non-stereoselective, bidirectional and saturable carrier-mediated mechanism with no influence of either active efflux mechanisms (e.g. Pgp) or monoamine oxidases.

Acknowledgements

The authors gratefully acknowledge the support of the Fonds national suisse de la recherche scientifique (BR) and Corning Costar for support of the Cellular Molecular Biopharmaceutics Handling Laboratory. We are grateful to Victoria Thiel, Tony Rice and Josh Snyder for skillful technical assistance for the isolation of endothelial cells and to Marlyse Amey for the stereoselective HPLC analysis of the citalopram enantiomers. We appreciate the help of

Professor Ronald T. Borchardt and Dr. Gian Camenisch for assistance with instrumentation.

References

1. Audus, K.L., Ng, L., Wang, W. and Borchardt, R.T., Brain microvessel endothelial cell culture systems, Pharm. Biotechnol., 8 (1996) 239-258.

2. Audus, K.L., Chikhale, P.J., Miller, D.W., Thompson, S.E. and Borchardt, R.T., Brain uptake of drugs: The influence of chemicals and biological factors, Adv. Drug Res., 23 (1992)1-64.

3. Baranczyk-Kuzma, A., Audus, K.L. and Borchardt, R.T., Catecholamine-metabolizing enzymes of bovine brain microvessel endothelial cell monolayers, J. Neurochem., 46 (1986) 1956-1960.

4. Bauer, M., Megret, C., Lamure, A., Lacabanne, C., and Fauran-Clavel, M.-J., Differential scanning calorimetry study of the interaction of antidepressant drugs, noradrenaline, and 5-hydroxytryptamine with a membrane model, J. Pharm. Sci., 79 1990) 897-901.

5. Baumann, P. and Rochat, B., Comparative pharmacokinetics of selective serotonin reuptake inhibitors: A look behind the mirror, Int. Clin. Psychopharmacol., 10 (Suppl 1) (1995) 15-21.

6. Bertilsson, L., Otani, K., Dahl, M.L., Nordin, C., and Aberg-Wistedt, A., Stereoselective efflux of (E)-10-hydroxynortriptyline enantiomers from the cerebrospinal fluid of depressed patients, Pharmacol. Toxicol., 68 (1991) 100-103.

7. Blakely, R., Berson, H., Fremeau, R., Caron, M., Peek, M., Prince, H., and Bradley, C., Cloning and expression of a functional serotonin transporter from rat brain, Nature, 354 (1991) 66-70.

8. Bondolfi, G., Chautems, C., Rochat, B., Bertschy, G. and Baumann, P., Non-response to citalopram in depressive patients: pharmacokinetic and clinical consequences of a fluvoxamine augmentation, Psychopharmacology, 128 (1996) 421-425.

9. Breidert, T., Spitzenberger, F., Grundemann, D. and Schomig, E., Catecholamine transport by the organic cation transporter type 1, Br. J. Pharmacol., 125 (1998) 218-224.

10. Brust, P., Bergmann, R. and Johannsen, B., High affinity binding of [^{3}H]paroxetine to caudate nucleus and microvessels from porcine brain, Neuroreport, 7 (1996) 1405-1408.

11. Brust, P., Bergmann, R. and Johannsen, B., Specific binding of [^{3}H]imipramine indicates the presence of a specific serotonin transport system on endothelial cells of porcine brain, Neurosci. Lett., 194 (1995) 21-24.

12. Conus, P., Bondolfi, G., Eap, C.B., Macciardi, F. and Baumann, P., Pharmacokinetic fluvoxamine-clomipramine interaction with favorable therapeutic consequences in therapy-resistant depressive patient, Pharmacopsychiatrv, 29 (1996) 108-110.

13. Fontaine, M., Elmquist, W.F. and Miller, D.W., Use of rhodamine 123 to examine the functional activity of P-glycoprotein in primary cultured brain microvessel endothelial cell monolayers, Life Sci., 59 (1996) 1521-1531.

