AIT-082, a Cognitive Enhancer, Is Transported into Brain by a Nonsaturable Influx Mechanism and out of Brain by a Saturable Efflux Mechanism

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ABSTRACT

A fundamental feature of any drug designed to treat a disease of the central nervous system is the ability to cross the blood-brain barrier. Passage across the blood-brain barrier of AIT-082, a cognitive enhancer, was investigated in mice. [14C]AIT-082 crossed the blood-brain barrier in young male Swiss-Webster mice with a mean influx constant ($K_i$) of 0.6 ± 0.2 μl g⁻¹ min⁻¹. Furthermore, [14C]AIT-082 was transported into brain of both young and old male C57BL/6 mice with a $K_i$ of 0.35 ± 0.06 and 0.33 ± 0.02 μl g⁻¹ min⁻¹, respectively. There was no significant effect of age or strain on the movement of [14C]AIT-082 across the blood-brain barrier in mice. When 110- or 650-fold excess unlabeled AIT-082 was included in the injection solution, the $K_i$ was not significantly changed in either Swiss-Webster or C57BL/6 mice. This indicated that [14C]AIT-082 crossed the blood-brain barrier by a nonsaturable mechanism. The passage of AIT-082 into brain extracellular fluid was confirmed with capillary depletion and microdialysis. The efflux of [14C]AIT-082 from brain also was examined. After i.c.v. injection, [14C]AIT-082 levels in brain decreased over time with a $t_{1/2}$ of 20.0 ± 1.0 min. Excess unlabeled AIT-082 (600-fold) increased the $t_{1/2}$ to 35.5 ± 3.6 min. Together, these data indicate that AIT-082 moves into brain via a nonsaturable mechanism and is actively transported out of brain.
NMAD administration (Caciagli et al., 1998; Di Iorio et al., 1999). Similarly, AIT-082 completely protected against glutamic acid decarboxylase activity loss in the hippocampus after systemic kainic acid administration (Di Iorio et al., 1999). Furthermore, AIT-082 ameliorates the neurodegeneration caused by an acute spinal cord crush injury in rats (Middlemiss et al., 1999). In this model of spinal cord injury, AIT-082 treatment resulted in fewer reactive glia, less tissue necrosis, less cavitary, an increase in nuclear staining, less increase in cellularity, and less swelling caudal to the lesion. These histological improvements were associated with improvement in a foot-orientating response test (segmental reflex recovery) and an open field walking task (gross locomotor recovery; Middlemiss et al., 1999).

The molecular basis for the neurotrophic actions of AIT-082 has not been fully elucidated; however, evidence is accumulating to suggest possible mechanisms. AIT-082 stimulates the synthesis and release of neurotrophic factors in vitro (Middlemiss et al., 1995; Ciccarelli et al., 1999; Rathbone et al., 1999) and in vivo (Chu-LaGraff et al., 1998; J. M. Conner and M. H. Tuszyński, unpublished data). Treatment with 100 μM AIT-082 for 6 h induces de novo synthesis and release of nerve growth factor and transforming growth factor-β from cultured rat astrocytes (Ciccarelli et al., 1999). Conditioned medium from these cells protect hippocampal and cortical neurons from glutamate-induced cell death and this neuroprotective effect is partially blocked by both anti-nerve growth factor and anti-transforming growth factor-β antibodies (Ciccarelli et al., 1999). In addition, treatment of astrocyte cultures with AIT-082 causes an increase in the extracellular concentration of purines such as adenosine and inosine (Caciagli et al., 1999). It has recently been shown that this is probably due to an inhibition of purine nucleotide phosphorylase and adenine deaminase (Caciagli et al., 1999).

In this study, we investigated the mechanism by which AIT-082 crosses the blood-brain barrier. It is known that a number of pyrimidine and purine transporters, including a hypoxanthine transporter, exist at the blood-brain barrier (Cornford and Olendorf, 1975; Betz, 1985; Spector, 1987; 1988) and we have investigated whether a specific, saturable mechanism mediates the transport of AIT-082 across the blood-brain barrier.

