Mechanisms of Nucleobase Transport in Rabbit Choroid Plexus

EVIDENCE FOR A Na⁺-DEPENDENT NUCLEOBASE TRANSPORTER WITH
BROAD SUBSTRATE SELECTIVITY*

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The overall goal of this study was to determine the mechanisms by which nucleobases are transported in the choroid plexus. Choroid plexus tissue slices were obtained from the lateral ventricles of rabbit brains and depleted of ATP with 2,4-dinitrophenol. In the presence of an initial inwardly directed Na⁺ gradient, hypoxanthine accumulated in the tissue slices against a concentration gradient. Na⁺-stimulated hypoxanthine uptake was saturable with a Kₘ of 31.1 ± 9.71 μM and a Vₘₐₓ of 2.69 ± 0.941 nmol/g/s (mean ± S.E.). Na⁺-stimulated hypoxanthine uptake was inhibited by (100) μM naturally occurring purine and pyrimidine nucleobases (adenine, cytosine, guanine, hypoxanthine, thymine, uracil, and xanthine) as well as by the nucleoside analog, dideoxycytosine. The stoichiometric coupling ratio between Na⁺ and hypoxanthine was 1.7:1. The data demonstrate the presence of a novel Na⁺-dependent nucleobase transporter in the choroid plexus, which is distinct from the previously described Na⁺-nucleoside transporter in choroid plexus and from Na⁺-nucleobase transporters in other tissues in terms of its kinetics, substrate selectivity, and Na⁺-nucleobase stoichiometry. This transporter may play a role in the targeting of both salvageable nucleobases and therapeutic nucleoside analogs to the central nervous system.

Nucleobases and their structural analogs have become important drugs in the treatment of a number of viral infections of the central nervous system such as cytomegalovirus retinitis, herpes simplex encephalitis, and AIDS-related dementia complex. Therefore, nucleobase transport mechanisms at the major barriers of the central nervous system, namely the blood-brain barrier and the blood-cerebrospinal fluid barrier (choroid plexus epithelium), may play a critical role in targeting nucleobase analogs to the affected tissues. Moreover, such transport systems may be important in the salvage of purines for nucleic acid synthesis in the brain.

Saturable, low affinity nucleobase transporters have been identified in the blood-brain barrier (1, 2). However, it appears that these transporters may not be sufficient to mediate the flux of important quantities of hypoxanthine, the principal salvageable nucleobase, or other nucleobases into the brain. As a result, attention has focused on the choroid plexus as a possible route of entry for hypoxanthine and other nucleobases into the brain (3).

In mammalian cells, transcellular flux of nucleobases is mediated by specific transporters in the plasma membranes. Two major classes of nucleobase transporters have been characterized: equilibrative and concentrative. Equilibrative transporters are present in a number of cell types and are broadly selective for purine and pyrimidine nucleobases. Recent studies with acyclovir and ganciclovir, nucleobase/nucleoside analogs, demonstrate that these agents permeate the human erythrocyte by way of equilibrative mechanisms (14, 15).

Recently, concentrative nucleobase transporters have been identified in LLC-PK₁ cells (4), guinea pig kidney (5, 13), rat jejunal tissue (6), and guinea pig placenta (7). These transporters are secondary active and Na⁺-dependent and mediate the influx of specific nucleobases. The substrate selectivity of the transporter in LLC-PK₁ cells has been studied in considerable detail and includes selected purine and pyrimidine nucleobases.

The goal of this study was to determine the mechanisms of nucleobase transport in the choroid plexus. Our data demonstrate the presence of a novel Na⁺-dependent nucleobase transporter in the choroid plexus, which is distinct from previously described Na⁺-driven nucleobase transporters in other tissues in terms of its substrate selectivity and kinetics. This transporter may play an important role in the delivery of both salvageable and therapeutic nucleobases to the central nervous system.

EXPERIMENTAL PROCEDURES

Preparation of ATP-depleted Choroid Plexus Tissue Slices—Choroid plexus tissue slices from rabbit were ATP-depleted by the method of Whitlisco (8). Choroid plexuses were obtained from the lateral ventricles of New Zealand White rabbits. The choroid plexus tissue was placed in KCl buffer (37 °C) of the following composition: KCl (150 mM), mannitol (40 mM), and HEPES (25 mM), pH 7.4, with 1 m Tris. Choroid plexus tissue was cut into 2-3-mm pieces and ATP-depleted by incubating at 37 °C for 20 min in 2,4-dinitrophenol (250 μM) in KCl buffer. Under these conditions, the ATP concentration is reduced to less than 10% of control (9). Following this incubation, the choroid plexus slices in buffer were stored on ice until uptake experiments were performed.