14. Fuller, R.W. and Snoddy, H.D., Drug concentrations in mouse brain at pharmacologically active doses of fluoxetine enantiomers, Biochem. Pharmacol., 45 (1993) 2355-2358.

15. Gan, L.S., Moseley, M.A., Khosla, B., Augustijns, P.F., Bradshaw, T.P., Hendren, R.W. and Thakker, D.R., CYP3A-like cytochrome P450-mediated metabolism and polarized efflux of cyclosporin A in Caco-2 cells, Drug Metab. Dispos., 24 (1996) 344-349.

16. Ghersi-Egea, J.F., Lehiinger-Muller, B., Cecchelli, R. and Fenstermacher, J.D., Blood-brain interfaces: relevance to cerebral drug metabolism, Toxicol. Lett., 82-83: (1995) 645-653.

17. Hyttel, J., Citalopram – Pharmacological profile of specific serotonin uptake inhibitor with antidepressant activity, Prog. Neuropsychopharmacol. Biol. Psychiat., 6 (1982) 277-295.

18. Hyttel, J., Bogeso, K.P., Perregaard, J. and Sanchez, C., The pharmacological effect of citalopram residues in the (S)-(+)-enantiomer, J. Neural Transm. Gen. Sect., 88 (1992) 157-160.

19. Joo, F., Endothelial cells of the brain and other organ systems: Some similarities and differences, Prog. Neurobiol., 48 (1996) 255-273.

20. Joo, F., The blood, brain barrier in vitro: the second decade, Neurochem. Int., 23 (1993) 499-521.

21. Koepsell, H., Organic cation transporters in intestine, kidney, liver, and brain, Ann. Rev. Physiol., 60 (1998) 243-260.

22. Lane, R. and Baldwin, D., Selective setotonin reuptake inhibitor, induced serotonin syndrome: Review, J. Clin. Psychopharmacol., 17 (1997) 208-221.

23. Martet, C.L., Mackic, J.B., Adams, J.D., Jr, McComb, J.G., Weiss, M.H. and Zlokovic, B.V., Transport of dopamine at the blood-brain barrier of the guinea pig: Inhibition by psychotropic drugs and nicotine, Pharm. Res., 13 (1996) 290-295.

24. Miller, D.W., Han, H.Y. and Carrney, D., Is the probenecid-sensitive transporter in the blood-brain barrier multidrug resistance associated protein (MRP)?, Pharm. Res., 14 (1997) S-332.

25. Minn, A., Ghersi-Egea, J.F., Perrin, R., Leininger, B. and Siest, G., Drug metabolizing enzymes in the brain and cerebral mictovessels, Brain Res., 16 (1991) 65-82.

26. Noble, S. and Benfield, P., Citalopram - A review of its pharmacology, clinical efficacv and tolerability in the treatment of depression, CNS Drugs, 8 (1997) 410-431.

27. Rochat, B., Amey, M., Gillet, M., Meyer, U.A. and Baumann, P., Identification of three cytochrome P450 isozymes involved in N-demethylation of citalopram enantiomers in human liver microsomes, Pharmacogenetics, 7 (1997) 1-10.

28. Rochat, B., Amey, M., Van Gelderen, H., Testa, B. and Baumann, P., Determination of the enantiomers of citalopram its demethylated and propionic acid metabolites in human plasma by chiral HPLC, Chirality, 7 (1995) 389-395.

29. Rochat, B., Kosel, M., Boss, G., Testa, B. and Baumann, P., Biotransformation of the selective serotonin re-uptake inhibitor citalopram by monoamine oxidases in human liver, Biochem. Pharmacol., in press (1998).

30. Sadee, W., Membrane transporters: Gateways to the future, Pharm. News, 4 (1997) 8-11.

31. Sandstrom, R., Karlsson, A., and Lennernas, H., The absence of stereoselective P-glycoprotein-mediated transport of R/S-verapamil across the rat jejunum, J. Pharm. Pharmacol., 50 (1998) 729-735.

