Formycin B Elimination from the Cerebrospinal Fluid of the Rat

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The goal of this study was to determine whether specific transport systems are involved in nucleoside elimination from the cerebrospinal fluid (CSF). First, *in vitro* studies were carried out in isolated choroid plexus tissue slices from rat to ascertain the mechanisms of transport of formycin B, a model nucleoside analogue. ^{3}H-Formycin B accumulated against a concentration gradient in the presence of an Na^+ gradient in the isolated ATP-depleted choroid plexus tissue slices. This accumulation was reduced by high concentrations of unlabeled formycin B. Nitrobenzylthioinosine (NBMPR), an equilibrative nucleoside transport inhibitor, inhibited the uptake of formycin B in the absence of an Na^+ gradient. These data suggest that both equilibrative and secondary active Na^+-nucleoside transport systems are present in rat choroid plexus. *In vivo,* formycin B, together with inulin as a bulk flow marker, was injected into the lateral ventricle of the anesthetized rat with the aid of a stereotaxic device, and CSF was sampled from the cisterna magna at various times after injection. Twelve rats were randomized and divided into a low- and a high-dose group. The CSF clearance (CL_{CSF}) of formycin B was significantly higher than the CL_{CSF} of inulin in both animal groups ($P < 0.01$), indicating that formycin B is cleared from CSF by a pathway(s) in addition to bulk flow. Formycin B CL_{CSF} was significantly lower in the high-dose group than in the low-dose group ($P < 0.05$), suggesting a saturable CSF elimination. The CL_{CSF} of formycin B was also significantly reduced in animals treated with NBMPR ($P < 0.05$). These data are consistent with the *in vitro* studies and collectively suggest that formycin B is eliminated from the CSF by a pathway(s) in addition to bulk flow. At least one pathway is saturable and may represent an equilibrative nucleoside transport system which can be inhibited by NBMPR.

KEY WORDS: formycin B, cerebrospinal fluid, choroid plexus, clearance, nucleoside transport.

INTRODUCTION

The choroid plexus functions to regulate the composition of the cerebrospinal fluid and thus to maintain homeostasis in the microenvironment of the central nervous system. A concentrative, nucleoside transport system in the choroid plexus which plays a role in nucleoside homeostasis in the brain was demonstrated by Reynold Spector and his colleagues (1–4). Recently, we further examined the mechanisms of nucleoside transport in isolated choroid plexus tissue slices from rabbit (5). Nucleosides are transported in choroid plexus from rabbit by both equilibrative and secondarily active Na^+-cotransport mechanisms. The Na^+-cotransport system is saturable and selective for naturally occurring purine and pyrimidine ribo- and deoxyribonucleosides but not for synthetic nucleoside analogs substituted on the ribose ring. The stoichiometric coupling ratio between Na^+ and nucleoside is 2:1. The Na^+-nucleoside cotransporter in rabbit choroid plexus differs from previously described Na^+-nucleoside transport systems in other tissues, which are generally selective for either purine or pyrimidine nucleosides and which have stoichiometric ratios of 1 (6–10).

Although there have been a number of studies characterizing nucleoside transport mechanisms in isolated choroid plexus tissue slices, the physiologic role of these transport systems in mediating nucleoside influx into or efflux from the CSF is not known. Studies from several laboratories have shown that transport systems in the choroid plexus are important in the elimination of many substances from the CSF (11–17). The goal of this study was to determine the routes by which nucleosides are eliminated from CSF *in vivo* and, particularly, to elucidate whether transport systems are involved in the CSF elimination of nucleosides. In this study, formycin B was used as a model nucleoside since this compound is considered a nonmetabolized nucleoside analogue and has been used in previous studies to examine Na^+-driven nucleoside transport in intact cells (6,10) and intact tissue (5). The rat was used as an experimental animal model since the ventricle and cisterna magna of the rat can be easily cannulated with the aid of a stereotaxic device. Furthermore, most studies of elimination of compounds from the CSF have been carried out in the rat (11,12,14,21,32–34).