N-Ethylmaleimide (NEM) Incubation—ATP-depleted choroid plexus slices were incubated at room temperature in NEM (1 mM) for 45 min as described previously (11).

Accumulation Studies—Uptake of [³H]hypoxanthine was studied by methods published previously (10). Briefly, individual choroid plexus tissue slices were incubated with 140 μl of reaction mixture containing [³H]hypoxanthine (0.24 μM), [¹⁴C]mannitol (25.4 μM), and 2,4-dinitrophenol (250 μM) in KCl (150 mM) or NaCl buffer (150 mM). The uptake was stopped by blotting on laboratory tissue. The choroid plexus tissue slices were then dried and weighed. Tissue slices were dissolved, and the corresponding reaction mixture by dual isotope liquid scintillation counting. Thin layer chromatography methods were used to determine

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¹ The abbreviations used are: NEM, N-ethylmaleimide; dDA, dideoxyadenosine.
FIG. 1. Hypoxanthine uptake (0.24 μM) in ATP-depleted rabbit choroid plexus tissue slices. The uptake of hypoxanthine (Vd) was examined in the absence (squares) and presence (circles) of an initial inwardly directed Na⁺ gradient. Each data point represents the uptake of hypoxanthine (mean ± S.E.) from three experiments.

whether metabolism or degradation of hypoxanthine had occurred.

Data Analysis—The radioactive content from each choroid plexus tissue slice was expressed as a volume of distribution (Vd) as described previously (8, 10).

\[ V_d = \frac{dpm[^3]H\text{hypoxanthine/g choroid plexus}}{dpm[^3]H\text{hypoxanthine/ml media}} - \frac{dpm[^4]C\text{mannitol/g choroid plexus}}{dpm[^4]C\text{mannitol/ml media}} \]

Statistical analysis was carried out by a Student's unpaired t test. A probability, p, of less than 0.05 was considered significant. Data points were determined in triplicate for each experiment. Data, unless mentioned otherwise, are expressed as the mean ± S.E. of data obtained from three experiments in choroid plexus tissue from separate animals.

Standard methods were used to determine IC_{50} values and Michaelis-Menten kinetics (10).

To determine the stochiometric coupling between Na⁺ and hypoxanthine, a modified version of the Hill equation was used,

\[ \text{Rate} = a \cdot C_{Na}^{n} \]

where a is \( V_{max}/K_{m} \) when \( K_{m} \) \( \gg \) \( C_{Na} \) in the reaction mixture. The data were transformed with a logarithm and linearly regressed to obtain a and n. This equation has been used previously in stoichiometry studies (12).

Materials—[^3]H-hypoxanthine (11.6 Ci/mmol),[^3]H-thymidine (65 Ci/mmol), and[^4]C-mannitol (56 mCi/mmol) were purchased from either Amersham Life Science, Arlington Heights, IL or Moravek Biochemicals, Inc., Brea, CA. Hypoxanthine, adenine, adenosine, deoxyadenosine (dA), guanine, xanthine, caffeine, cytosine, thymine, uracil, thymidine, and proline were purchased from either Sigma or Aldrich. All other chemicals were purchased from either Sigma, Fisher Scientific, or Aldrich. New Zealand White rabbits were purchased from Nitabell Rabbitry, Hayward, CA. Cytoscint ES scintillation fluid was purchased from ICN Biomedical Inc.

RESULTS

In the absence of a Na⁺ gradient, hypoxanthine accumulated in the tissue slices and reached an equilibrium (Vd = 2.57 ± 0.29) in approximately 5 min (Fig. 1). This uptake was not reduced by any compound (including unlabeled hypoxanthine) in the concentration range used in these studies. These results suggest that in the absence of a Na⁺ gradient, the uptake of hypoxanthine represents a nonselective or low affinity binding process or transport process.

In contrast, in the presence of an initial inwardly directed Na⁺ gradient (150 mM), hypoxanthine accumulated temporarily ("overshoot phenomenon") in the tissue slices above the equilibrium value (Vd = 6.11 ± 0.91 at 1 min) (Fig. 1). Thin layer chromatography (TLC) studies indicated that hypoxanthine was not significantly metabolized at 30 s or 5 min (data not shown).