32. Scriba, G.K. and Borchardt, R.T., Metabolism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by bovine brain microvessel endothelial cells, Brain Res., 501 (1989) 175-178.

33. Seelig, A., A general pattern for substrate recognition by P-glycoprotein, Eur. J. Biochem., 251 (1998) 252-261.

34. Sidhu, J., Priskorn, M., Poulsen, M., Segonzac, A., Grollier, G., and Larsen, F., Steady-state pharmacokinetics of the enantiomers of citalopram and its metabolites in humans, Chirality, 9 (1997) 686-692.

35. Sur, C., Betz, H. and Schloss, P., Distinct effects of imipramine on 5-hydroxytryptamine uptake mediated by the recombinant rat serotonin transporter SERT1, J. Neurochem., 70 (1998) 2545-2553.

36. Van Asperen, J., Mayer, U., Van Tellingen, 0. and Beijnen, J.H., The functional role of P-glycoprotein in the blood-brain barrier, J. Pharm. Sci., 86 (1997) 881-884.

FIGURE LEGENDS

Figure 1. Chemical structure of citalopram.

Figure 2. Concentration dependence of apical to basolateral permeation of citalopram (CIT) at 37°C across BMEC monolayers in the Side-bi-Side™ system. Each data point represents the mean ± SD ($n \geq 3$) of CIT flux or apparent permeability coefficient. **(A)** Concentration-dependence of the flux of citalopram permeation across BMEC monolayers. In the concentration range of 1 to 1000 μM data follow a Michaelis-Menten model with K_m and J_{max} values of 487 μM and 7.4 nmol/min, respectively. *Inset :* regression curve fit linearly the concentration range of 1 to 100 μM. **(B)** Concentration-dependence of the apparent permeability coefficients for citalopram permeation across BMEC monolayers.

Figure 3. Temperature dependence of apical to basolateral transport of 5 μM citalopram (CIT) across BMECs monolayers in Side-bi-Side™ systems. Each data point represents the mean ± SD ($n \geq 3$). **(A)** Squares represent control transport performed at 37°C through 90 min*;* empty squares represent transport at 37°C through 50 min and when (*arrow*) the temperature was reduced to 4°C through 90 min. **(B)** Apparent

permeability coefficients were calculated from the flux data for CIT permeation across BMEC monolayers at 37°C and 4°C.

Figure 4. Apparent permeability coefficients for passage of 5 μM citalopram (CIT) or 10 μM rhodamine 123 (Rho123) across BMECs monolayers. Studies were performed in Side-bi-Side™ systems, with substrates permeating apical to basolateral (A to B), or basolateral to apical (B to A), or apical to basolateral in presence of 1 μM cyclosporin A (A to B + CsA). Controls were studies performed with CIT or Rho123 alone. Each data point represents the mean ± SD ($n \geq 3$).