Since mechanistic studies of nucleoside transport had been carried out previously in rabbit choroid plexus (1–5), initial studies in isolated choroid plexus tissue slices were carried out to verify that nucleoside transport systems are present in rat choroid plexus. Choroid plexus tissue slices were ATP-depleted to allow us to experimentally control the intra- and extracellular environment (5,18–20). Our data suggested that nucleosides are transported in the choroid plexus of the rat by mechanisms similar to those observed in the rabbit.

After the initial *in vitro* studies, *in vivo* studies were carried out using the technique of bolus injection into the lateral ventricle of the anesthetized rat and sampling of CSF from the cisterna magna to estimate the clearance of formycin B and inulin, a marker for bulk flow of CSF through the arachnoid villi (11,21). The study demonstrated that formycin B is eliminated from CSF by a pathway(s) in addition to bulk flow, and at least one pathway is saturable, suggesting that a facilitated and/or an active transport system(s) in the rat choroid plexus plays a role in nucleoside elimination from the CSF. An equilibrative nucleoside transport system which can be inhibited by NBMPR (nitrobenzylthioinosine) appears to be partially responsible for the elimination of formycin B from the CSF.

MATERIALS AND METHODS

Preparation of ATP-Depleted Choroid Plexus

ATP-depleted choroid plexuses from rat were prepared by the method of Carter-Su and Kimmich (18) as modified

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previously (5,19,20). Following sedation with ketamine (130 mg/kg), male Sprague Dawley rats were quickly sacrificed by decapitation. Choroid plexuses were obtained from the lateral ventricles and placed in the following buffer (m*M*): KCl (120), mannitol (40), HEPES [*N*-2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)] (25), pH 7.4, with 1 *M* Tris. Choroid plexuses were cut into 2- to 3-mm pieces and incubated for 20 min at 37°C in the buffer mentioned above containing 0.25 m*M* 2,4-dinitrophenol. After the incubation period the plexuses in the dinitrophenol solution were placed on ice until use.

Accumulation Study

Uptake of formycin B was studied by incubating a tissue slice with 140 μL of reaction medium containing ^{3}H-formycin B (0.18 μ*M*) and ^{14}C-mannitol (25.4 μ*M*), an extracellular marker, in Na^+ or Na^+-free buffer as described previously (5). Other constituents are specified in the figure captions. ^{14}C and ^{3}H in the dried tissue slice and in the corresponding reaction medium were determined by dual-isotope liquid scintillation counting on a Beckman Model 1801 liquid scintillation counter. The counting efficiency of ^{3}H ranged between 45–47% and that of ^{14}C ranged between 92 and 94%.

Cannula Implantation

Male Sprague Dawley rats (280–320 g) were anesthetized with an intramuscular dose of acepromazine (3 mg/kg) and ketamine (130 mg/kg). Full surgical anesthesia was maintained by supplementary doses of acepromazine (1.5 mg/kg) and ketamine (65 mg/kg) throughout the experiment. Normal body temperature was maintained with heating pads. Methods previously described by Whittico and Giacomini in our laboratory for ventriculocisterna study were used (11). Briefly, the rats' heads were fixed in a stereotaxic device. The frontal, parietal, and occipital bones were exposed through a linear midline incision. Coordinates (posterior, 1.8 mm, laterally, 3.8 mm, and ventrally, 4 mm, from bregma, respectively) obtained from a stereotaxic atlas of the rat brain (22,23) were used to locate the lateral ventricle, and a cannula was inserted into the right lateral ventricle through the electrode manipulator of the stereotaxic device. Another cannula was placed into the cisterna magna for CSF sampling (11,21). Cannulae were fixed to the skull with anchoring screws, super glue, and dental cement. Screw-capped cannula-dummy wires were screwed into the cannula guides to maintain a closed system. The CSF pressure was allowed to equilibrate for 45 min before ventricular bolus injection.