Kinetic experiments (Fig. 2) were performed in which the rate of hypoxanthine uptake (at 30 s) as a function of concentration was determined in the presence of an inwardly directed Na⁺ gradient (150 mM). The data are consistent with a single saturable process. The data from each of the three experiments were fit to an appropriate Michaelis-Menten equation, which included a linear component (8). The K_{m} and V_{max} (mean ± S.E.) for Na⁺-stimulated hypoxanthine uptake were 31.1 ± 9.71 μM and 2.69 ± 0.94 nmol/g/s, respectively.

To determine the stoichiometry (Fig. 3) of the Na⁺-dependent nucleobase transport system, the Na⁺-dependent uptake of hypoxanthine (0.24 μM) was examined in the presence of increasing Na⁺ concentrations (0–140 mM). The uptake of hypoxanthine (Vd) was sensitive to Na⁺ concentration. The data were fit to a Hill equation as described under "Experimental Procedures." The Hill coefficient was 1.7 ± 0.4 (mean ± S.E.) for Na⁺-hypoxanthine and was significantly different from 1 but not from 2.

The effect of various purines on Na⁺-stimulated hypoxanthine uptake was examined (Fig. 4). At concentrations of 100 μM, hypoxanthine, adenine, adenosine, dA, guanine, and xanthine significantly inhibited Na⁺-dependent hypoxanthine uptake (Vd) at 30 s (p < 0.05). Hypoxanthine uptake was slightly inhibited by 2′-deoxyadenosine (29%) but not 3′-deoxyadenosine (data not shown). Caffeine (100 μM) did not significantly inhibit Na⁺-dependent hypoxanthine transport. At concentrations of 100 μM, the pyrimidine nucleobases, cytosine, thymine, and uracil, significantly inhibited Na⁺-dependent hypoxanthine uptake at 30 s (p < 0.05) whereas the pyrimidine nucleoside, cytidine, did not (Fig. 5). The amino acid, proline, also did not inhibit Na⁺-dependent hypoxanthine uptake (data not shown). A range of concentrations (0–1 mm) was used to determine IC_{50} values for xanthine, uracil, and adenine. Xanthine (65.7 ± 5 μM) (Fig. 6), uracil (64.6 ± 11.2 μM), and adenine (77.9 μM) were potent inhibitors of IC_{50} values in the low micromolar range of Na⁺-dependent hypoxanthine transport. Consistent with a single transport mechanism, inhibition curves were monophasic.

The effect of the nucleobases, hypoxanthine and thymine, on the Na⁺-dependent uptake of the nucleoside, thymidine, was examined (Fig. 7). At concentrations of 100 μM, hypoxanthine and thymine did not significantly inhibit Na⁺-dependent thy-
The data in this study suggest that the Na\(^+\)-dependent hypoxanthine transporter in the choroid plexus is distinct from Na\(^+\)-dependent hypoxanthine transporters in other tissues. First, Na\(^+\)-dependent hypoxanthine uptake in the choroid plexus has a lower affinity (31.1 \(\mu\)M) than Na\(^+\)-stimulated hypoxanthine uptake in guinea pig kidney (4.4 \(\mu\)M) (5, 13) and LLC-PK\(_1\) cells (0.79 \(\mu\)M) (4) but a higher affinity than the saturable uptake system for hypoxanthine (400 \(\mu\)M) identified in the blood brain barrier (2).

Second, the substrate selectivity of the Na\(^+\)-stimulated hypoxanthine transporter in the choroid plexus differs from that of Na\(^+\)-stimulated hypoxanthine transporters in other tissues. In the choroid plexus, the purine nucleobases, adenine, guanine, and xanthine, and the pyrimidine nucleobases, cytosine, thymine, and uracil, were potent inhibitors of Na\(^+\)-dependent hypoxanthine uptake (4). The transporter also appears to differ in its substrate selectivity from the Na\(^+\)-dependent nucleobase transporter in renal brush border membrane vesicles from guinea pig, which excludes adenine (5, 13). A Na\(^+\)-dependent nucleobase transporter for pyrimidines in rat jejunal tissue has been characterized (6); however, the interactions of purines with the transporter were not examined. The interaction of ddA with the Na\(^+\)-dependent nucleobase transporter suggests that the choroid plexus may play a role in the transport of certain clinically relevant nucleoside analogs.