Figure 1

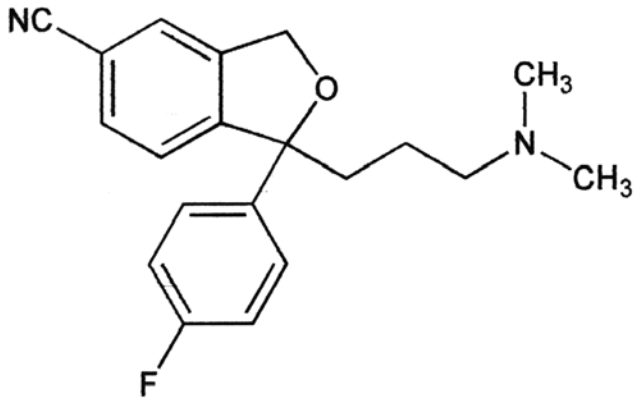


Figure 2A

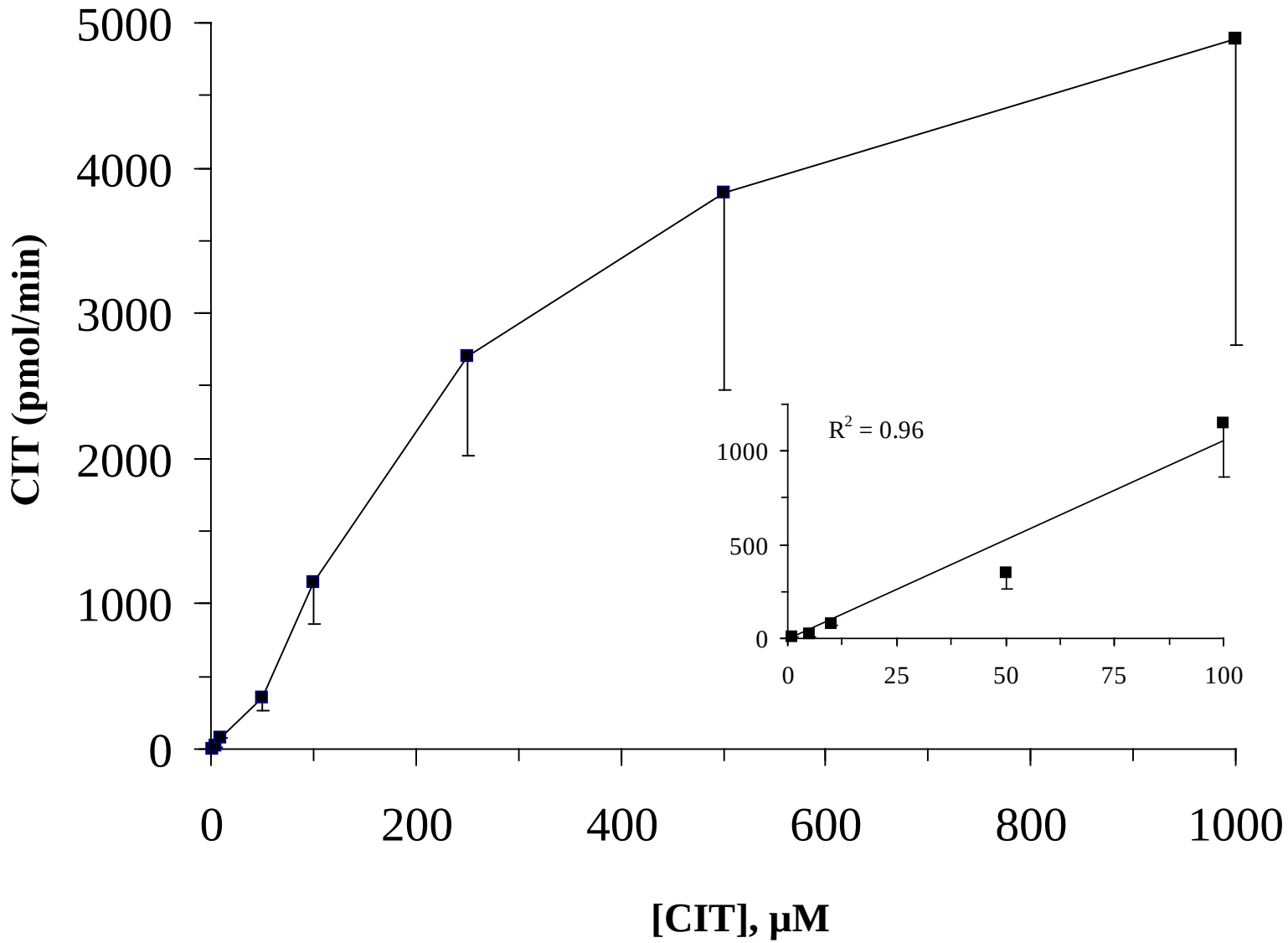

5000
4000
3000
2000
1000
0
CIT (pmol/min)
0
200
400
600
800
1000
[CIT], µM
R2 = 0.96
1000
500
0
0
25
50
75
100