Ventriculocisternal Procedure

Twelve rats were randomized and divided into three groups. Three rats received a high dose and six rats received a low dose of formycin B. Another three rats received a low dose of formycin B plus NBMPR. Five microliters of a solution containing ^{3}H-formycin B (4–12 ng), ^{14}C-inulin (63–77 μg), and unlabeled formycin B (74–81 μg) in mock CSF (127.6 m*M* Na^+, 2.5 m*M* K^+, 1.3 m*M* Ca^{2+}, 1 m*M* Mg^{2+}, and 134.7 m*M* Cl^-, pH 7.33 with 0.1 *N* NaOH) (11,14) was administered to the three rats in the high-dose group. The six rats in the low-dose group received 5 μL of a solution containing ^{3}H-formycin B (4–12 ng), ^{14}C-inulin (63–77 μg), and mock CSF without unlabeled formycin B. Ten microliters of a solution containing ^{3}H-formycin B (8–9 ng), ^{14}C-inulin (58–66 μg), and NBMPR (6.8 μg, 16.3 nmol) in mock CSF was administered to the three rats in the low dose with NBMPR group. Solutions were injected through the cannula guide into the right lateral ventricle using a Hamilton microsyringe modified as previously described (11). Samples of 5 μL of CSF were drawn from the cisterna magna, at 2, 5, 10, 20, 40, 60, 90, 120, 150, and 180 min after injection, using another modified Hamilton microsyringe (11). CSF samples were placed into scintillation vials and 5 mL of scintillant was added into each vial. ^{14}C and ^{3}H were determined by dual-isotope liquid scintillation counting as mentioned earlier.

Data Analysis

Uptake of formycin B in each choroid plexus tissue slice was expressed as a volume of distribution (V_d) and was corrected with the V_d of mannitol, an extracellular marker, as previously described (5,20,25):

$$V_d = \frac{\text{dpm of [}^3\text{H]formycin B/g of tissue}}{\text{dpm of [}^3\text{H]formycin B/ml of media}}$$

In each experiment the V_d was determined in triplicate to generate each data point.

A model-independent method in which $CL_{CSF} = D/AUC$ was used to calculate the CL_{CSF} of formycin B and the CL_{CSF} of inulin. *D* represents the dose of the compound and AUC represents the area under the CSF concentration–time curve from time 0 to time infinity. AUC was calculated from the data by the trapezoidal rule (26) and extrapolated to time infinity by C_z/k, where C_z is the concentration of the last sample and k is the terminal elimination rate constant estimated by loglinear regression analysis of the last three points (CSF concentration versus sampling time). In these studies the extrapolated AUC was less than 15% of the total AUC. CL_{CSF} values of formycin B and inulin were compared statistically by groups. Statistical analysis for differences between formycin B and inulin CL_{CSF} within each group was performed using Student's paired *t* test. Clearance differences of formycin B between groups were determined by Student's unpaired *t* test. A *P* value less than 0.05 was considered statistically significant.

Materials

^{3}H-Formycin B (7 Ci/mmol) was obtained from Moravek Biochemicals, Inc., Brea, California. ^{14}C-Inulin (1.62 mCi/g) was obtained from Dupont, New England Nuclear, Boston, Massachusetts. Formycin B and NBMPR (nitrobenzylthioinosine) were obtained from Sigma Chemical Co., St. Louis, Missouri. All other chemicals were obtained from either Sigma or Fisher Scientific Co. Cytoscint ES scintillation fluid was obtained from ICN Biomedical Inc., Irvine, California. Acepromazine was obtained from Aveco Co., Inc., Fort Dodge, Iowa. Ketamine was obtained from Division of Warner-Lamber Co./Morris Plains, New Jersey. The stereotaxic device was a product of David Kopf Instru-

ments, Tujunga, California. Stainless-steel screws, cannula guides, and cannula-guide dummy wires were purchased from Plastic Products Co., Inc., Roanoke, Virginia. Krazy glue was obtained from Division of B. Jadow & Sons Inc., New York (made in Japan). Dental cement was obtained from Lang Dental Manufacturing Co., Inc., Chicago, Illinois. Hamilton syringes were obtained from Hamilton Co., Reno, Nevada. Sprague Dawley rats were obtained from Simomson Inc., Gilroy, California.