**Materials and Methods**

**Animals.** Male Swiss-Webster CFW and C57BL/6 mice were supplied by Charles River Laboratories (Hollister, CA) and all experiments were conducted according to the National Institutes of Health Guide on Care and Use of Laboratory Animals. Young male mice were 2 to 3 months old at the time of use. Aged male mice were 13 to 25 months old. This large range in age was due to a supply shortage.

**AIT-082 and Other Reagents.** AIT-082 (>99.5% pure) was synthesized by Eprova (Schaffhausen, Switzerland). The free acid of AIT-082 was produced by preparing a concentrated solution of AIT-082, adjusting to pH 1 to 2 with HCl, filtering the solution, washing and then drying the precipitate. The precipitate was then dissolved in PBS containing 160 mM NaOH. [14C]AIT-082 (51.5 mCi/mmol; 98% pure) was synthesized by Chemsyn Laboratories (Lenexa, KS). [125I]-BSA (300 Ci/mmol) was supplied by NEN (Boston, MA) and [3H]sucrose (5–15 Ci/mmol) was supplied by Amersham (Arlington Heights, IL).

**Octanol/PBS Partition Coefficients.** A 400-μl aliquot of PBS (pH 7.4) was combined with 400 μl of octanol and 5 μl of [14C]AIT-082 (0.1 mg/ml) and mixed for 24 h. After 24 h, radioactivity was measured in 100 μl of the octanol layer and 100 μl of the aqueous layer. The partition coefficient (P) was calculated as disintegrations per minute in octanol/disintegrations per minute in PBS and log P was determined.

**Influx Experiments.** These experiments were conducted as described in Blasberg et al. (1983), Patlak et al. (1983), and Banks and Kastin (1993). Under urethane anesthesia (2.25 g/kg i.p.), the right jugular vein and the left carotid artery were exposed. [14C]AIT-082 (1 μCi/animal) and 125I-albumin (0.25 μCi/animal) were coadministered via the jugular vein in a volume of 160 μl of PBS. At various times thereafter, brain and blood from the carotid artery were collected. Radioactivity in the weighed brain and 50 μl of serum was measured with an LS6500 liquid scintillation counter (Beckman Instruments, Fullerton, CA). The brain/serum ratios for [14C]AIT-082 and 125I-albumin were calculated and plotted against exposure time (Blasberg et al., 1983; Patlak et al., 1983; Banks and Kastin, 1993).

**In Vivo Stability of [14C]AIT-082 in Brain and Serum.** Mice were anesthetized with 2.25 g/kg urethane. [14C]AIT-082 in PBS (10 μCi/mouse) was injected into the jugular vein in a volume of 160 μl. At various times after injection, blood was collected from the inferior vena cava and the animal was perfused through the left ventricle with 30 ml of ice-cold PBS. Thereafter, the brain was removed. Whole brain was homogenized in 300 μl of ice-cold water and the homogenate was diluted with a further 600 μl of water before centrifugation for 15 min at 12,000 g at 4°C; the supernatant was retained. A 100-μl aliquot of serum was diluted with 300 μl of ice-cold water. To 500 μl of brain supernatant and 400 μl of diluted serum, 1000 and 800 μl of 2:1 chloroform:methanol were added, respectively. The samples were vortexed for 15 s and then centrifuged for 10 min at room temperature. The upper aqueous phase was lyophilized, resuspended in 160 μl of water, and filtered before reversed phase HPLC analysis.

**Processing controls were obtained by adding 3 × 10^4 dpm [14C]AIT-082 in serum and brain was resolved with a 250-× 4.6-mm, 5-μm ODS reversed phase column (Phenomenex, Torrance, CA) coupled to a 30- × 4.6-mm guard column. The solvent system used was 0.2% o-phosphoric acid in water and acetonitrile. [14C]AIT-082 and metabolites were eluted from the column with a 10 to 12% gradient over 20 min followed by a 12 to 65% gradient over 5 min. With this method, both AIT-082 and [14C]AIT-082 elute at 19 to 20 min. Fractions were collected at 1-min intervals for 25 min and the radioactivity in each fraction measured.**

Processing controls were obtained by adding 3 × 10^4 dpm [14C]AIT-082 to brain or serum samples in vitro and processing as described above. These controls were used to correct in vivo data for degradation that occurred during processing.