Figure 2B

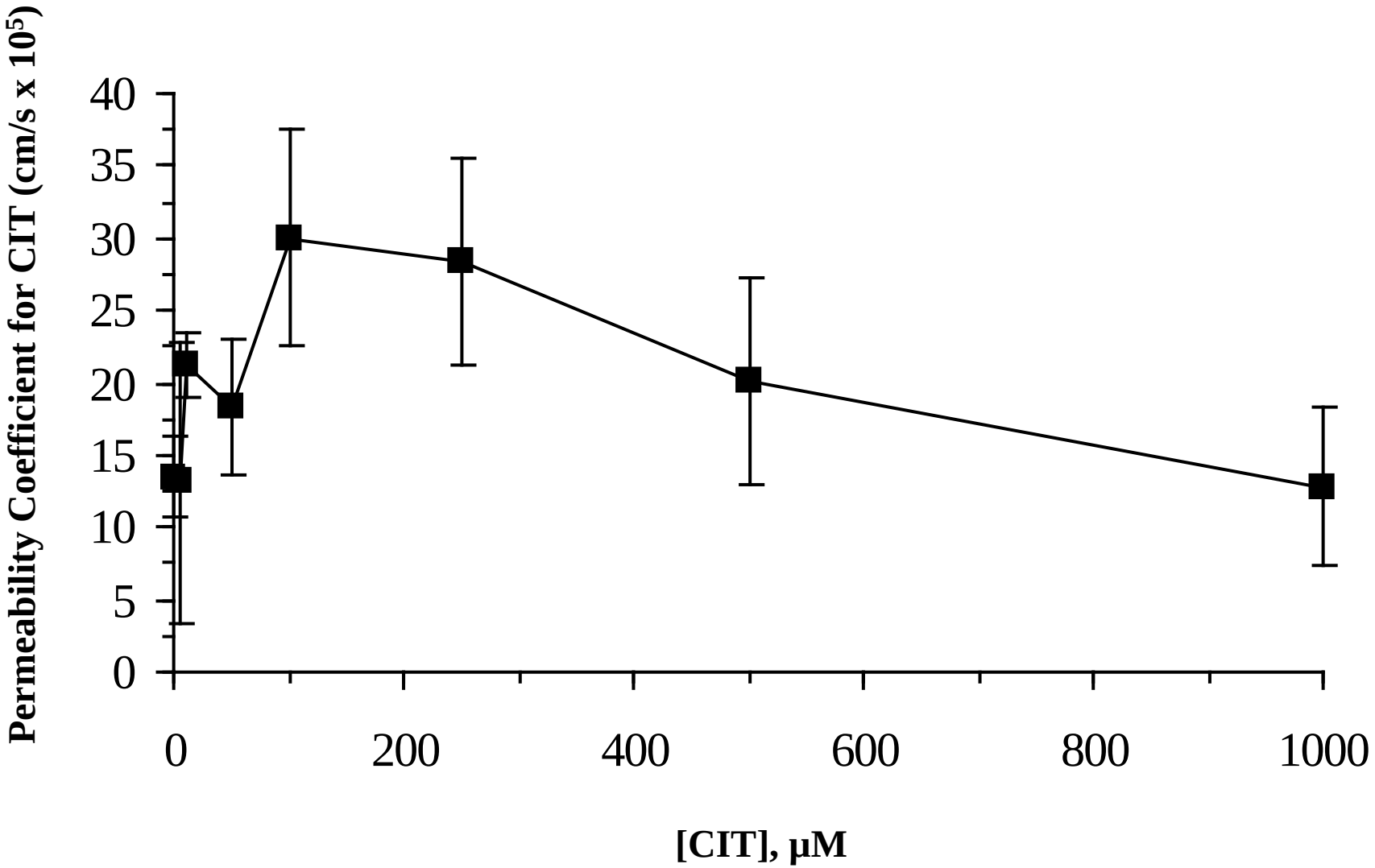

Permeability Coefficient for CIT (cm/s x 10^5)
40
35
30
25
20
15
10
5
0
0
200
400
600
800
1000
[CIT], µM