RESULTS AND DISCUSSION

The goal of this study was to determine whether nucleoside transport systems play a role in the elimination of nucleosides from the cerebrospinal fluid in the intact animal. The rat was selected as an animal model because of the ease of conducting precise *in vivo* studies with the aid of a stereotaxic device. Since transport systems have been characterized previously in choroid plexus from rabbit (4,5), it was first important to document that such transport systems are also present in the choroid plexus of the rat. In the presence of an initial inwardly directed Na^+ gradient, formycin B accumulated temporarily against a concentration gradient ("overshoot phenomenon") in isolated choroid plexus tissue slices from rat (Fig. 1). These data, which are consistent with previous data in rabbit choroid plexus (4,5), suggest that formycin B transport in the rat choroid plexus is coupled to an Na^+ gradient. The uptake of ^{3}H-formycin B was significantly inhibited by a high concentration of unlabeled formycin B (data not shown), suggesting that the Na^+-cotransport system is saturable.

The data also suggest that an equilibrative transport system sensitive to NBMPR was present in isolated choroid plexus tissue from rat (Fig. 2). In the absence of an Na^+ gradient the V_d (mean ± SD) of formycin B at 5 min was 2.10 ± 0.36 mL/g and was inhibited significantly by NBMPR (10 μ*M*), to 1.33 ± 0.1 mL/g ($P < 0.001$). In the presence of an initial inwardly directed Na^+ gradient, NBMPR significantly increased the uptake of formycin B from 2.71 ± 0.37 to 5.16 ± 0.61 mL/g (mean ± SD) ($P < 0.01$), presumably by inhibiting efflux. These data are analogous to data obtained previously in rabbit choroid plexus and suggest that, in rat choroid plexus, nucleosides are transported by similar mechanisms to those in rabbit choroid plexus.

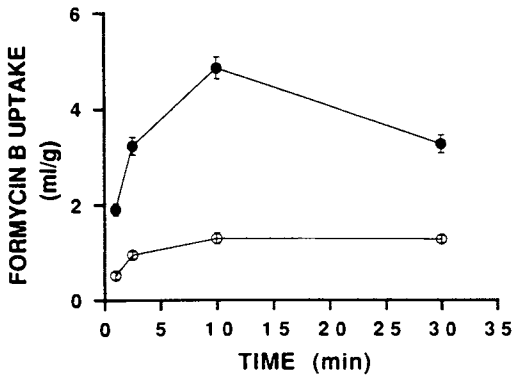

Fig. 1. The uptake of formycin B (2.18 μ*M*) in ATP-depleted choroid plexus slices from rat in the presence (filled circles) and absence (open circles) of an initial inwardly directed Na^+ gradient as a function of time. NBMPR (10 μ*M*) was present in the reaction media. Data represent the mean (±SE) V_d from three experiments.

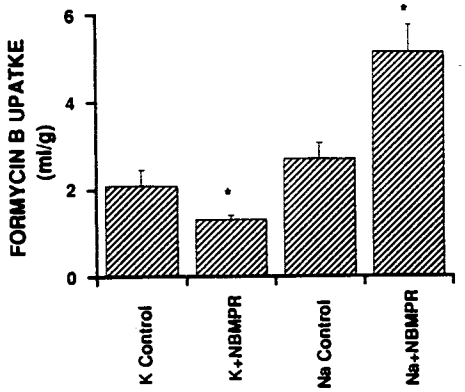

Fig. 2. The uptake of formycin B (2.18 μ*M*) in ATP-depleted choroid plexus slices from rat. Data were obtained at 5 min in the absence (K) and presence (Na) of an initial inwardly directed Na^+-gradient. Bars represent the mean (±SD) of data obtained in three separate experiments in the presence and absence of NBMPR. NBMPR (10 μ*M*) significantly reduced the V_d of formycin B in the absence of Na^+ gradient ($P < 0.01$), and significantly increased the V_d of formycin B in the presence of an Na^+ gradient ($P < 0.01$).