Our data are consistent with a 2:1 coupling ratio for Na\(^+\)-
hypoxanthine transport. Na^+hypoxanthine transport exhibits a 1:1 stoichiometry in LLC-PK1 cells and a 2:1 stoichiometry in the guinea pig kidney (13). Similar data have been obtained for Na^+-nucleoside transport in choroid plexus. That is, a 2:1 Na^+-nucleoside stoichiometry has been obtained for the N-3, Na^+-nucleoside transporter in choroid plexus (10).

The Na^+-nucleobase transporter in the choroid plexus also appears to be distinct from the Na^+-nucleoside transporter in the same tissue. Neither cytidine (Fig. 5) nor thymidine (data not shown) inhibited Na^+-stimulated hypoxanthine uptake in the choroid plexus. Conversely, neither hypoxanthine (100 μM) nor thymine (100 μM) inhibited Na^+-stimulated thymidine uptake (Fig. 7). Moreover, previous studies in this laboratory have demonstrated that the sulfhydryl modifier, NEM, irreversibly inhibits Na^+-nucleoside transport in choroid plexus (11). However, NEM did not inhibit Na^+-hypoxanthine uptake in rabbit choroid plexus.

In conclusion, a Na^+-dependent nucleobase transporter in choroid plexus from rabbit has been characterized. This transporter is broadly selective for both purine and pyrimidine nucleobases and appears to differ from the recently characterized Na^+-nucleobase transporters in guinea pig kidney and LLC-PK1 based on its kinetics and substrate selectivity. However, this cannot be confirmed until the transport has been cloned and sequenced. Further studies are also needed to determine the relative contribution of the choroid plexus (in comparison with the blood brain barrier) in transporting physiologically relevant quantities of nucleobases into the brain and the role of the transporter in targeting nucleobase and nucleoside analogs to the central nervous system.

Fig. 7. The effect of nucleobases on Na^+-nucleoside transport in ATP-depleted rabbit choroid plexus tissue slices. [3H]Thymidine (6 μM) uptake (30 s) was determined in ATP-depleted rabbit choroid plexus tissue slices in the presence of unlabeled thymidine, thymine, or hypoxanthine (100 μM) and an inwardly directed Na^+ gradient. Bars represent the mean (± S.E.) of data obtained in three separate experiments. Solid and hatched bars represent data in the presence and absence of an inwardly directed Na^+ gradient, respectively. Only unlabeled thymidine significantly (p < 0.05) inhibited Na^+-dependent thymidine uptake.

REFERENCES

1. Betz, A. L. (1985) J. Neurochem. 44, 574–579
2. Spector, R. (1987) Neurochem. Res. 12, 791–796
3. Spector, R. (1988) J. Neurochem. 50, 969–978
4. Griffith, D. A., and Jarvis, S. M. (1993) J. Biol. Chem. 268, 20085–20090
5. Griffith, D. A., and Jarvis, S. M. (1993) Biochem. Soc. Trans. 22, 815
6. Bronk, L. F., and Hastewell, J. G. (1987) J. Physiol. (Lond.) 382, 475–488
7. Barros, L. F. (1994) Am. J. Obstet. Gynecol. 171, 111–117
8. Whittico, M. T., Yuan, G., and Giacomini, K. M. (1990) J. Pharmacol. Exp. Ther. 255, 615–623
9. Wu, X., Gutierrez, M. M., and Giacomini, K. M. (1994) Biochim. Biophys. Acta 1191, 190–196
10. Wu, X., Yuan, G., Brett, C. M., Hui, A. C., and Giacomini, K. M. (1992) J. Biol. Chem. 267, 8813–8818
11. Washington, C. B., Brett, C. M., Wu, X., and Giacomini, K. M. (1995) J. Pharmacol. Exp. Ther. 274, 110–114
12. Chung, S. J., Ramanathan, V., Giacomini, K. M., and Brett, C. M. (1994) Biochim. Biophys. Acta 1193, 10–16
13. Griffith, D. A., and Jarvis, S. M. (1994) Biochem. J. 303, 901–905
14. Mahony, W. B., Domín, B. A., McConnell, R. T., and Zimmerman, T. P. (1988) J. Biol. Chem. 263, 9285–9291
15. Mahony, W. B., Domín, B. A., and Zimmerman, T. P. (1991) Biochem. Pharmacol. 41, 263–271
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