**Capillary Depletion.** These experiments were conducted with the method of Triguero et al. (1990) as modified by Gutierrez et al. (1993). [14C]AIT-082 (10 μCi/animal) and 125I-albumin (1 μCi/animal) were administered as described for influx experiments. At various times after administration, blood was collected from the inferior vena cava and the animal was perfused through the left ventricle with 30 ml of ice-cold PBS. The mouse was decapitated immediately, and the brain was dissected on ice to collect the cerebral cortex. Cortex was weighed and homogenized in 0.7 ml of ice-cold capillary buffer (10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl_2, 1...
26% dextran (1.7 ml) was added and the homogenate centrifuged at
5400g for 15 min at 4°C in a Beckman TL-100 centrifuge with a
TLS-55 swinging bucket rotor. The pellet, containing the capillaries,
and the supernatant, containing brain parenchyma, were separated
and radioactivity in each compartment was measured with a liquid
scintillation counter (LS6500; Beckman, Fullerton, CA).

Pharmacokinetic Analysis. AIT-082 at 60 mg/kg was adminis-
tered i.p. to 2- to 3-month-old mice. At various times thereafter,
trunk blood was collected into heparinized tubes and plasma was
prepared. Plasma AIT-082 concentration was measured by HPLC as
described above.

Microdialysis. The surgical procedures were divided into two
parts. Initially, mice were anesthetized with a ketamine/xylazine
cocktail (ketamine 150 mg/kg; xylazine 10 mg/kg, 10 ml/kg i.p.) and
placed in a stereotaxic apparatus (ASI Instruments, Eugene, OR). A
sagittal incision was made over the skull and a small hole drilled to
expose the dura on the right side to allow for implantation of a guide
cannula for the subsequent insertion of a precalibrated concentric
microdialysis probe (CMA, Acton, MA) into the right cortex (coordi-
nates: AP, 1.94 mm; LR, 2.00 mm relative to bregma; DV, 1.00 mm
relative to the dura surface). Two additional burr holes were made
for skull screws and the guide cannula (CMA) was secured using
epoxy glue. After surgery, animals were returned to a home cage and
allowed to recover overnight.

After recovery, mice were removed from their home cage and
placed into a Plexiglas rodent dialysis box. A concentric dialysis
probe was inserted unilaterally into the cortex, to a depth of 2 mm
below the end of a guide cannula. The probe was 0.24 mm in diam-
eter and had 2 mm of active membrane exposed at the tip. The probe
was perfused with artificial cerebrospinal fluid [145 mM NaCl, 2.7
mM KCl, 1.0 mM MgCl2(6H2O), 1.2 mM CaCl2(2H2O), 2.0 mM
NaHPO4, pH 7.4, with 85% H3PO4], at a rate of 1.5
ml/min with a
Harvard infusion pump (Harvard Apparatus, Holliston, MA), for 2
h to allow stabilization of injury-mediated neurotransmitter release
(Benveniste and Huttemeier, 1990). Two 20-min baseline samples
were then collected and AIT-082 (60 mg/kg, dissolved in 0.9% saline)
was then administered i.p. and microdialysate collected every 20 min
for 120 min. The microdialysate was immediately frozen at
−80°C and subsequently the AIT-082 concentration in each sample was
measured with liquid chromatography-tandem mass spectrometry
(LC-MS-MS) described below.

To assess in vitro recovery of AIT-082 by the microdialysis probe,
the probe was placed in a 1 mg/ml solution of AIT-082 and microdi-
alysis was performed. The AIT-082 concentration was measured in
collected samples with the method described below.

The AIT-082 concentration in both in vivo and in vitro microdia-
laysate samples was measured by LC-MS-MS with an LC-MS-MS
column fitted with a Supelcosil LC-ABZ guard column and a Micromass Quattro LC triple quadrupole spectrometer.