The elimination of a substance from CSF may include the following pathways. First, any substance, regardless of molecular size or lipid solubility, will be eliminated with the normal turnover of CSF via bulk flow passage by filtration across the arachnoid villi (27). Second, nonionized, lipid-soluble compounds can leave the CSF by passive diffusion into brain cells and then across the lipid-like blood–brain barrier. Third, facilitated or active transport systems located in the choroid plexus epithelium of the ventricles will contribute to the elimination of substances with specific physical and chemical properties (11–17,27–29). Finally, an additional elimination pathway from CSF is biotransformation. We hypothesized that the elimination of formycin B from the CSF may include most of these pathways. Since formycin B has been used as a nonmetabolized substrate (5,6,10), the biotransformation pathway is considered negligible.

The CSF concentrations of formycin B and inulin declined biexponentially in all animals (Fig. 3). The AUC of formycin B obtained in the animals that received a low dose of the drug versus dose increased linearly with dose (r = 0.92), suggesting that linear pharmacokinetic principles are applicable to this model in the low dosage range (Fig. 4). The CL_{CSF} of formycin B, calculated as the reciprocal of the slope of the regression line, was 16.6 μL/min.

Inulin, a macromolecular (MW 5000) polar compound, has been used as a bulk flow marker in previous studies (13,27,30,31). The mean (±SE) inulin clearance obtained in this study of 4.6 (±0.2) μL/min is in the range of CSF inulin clearances obtained previously in anesthetized rats, which include 2.2 μL/min (14,24,30), 3.7 μL/min (32), 2–4 μL/min (33), and 5.7 μL/min (34).

In this study, formycin B was cleared significantly ($P <$ 0.05) faster than inulin in these three groups (Table I). These results suggest that formycin B is eliminated from CSF by a pathway(s) in addition to bulk flow. The net clearance of formycin B (CL_{CSF} of formycin B − CL_{CSF} of inulin) reflects the clearance by this additional pathway(s).

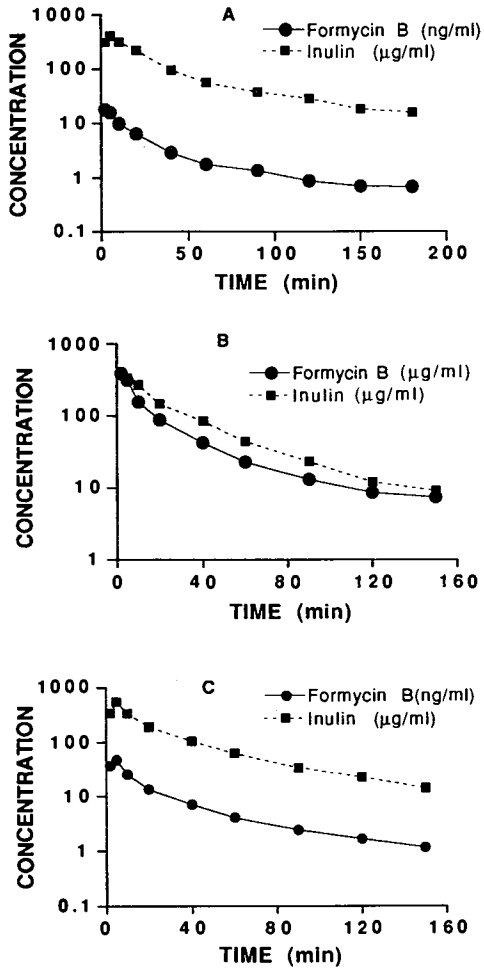

Fig. 3. The semilogarithmic plots of the CSF concentration of formycin B and inulin versus time in a representative rat that received a low dose of formycin B (A), in a representative rat that received a high dose of formycin B (B), and in a representative rat that received a low dose of formycin B with NBMPR (C).