Figure 3A

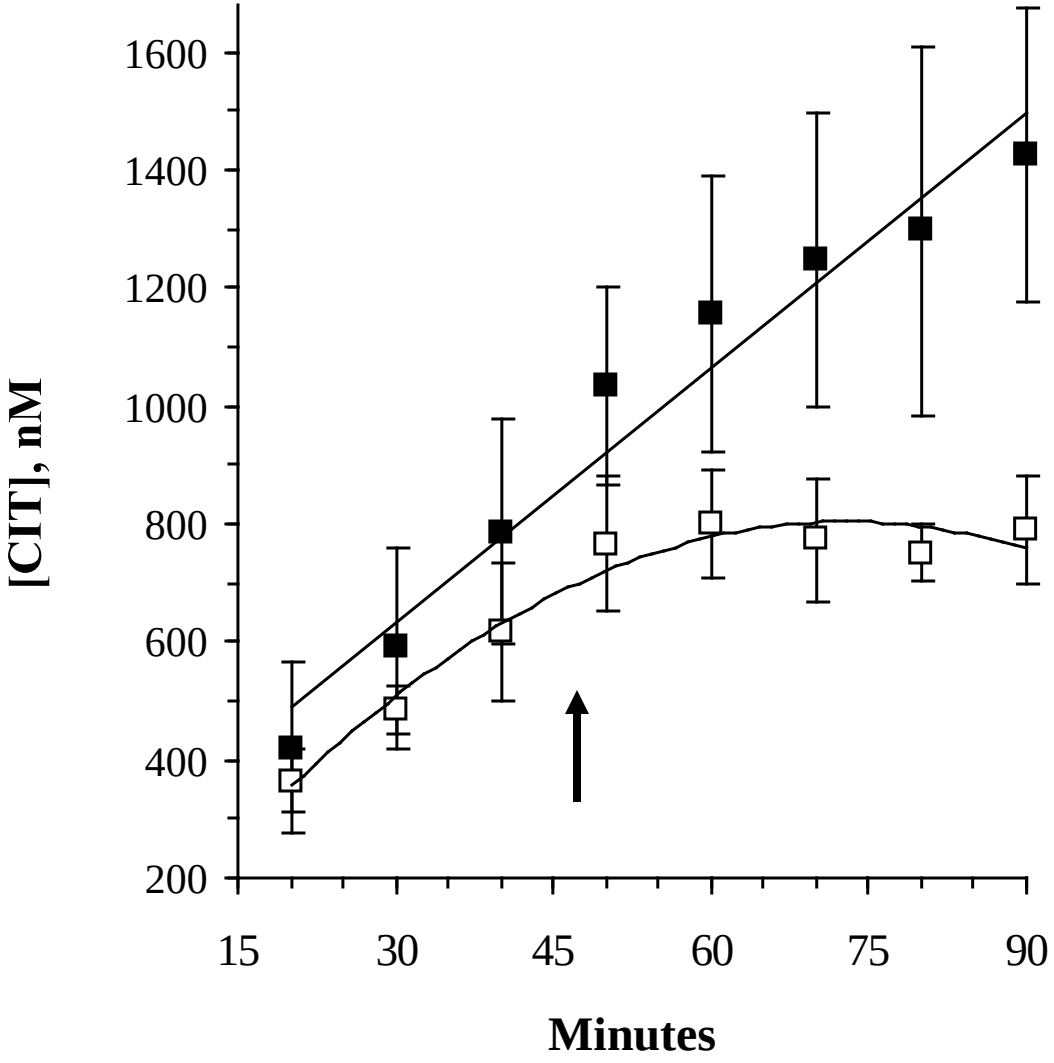

[CIT], nM
1600
1400
1200
1000
800
600
400
200
15
30
45
60
75
90
Minutes

Figure 3B