Efflux Experiments. These experiments were conducted accord-
ing to the method of Banks et al. (1997) with minor modifications.
[14C]AIT-082 or [3H]sucrose in PBS was injected i.c.v. into mice at 1
mm posterior, 1 mm lateral to Bregma and 3.5 mm in depth relative
to the skull surface, in a volume of 1 µl. After injection and on
withdrawing the needle, there was often back flux of fluid; this was
collected. At various times thereafter, the amount of radioactivity in
brain, back flux, and a 10-µl aliquot of injection mixture was deter-
mined. The amount of radioactivity in brain was corrected for the
back flux and the log of this corrected value (disintegrations per
minute) was plotted against time. The t1/2 was calculated as de-
scribed previously (Banks et al., 1997). To investigate the saturabil-
ity of [14C]AIT-082 efflux from brain, 100- or 600-fold excess unla-
beled K+-AIT-082 was included in the injection mixture.

Fig. 1. Structure of AIT-082 (Neotrofin, leteprinim potassium)

Fig. 2. Influx of [14C]AIT-082 into brain in young Swiss-Webster mice.
[14C]AIT-082 and 125I-albumin were injected i.v. and at various times
thereafter, radioactivity was measured in serum and brain. The brain/
blood ratios for 14C(n) and 125I(V) were plotted against exposure time
(A). The 14C brain/blood ratio was corrected for serum in brain by sub-
tracting the 125I brain/blood ratio and these corrected data were plotted
against the exposure time (B). A linear portion of the curve was identified
and the slope was determined; this gave the rate constant (K) for trans-
port of [14C]AIT-082 across the blood-brain barrier. A representative
of seven experiments is shown.
TABLE 1

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Influx Constant (K_i)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Swiss-Webster</td>
<td>C57BL/6</td>
</tr>
<tr>
<td>Control</td>
<td>0.60 ± 0.20</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>Excess AIT-082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>110-fold</td>
<td>0.40 ± 0.11</td>
<td>0.25 ± 0.08</td>
</tr>
<tr>
<td>650-fold</td>
<td>0.40 ± 0.02</td>
<td>0.24 ± 0.08</td>
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</tbody>
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ND, not determined.
* Aged animals were 13 to 25 months of age.
$ Data shown are mean ± S.E.

Data Analysis. To analyze whether there was a significant effect of age, mouse strain, or excess unlabeled AIT-082 on influx or efflux of [14C]AIT-082, one-way ANOVA was used with a Scheffe’s post hoc analysis.

Results

Figure 1 shows the structure of AIT-082. It has a molecular weight of 365 and an octanol:PBS partition coefficient of 0.0029 (log P is −2.5). Figure 2 shows a representative experiment demonstrating the influx of [14C]AIT-082 into brain of young Swiss-Webster mice. Clearly, the brain/blood ratio of [14C]AIT-082 was increased above that for 125I-albumin (Fig. 2A). The brain/blood ratio for 125I-albumin showed little change over time, whereas the brain/blood ratio of [14C]AIT-082 increased over time. When [14C]AIT-082 uptake into brain was corrected for blood content of brain, it was evident that the levels of [14C]AIT-082 in brain increased rapidly over the first 5 to 10 min of exposure time, thereafter reaching a plateau (Fig. 2B). The variability was large in the influx experiments and this was presumably due to the low influx rate. However, there was convincing evidence that uptake across the blood-brain barrier occurred and that a linear uptake phase existed. For consistency, the linear portion of the curve was identified with time points up to and including 5 min of experimental time in all experiments. Due to the large variability in the data, the r^2$ value for many of the fitted lines was very low. To assess whether there was a statistically significant linear uptake phase, the data in each treatment group were averaged and a one-sample t test performed to assess whether the mean slope was significantly different from zero. The mean slope was significantly different from zero in all animal and treatment groups (P < .05).

The K_i for uptake of [14C]AIT-082 into brain of Swiss-Webster mice was 0.6 ± 0.2 μl g^{-1} min^{-1} (Table 1). Figures 3 and 4 show that the influx of [14C]AIT-082 into brain of both young (Fig. 3) and aged (Fig. 4) C57BL/6 mice was qualitatively similar to that in young Swiss-Webster mice. The K_i for uptake was 0.35 ± 0.05 and 0.33 ± 0.02 μl g^{-1} min^{-1} for young and aged mice, respectively (Table 1). There were no statistically significant differences between the K_i in Swiss-Webster mice and C57BL/6 or between the K_i in young and aged C57BL/6 mice.