The net CL_{CSF} of formycin B was significantly ($P < 0.05$) lower in the animals that received the high dose of formycin B than in the animals that received the low dose of formycin B, whereas the CL_{CSF} of inulin was not affected by the high dose of formycin B ($P > 0.05$) (Table I). These results suggest that at least one pathway of elimination of formycin B is saturable. The results are consistent with the results from our *in vitro* study demonstrating that the uptake of ^{3}H-formycin B was significantly reduced by a high concentration of unlabeled formycin B (data not shown), and with results from previous *in vitro* studies demonstrating that nucleosides are transported by saturable mechanisms in rabbit choroid plexus (4,5). The data suggest that nucleoside transport systems in choroid plexus play a role in the elimination of formycin B from the CSF.

To determine the nature of the transport system(s) involved in formycin B elimination from the CSF, we examined the effect of NBMPR on the CSF clearance of formycin B. NBMPR significantly reduced the clearance of formycin B while not significantly affecting the clearance of inulin (Table I). Our data (not shown) demonstrated that the clearance of cimetidine, a compound which is transported by a saturable mechanism in the choroid plexus (20), was not affected by NBMPR, suggesting that NBMPR did not reduce formycin B CSF clearance by nonspecific cytotoxic effects. Thus, the data suggest that an equilibrative nucleoside transporter sensitive to NBMPR is responsible in part for the elimination of formycin B from the CSF. Although NBMPR concentrations in CSF were not measured, the dose of NBMPR used in this study was calculated based upon the total volume of CSF (400 μL) to achieve an initial concentration of 40 μ*M*. Since NBMPR did not reduce formycin B clearance to bulk flow, the data suggest quantitatively that other clearance mechanisms also contribute to the elimination of formycin B from the CSF. These may include diffusional pathways, secondarily active Na^+-cotransport mechanisms, and equilibrative nucleoside transport systems that are insensitive to NBMPR.

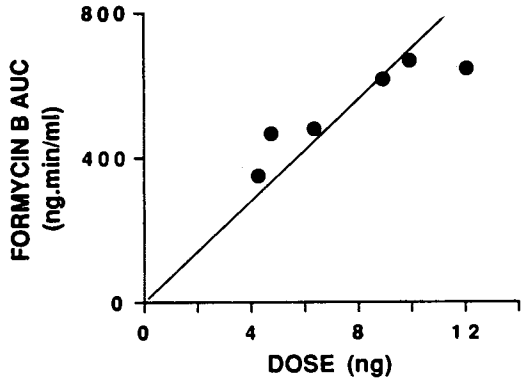

Fig. 4. The area under the CSF concentration–time curve (AUC) of formycin B versus the dose of formycin B in the six animals that received the low dose of the drug. The line is drawn from a fit of the data using linear least-squares regression analysis forced through the origin.

In summary, the results of this study suggest that transport systems are involved in the elimination of nucleosides from the CSF. However, it is important to point out that many endogenous nucleosides are highly biotransformed in the central nervous system (35,36). For such compounds, transport systems in choroid plexus epithelium would play a minor role in their overall elimination from the CSF. However, many nucleoside analogues such as formycin B are not metabolized extensively. For these compounds, transport systems in the choroid plexus may play a major role in their overall CSF elimination and thus, their concentrations in CSF. These transport systems may be important in nucleoside analogue targeting to or away from the central nervous system.