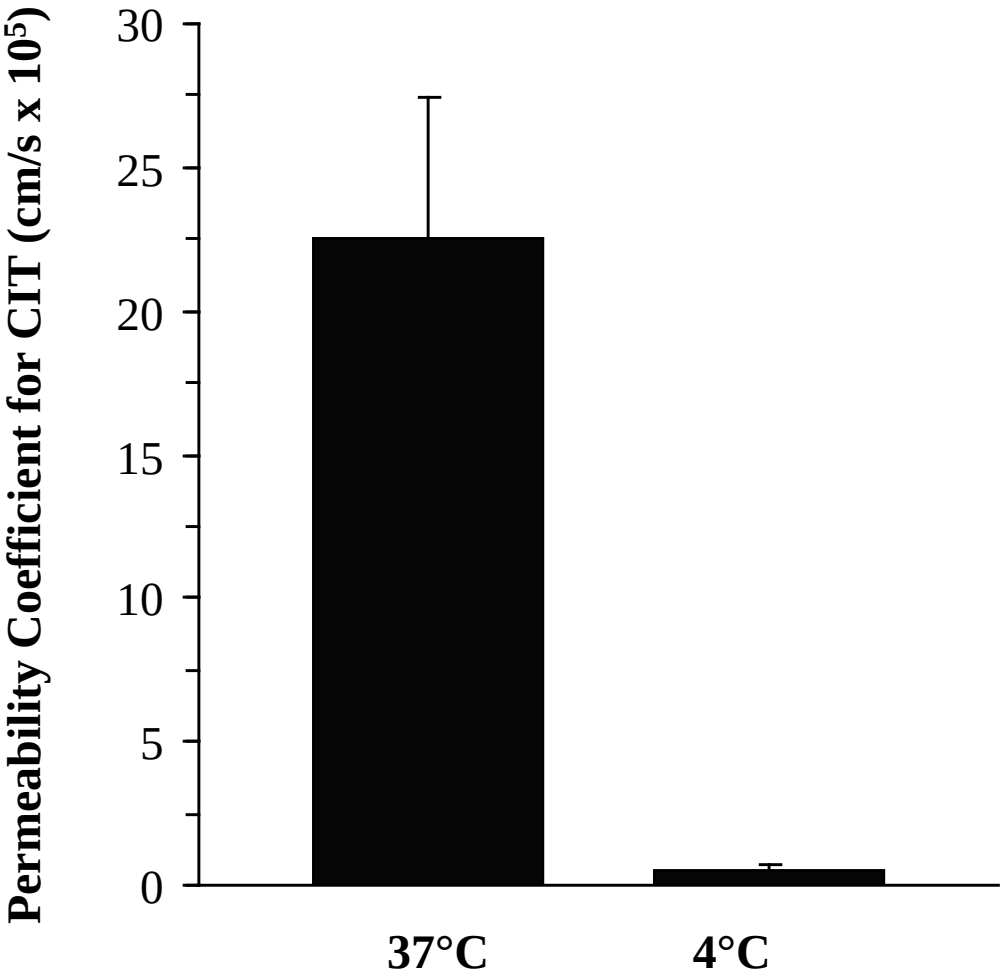
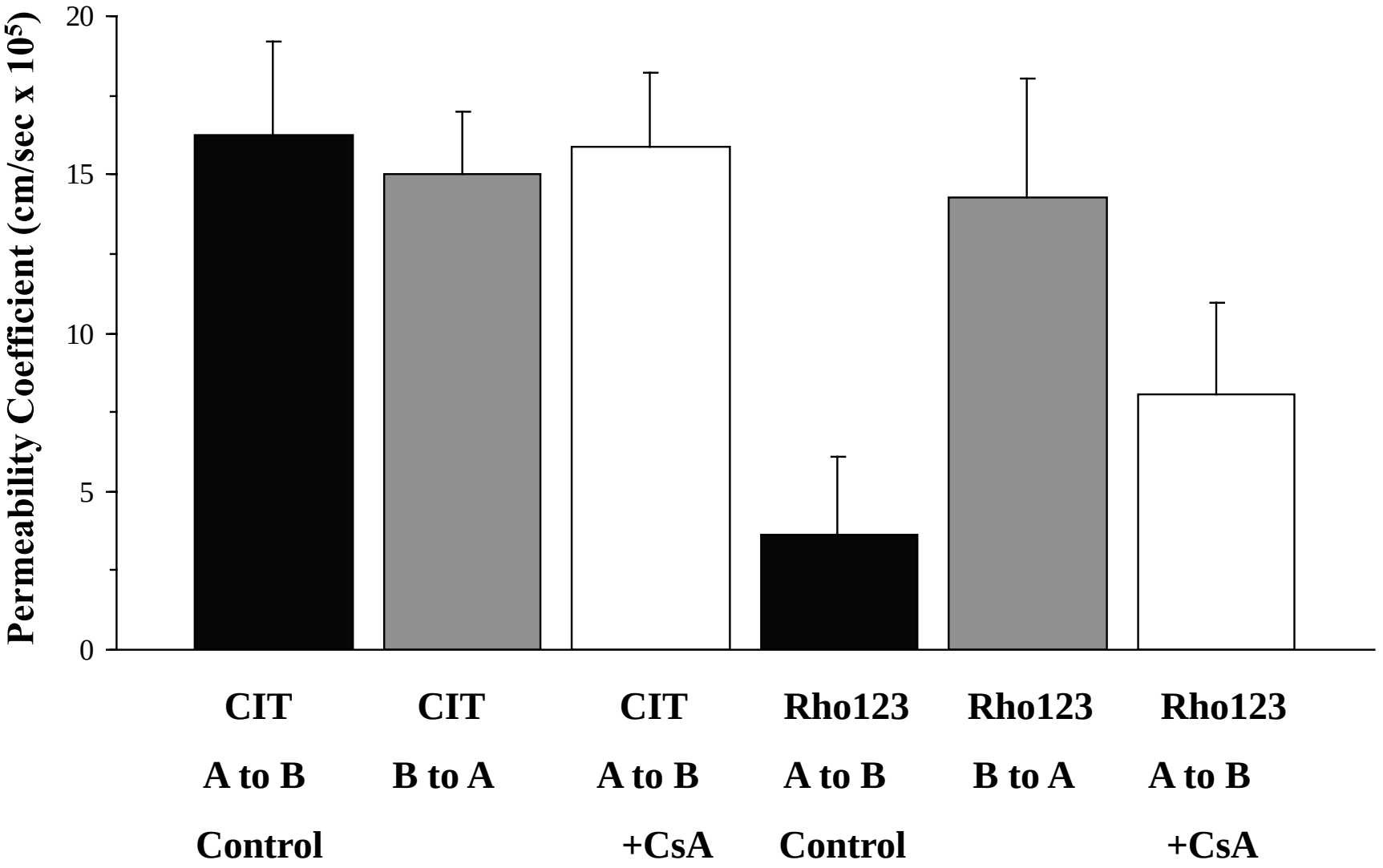

Permeability Coefficient for CIT (cm/s x 10^5)
30
25
20
15
10
5
0
37°C
4°C

Figure 4


Permeability Coefficient (cm/sec x 10^5)
20
15
10
5
0
CIT
A to B
Control
CIT
B to A
CIT
A to B
+CsA
Rho123
A to B
Control
Rho123
B to A
Rho123
A to B
+CsA