The saturability of transfer across the blood-brain barrier was examined by including 110- or 650-fold excess unlabeled AIT-082 in the i.v. administration of [14C]AIT-082. There was no significant effect of 110- or 650-fold excess unlabeled AIT-082 on the K_i in either Swiss-Webster or C57BL/6 mice (Table 1).

To confirm that the uptake data represented uptake of [14C]AIT-082 and not uptake of a 14C-metabolite, the in vivo stability of [14C]AIT-082 was examined. Figure 5 shows that the proportion of 14C that was intact AIT-082 was >90% in serum at all time points examined. It decreased slightly from 100 ± 0.4% at 2 min to 94 ± 2.6% at 60 min. Similarly, the percentage of 14C in brain that was intact [14C]AIT-082 decreased slowly with time from 97 ± 2.9% at 2 min to 88 ± 11.8% at 60 min.

The above-mentioned influx data revealed that AIT-082 is taken up from the circulation by the brain. However, it did not describe whether the AIT-082 was simply sequestered by brain capillary endothelial cells or passed through them to enter the brain parenchyma. Figure 6A demonstrates that at all time points examined, ≥90% of the [14C]AIT-082 detected in brain after perfusion passed into the brain parenchyma, whereas <10% remained in the capillaries. The level of [14C]AIT-082 in brain parenchyma increased over time to a peak at 30 min, after which it declined (Fig. 6B).

The passage of AIT-082 into brain parenchyma was further confirmed with microdialysis. Figure 7 shows the plasma and brain levels of AIT-082 after i.p. administration of 60 mg/kg. The peak in plasma levels (63.4 μg/ml) occurred at 10 min (Fig. 7A). The concentration of AIT-082 in microdialysate of cortical extracellular fluid increased with time to a peak of 114 ± 70.4 ng/ml at 40 ± 3.3 min and then declined (Fig. 7B). Based on the in vitro recovery of the microdialysis probe (9.5 ± 3.2%), the peak concentration of AIT-082 in vivo was 1173.1 ± 740.7 ng/ml or 3.2 ± 2.2 μM. However, caution must be exercised when extrapolating the in vivo concentration from in vitro recovery data because a number of confounding factors might influence in vivo recovery.

Figure 8 shows data from a representative experiment in which the efflux of [14C]AIT-082 and [3H]sucrose was examined after i.c.v. administration. [14C]AIT-082 and [3H]sucrose were cleared from brain exponentially with a t_{1/2} of 20.0 ± 1.0 and 65.9 ± 16.4 min, respectively. The efflux of [14C]AIT-082 from brain was not inhibited by 100-fold molar excess unlabeled AIT-082; however, 600-fold molar excess unlabeled AIT-082 significantly inhibited [14C]AIT-082 efflux (Fig. 9; P < .01). In the presence of 600-fold excess unlabeled AIT-082, the t_{1/2} increased to 35.5 ± 3.6 min. Given that AIT-082 is a potassium salt, the effect of 600-fold excess K^+ was examined. There was no significant effect of excess K^+ on the efflux of [14C]AIT-082 from brain (Fig. 9).

Discussion

The design of drugs that are targeted at the central nervous system is commonly based on pharmacological ap-
approaches to disease mechanisms. However, as stated by others, such drug development programs are at a high risk of failure if the drug does not cross the blood-brain barrier in useful quantities (Pardridge, 1998). As part of a program to develop AIT-082 as a therapy for Alzheimer's disease, we investigated the transport of this purine derivative across the blood-brain barrier. Herein, we present data to support the transfer of AIT-082 across the blood-brain barrier in useful quantities.

Using multiple-time regression analysis, we have demonstrated that AIT-082 crosses the blood-brain barrier and that this transport is not inhibited by up to a 650-fold excess of unlabeled AIT-082. These experiments indicate that AIT-082 crosses the blood-brain barrier by a nonsaturable mechanism. In the absence of a specific, saturable transport mechanism, compounds may cross the blood-brain barrier by leakage or by simple diffusion (Banks and Kastin, 1993). Leakage is demonstrated by the entry of albumin into the brain at a very slow rate of $-1.5 \times 10^{-5}$ ml g$^{-1}$ min$^{-1}$ (Banks and Kastin, 1993). The rate of diffusion of a molecule across the blood-brain barrier is determined by its lipid solubility, hy-
drogen bonding potential, and molecular weight (Audus et al., 1992). If diffusion is the main mechanism by which AIT-082 enters the brain, the rate of entry would be similar to that of a hydrophilic molecule, of similar molecular weight, that is not transported by any saturable mechanism. In experiments not presented herein, we examined the passage of sucrose (mol. wt. 5350) across the blood-brain barrier and showed that the rate of transfer was similar to AIT-082 (10.4 mg/21 min). This supports the suggestion that [14C]AIT-082 crosses the blood-brain barrier by simple diffusion.