Table I. Clearances of Formycin B and Inulin from Rat CSF

Group	CL_{CSF} (μL/min, mean ± SD)		
	Formycin B	Inulin	Net clearance
Low dose (n = 6)[a]	12.1 ± 2.8	4.4 ± 0.6	7.7 ± 2.3
NBMPR (n = 3)	7.8 ± 0.6	4.4 ± 0.02	3.4 ± 0.6
High dose (n = 3)	8.8 ± 2.3	5.1 ± 1.6	3.6 ± 0.8

[a] n refers to the number of rats in the group.

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REFERENCES

1. R. Spector. Thymidine accumulation by choroid plexus *in vitro*. *Arch. Biochem. Biophys.* 205:85–93 (1980).
2. R. Spector. Nucleoside transport in choroid plexus: Mechanism and specificity. *Arch. Biochem. Biophys.* 216:693–703 (1982).
3. R. Spector and W. G. Berlinger. Localization and mechanism of thymidine transport in central nervous system. *J. Neurochem.* 39:837–841 (1982).
4. R. Spector and S. Huntoon. Specificity and sodium dependence of the active nucleoside transport system in choroid plexus. *J. Neurochem.* 42:1048–1052 (1984).
5. X. Wu, G. Yuan, C. M. Brett, A. C. Hui, and K. M. Giacomini. Sodium-dependent nucleoside transport in choroid plexus from rabbit: Evidence for a single transporter for purine and pyrimidine nucleosides. *J. Biol. Chem.* 267:8813–8818 (1992).
6. D. Vijayalakshmi and J. A. Belt. Sodium-dependent nucleoside transport in mouse intestinal epithelial cells: Two transport systems with differing substrate specificities. *J. Biol. Chem.* 263:19419–19423 (1988).
7. C. R. Crawford, C. Y. C. Ng, L. D. Noel, and J. A. Belt. Nucleoside transport in L1210 murine leukemia cells: Evidence for three transporters. *J. Biol. Chem.* 265:9732–9736 (1990).
8. L. Dagnino, L. Lee Bennett, and A. R. P. Paterson. Substrate specificity, kinetics, and stoichiometry of sodium-dependent adenosine transport in L1210/AM mouse leukemia cells. *J. Biol. Chem.* 266:6312–6317 (1991).
9. M. L. Hir. Evidence for separate carriers for purine nucleosides and pyrimidine nucleosides in the renal brush border membrane. *Renal Physiol. Biochem.* 13:154–161 (1990).
10. E. S. Jakobs and A. R. P. Paterson. Sodium-dependent, concentrative nucleoside transport in cultured intestinal epithelial cells. *Biochem. Biophys. Res. Commun.* 140:1028–1035 (1986).
11. M. T. Whittico and K. M. Giacomini. Cimetidine elimination from cerebrospinal fluid of the rat. *Pharm. Res.* 5:625–633 (1988).
12. J. T. Huang. Accumulation of peptide tyr-D-ala-gly by choroid plexus during ventriculocisternal perfusion of rat brain. *Neurochem. Res.* 7:1541–1548 (1982).
13. J. R. Pappenheimer, S. R. Heisey, and E. F. Jordan. Active transport of diodrast and phenol-sulfonphthalein from cerebrospinal fluid to blood. *Am. J. Physiol.* 200:1–10 (1961).
14. J. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga, and M. Hanano. Saturable transport of cimetidine from cerebrospinal fluid to blood in rats. *J. Pharmacobiodyn.* 8:73–76 (1985).
15. H. Davson, J. G. Hollingsworth, M. B. Carey, and J. D. Fenstermacher. Ventriculocisternal perfusion of twelve amino acids in the rabbit. *J. Neurobiol.* 13:293–318 (1982).
16. S. R. Snodgrass, R. W. P. Cutler, E. S. Kang, and A. V. Lorenzo. Transport of neutral amino acids from feline cerebrospinal fluid. *Am. J. Physiol.* 217:974–980 (1969).
17. L. S. Schanker, L. D. Prockop, J. Schou, and P. Sisodia. Rapid efflux of some quaternary ammonium compounds from cerebrospinal fluid. *Life Sci.* 10:515–521 (1962).
18. C. Carter-Su and G. A. Kimmich. Membrane potentials and sugar transport by ATP-depleted intestinal cells: Effect of anion gradients. *Am. J. Physiol.* 237:C67–C74 (1979).
19. H. Suzuki, Y. Sawada, Y. Sugiyama, T. Iga, and M. Hanano. Anion exchanger mediates benzyl penicillin transport in rat choroid plexus. *J. Pharmacol. Exp. Ther.* 243:1147–1152 (1987).
20. M. T. Whittico, G. Yuan, and K. M. Giacomini. Cimetidine transport in isolated brush border membrane vesicles from bovine choroid plexus. *J. Pharmacol. Exp. Ther.* 255:615–623 (1990).
21. H. J. Bouman and T. B. Van Wimersma Greidanus. A rapid and simple cannulation technique for repeated sampling of cerebrospinal fluid in freely moving rat. *Brain Res. Bull.* 4:575–577 (1979).
22. L. J. Pellegrino and A. J. Cushman. Use of the stereotaxic technique. In R. D. Myers (ed.), *Methods in Psychobiology, Vol. I*, Academic Press, New York, 1971, pp. 67–89.
23. L. J. Pellegrino, A. S. Pellegrino, and A. J. Cushman. *A Sereotaxic Atlas of the Rat Brain*, Plenum Press, New York, 1979.
24. H. F. Cserr. Physiology of the choroid plexus. *Physiol. Rev.* 51:273–311 (1971).
25. V. A. Murphy and C. E. Johanson. Alteration of sodium transport by the choroid plexus with amiloride. *Biochim. Biophys. Acta* 979:187–192 (1989).
26. M. Gibaldi and D. Perrier. *Pharmacokinetics*, 2nd ed., Marcel Dekker, New York, 1982.
27. L. D. Prockop and L. S. Schanker. On the mode of exit of substances from cerebrospinal fluid. *Life Sci.* 4:141–149 (1962).
28. H. J. Ross and E. M. Wright. Neutral amino acid transport by plasma membrane vesicles of the rabbit choroid plexus. *Brain Res.* 295:155–160 (1984).
29. A. V. Lorenzo and R. Spector. Transport of salicylic acid by the choroid plexus *in vitro*. *J. Pharmacol. Exp. Ther.* 184:465–471 (1973).
30. H. Cserr. Potassium exchange between cerebrospinal fluid, plasm and brain. *Am. J. Physiol.* 209:C482–C489 (1965).
31. S. R. Heisey, D. Held, and J. R. Pappenheimer. Bulk flow and diffusion in the cerebrospinal fluid system of the goat. *Am. J. Physiol.* 203:775–781 (1962).
32. P. P. Harnish and K. Samul. Reduced cerebrospinal fluid production in the rat and rabbit by diatrizoate: Ventriculocisternal perfusion. *Invest. Radiol.* 23:534–536 (1988).
33. N. H. Bass and P. Lundborg. Postnatal development of bulk flow in the cerebrospinal fluid system of the albino rat: Clearance of carboxy-(14C) inulin after intrathecal injection. *Brain Res.* 52:323–332 (1973).
34. M. Manin, Y. Broer, M. Balage, W. Rostene, and J. Grizard. Metabolic clearance of insulin from the cerebrospinal fluid in the anesthetized rat. *Peptides* 11:5–12 (1990).
35. A. S. Suleiman and R. Spector. Metabolism of deoxyuridine in rabbit brain. *J. Neurochem.* 39:8224–8230 (1982).
36. R. Spector. Thymidine transport in the central nervous system. *J. Neurochem.* 35:1092–1098 (1980).