The specific activity of [14C]AIT-082 was such that 1 µCi of radioactivity represented 7 µg of compound. Given this high level, there is a possibility that self-inhibition of a transport system may have occurred in the experiments presented. Self-inhibition would effectively reduce the $K_i$ and preclude demonstration of a saturable mechanism by inclusion of excess unlabeled AIT-082. This possibility was investigated with [3H]AIT-082 that had a 12.5-fold higher specific activity (4 Ci/mmol) than [14C]AIT-082; therefore, administration of 1 µCi/animal was equivalent to 90 ng/animal. There was no change in the $K_i$ (data not shown). Thus, there is no evidence that AIT-082 crosses the blood-brain barrier via a saturable mechanism.

It has been demonstrated that the purine hypoxanthine is transported across the blood-brain barrier via a high-capacity, low-affinity transporter in rats (Cornford and Olendorf, 1975; Betz, 1985; Spector, 1987, 1988). The rate of transport of hypoxanthine was 100-fold faster than for the hypoxanthine derivative AIT-082 and was significantly diminished by increasing doses of unlabeled hypoxanthine, adenine, theophylline, and uracil (Spector, 1987). Given that [14C]AIT-082 influx is slower than that of hypoxanthine and is not saturable, it is unlikely that AIT-082 is transported across the blood-brain barrier by the hypoxanthine transporter.

There was no significant difference in either the quantitative or qualitative uptake of AIT-082 into brain between an outbred strain of mouse (Swiss-Webster) and an inbred strain of mouse (C57BL/6), both of which have been commonly used in AIT-082 studies. Nor was there a significant effect of age on the transfer of AIT-082 across the blood-brain barrier. These results are consistent with the literature. Although age induces significant histological and biochemical transference of 

Fig. 5. Stability of [14C]AIT-082 in vivo. [14C]AIT-082 was injected i.v. into young adult mice and at various times serum and brain collected. Extracts of serum and brain were applied to HPLC and fractions collected. Elution of [14C]AIT-082 was monitored by measuring radioactivity in fractions collected at 1-min intervals. A sample elution profile (A) shows that [14C]AIT-082 eluted at 20 min. The percentage of intact [14C]AIT-082 in serum (○) and brain (■) was calculated, corrected for degradation due to processing, and plotted against time (B). Data presented are mean ± S.E. ($n = 7$).
changes in the blood-brain barrier, including loss of endothelial cells and decreased capillary diameter in rats, there is no apparent change in blood-brain barrier permeability to hydrophilic and high molecular-weight substances in healthy, aged animals (Shah and Mooradian, 1997; Banks et al., 1999). In contrast, there may be a change in blood-brain barrier permeability associated with Alzheimer’s disease. A study of sixty-five 85-year-old subjects revealed that in those that had dementia, there was an increase in the cerebral spinal fluid/plasma protein ratio suggestive of an increase in blood-brain barrier permeability (Skoog et al., 1998). This may have implications for the design and administration of compounds, such as AIT-082, which are targeted at the treatment of Alzheimer’s disease and other neurodegenerative disorders.

All of these studies might be subject to complication by the possibility that [14C]AIT-082 is metabolized and that 14C measurements may represent measurement of a 14C-metabolite. Although degradation/metabolism was minimal in brain, it was slightly increased compared with serum and so suggests that there may be some brain-specific degradation/metabolism of [14C]AIT-082. It is known that the brain endothelium is enriched in degradative enzymes, including drug-metabolizing and purine-metabolizing enzymes (Audus et al., 1992). Specifically, adenine deaminase and purine nucleotide phosphorylase are highly enriched in brain capillary endothelial cells (Johnson and Anderson, 1996). It is, however, unlikely that these enzymes are responsible for the slight increase in degradation in brain because it has recently been shown that AIT-082 inhibits the action of these enzymes (Caciagli et al., 1999).

The influx data discussed above demonstrate that [14C]AIT-082 crosses the blood-brain barrier. To confirm the transport of [14C]AIT-082 into brain parenchyma, we used the capillary depletion technique and demonstrated that >80% of the [14C]AIT-082 that remained in brain after per-
fusion was present in the brain parenchyma. This clearly demonstrates that \(^{14}\text{C}\)AIT-082 is not sequestered by endothelial cells and passes into the brain parenchyma where it is presumably available to interact with neurons, astrocytes, and other cells of the central nervous system. \(^{14}\text{C}\)AIT-082 levels in brain parenchyma peaked at 30 min and from these data, the maximal concentration of AIT-082 in cortical extracellular fluid was estimated to be in the low micromolar range. To further confirm the passage of AIT-082 into brain interstitial fluid and to quantify the concentration more accurately, microdialysis experiments were performed. AIT-082 was readily detectable in cortical extracellular fluid and the concentration increased over time to a peak of \(\sim 3\) \(\mu\)M at 40 min. This confirmed the previous estimate. When comparing the data obtained with the two techniques, it is apparent that there was a shift in the time at which peak levels of AIT-082 were detected in parenchyma. Peak levels of \(^{14}\text{C}\)AIT-082 were detected at 30 min with the capillary depletion technique, whereas peak levels of AIT-082 were detected at 40 min with the microdialysis technique. This shift is likely due to the shift in peak plasma levels from 0 min, when \(^{14}\text{C}\)AIT-082 was given i.v. (capillary depletion), to 10 min, when AIT-082 was administered i.p. (microdialysis); Fig. 7). The apparent parallel between plasma and brain AIT-082 concentrations further supports the suggestion that AIT-082 crosses into brain by a nonsaturable mechanism.

The concentration of AIT-082 that has been used in in vitro studies ranges from 1 to 100 \(\mu\)M. At these concentrations, AIT-082 stimulates neurite outgrowth and an increase in extracellular neurotrophic factors (Middlemiss et al., 1995; Caciagli et al., 1998, 1999; Juurlink and Rathbone, 1998; Caciagli et al., 1998, 1999; Bintner et al., 1999; Cacciarelli et al., 1999). In vivo, 60 mg/kg AIT-082, administered i.p., ameliorates NMDA-induced (Caciagli et al., 1998; Di Iorio et al., 1999) and kainic acid-induced neurodegeneration in striatum and hippocampus (Di Iorio et al., 1999), and the neurodegeneration and motor deficits caused by spinal cord crush injury (Middlemiss et al., 1999). Given that the concentration of AIT-082 in brain reaches low micromolar concentrations when 60 mg/kg AIT-082 is administered i.p., these data suggest that there is parity between the in vitro and in vivo studies of AIT-082 neurotrophic properties.

The fact that uptake of AIT-082 into brain rapidly reached a plateau (Fig. 2–4) suggests that equilibrium was reached between the nonsaturable influx of AIT-082 and efflux of AIT-082 from brain to blood. Indeed, after i.c.v. administration, \(^{14}\text{C}\)AIT-082 was transported out of brain with a \(t_{1/2}\) of \(\sim 20\) min. In contrast \(^{3}\text{H}\)sucrose was transported out of brain with a \(t_{1/2}\) of \(\sim 60\) min. This is similar to the efflux of albumin (Banks et al., 1997) and suggests that sucrose is transported out of brain via passive mechanisms, including cerebral spinal fluid reabsorption. The faster rate of efflux of \(^{14}\text{C}\)AIT-082 from brain, compared with sucrose, and the fact that efflux was inhibited by 600-fold excess unlabeled AIT-082 indicate that AIT-082 is transported out of brain by an active mechanism.

In conclusion, AIT-082, a hypoxanthine derivative, crosses the blood-brain barrier and is available to astrocytes and neurons at significant concentrations. Together with the demonstration of an active efflux mechanism, the results suggest that there are two pools of AIT-082; one that is transported into brain parenchyma and one that is rapidly returned to the circulation via a specific, saturable efflux mechanism.

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### References


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