Differential Transport of a Secretin Analog across the Blood-Brain and Blood-Cerebrospinal Fluid Barriers of the Mouse

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ABSTRACT
Secretin is a gastrointestinal peptide belonging to the vasoactive intestinal peptide (VIP)/glucagon/pituitary adenylate cyclase-activating polypeptide (PACAP) family recently suggested to have therapeutic effects in autism. A direct effect on brain would require secretin to cross the blood-brain barrier (BBB), an ability other members of the VIP/PACAP family have. Herein, we examined whether a secretin analog (SA) radioactively labeled with $^{131}$I could cross the BBB of 4-week-old mice. We found SA was rapidly cleared from serum with fragments not precipitating with acid appearing in brain and serum. Levels of radioactivity were corrected to reflect only intact SA as estimated by acid precipitation. After i.v. injection, SA was taken up by brain at a modest rate of 0.9 to 1.5 $\mu$l/g-mm. Capillary depletion, brain perfusion, and high-performance liquid chromatography were used to confirm the passage of intact SA across the BBB. SA entered every brain region, with the highest uptake into the hypothalamus and cerebrospinal fluid (CSF). Unlabeled SA (10 $\mu$g/mouse) did not inhibit uptake by brain but did inhibit clearance from blood and uptake by the CSF, colon, kidney, and liver. The decreased clearance of SA from blood increased the percentage of the i.v. injected dose taken up per brain (%Inj/g) from about 0.118 to 0.295%Inj/g. In conclusion, SA crosses the vascular barrier and provides a pathway through which peripherally administered SA could affect the CNS.

Secretin was the first hormone to be discovered, its action being elucidated by Bayliss and Starling (1902). It is released into the blood from the proximal small intestine and stimulates the pancreas to secrete bicarbonate and water. Secretin also inhibits gastric acid secretion and may influence the release of other hormones (Strand, 1999). It is a member of a large and important family of hormones that includes vasoactive intestinal peptide, glucagon, growth hormone-releasing hormone, and pituitary adenylate cyclase-activating polypeptide (PACAP). To date, only a single receptor unique to secretin has been cloned (Ishihara et al., 1991).

Secretin was first found in the brain (Mutt et al., 1979; O’Donohue et al., 1981) and shown to have effects on the central nervous system (Ishibashi et al., 1979; Becker et al., 1982; Charlton et al., 1983) over 20 years ago. Brain receptors specific for secretin are coupled to adenylate cyclase and occur in many brain regions (Fremeau et al., 1983, 1986; Yung et al., 2001). The cAMP levels in the hippocampus and hypothalamus especially are stimulated by secretin (Karelsen et al., 1995). Secretin (Strand, 1999) is much more potent in stimulating pancreatic secretion when given directly into the brain than when given intravenously (Cotner et al., 1996). Additionally, secretin may affect cerebellar neural transmission and stabilize Purkinje cells (Yung et al., 2001).

Recently, secretin has been suggested to have beneficial effects in autism. Autism is characterized by a markedly abnormal or impaired development in social interactions and communication that begins in childhood. Autistic children also tend to have gastrointestinal complaints. Autism has strong but complex patterns of inheritance, with the distal q region of chromosome 7 likely involved (Wassink and Piven, 2000). A spectrum of disease is thought to occur, with autism being the most severe form and Asperger’s syndrome a much milder form. In an open trial, three children were noted to have a significant improvement in gastrointestinal symp-

**ABBREVIATIONS:** PACAP, pituitary adenylate cyclase-activating polypeptide; BBB, blood-brain barrier; CNS, central nervous system; SA, secretin analog; HPLC, high-performance liquid chromatography; I-SA, secretin analog radioactively labeled with $^{131}$I; T-Alb, albumin radioactively labeled with $^{99m}$Tc; $K_i$, unidirectional influx rate; $V_i$, initial volume of distribution within brain; %Inj/g, percentage of the intravenously injected dose present in 1 g of brain; PE, polyethylene; CSF, cerebrospinal fluid.
tomato and social interaction skills within 5 weeks of a single secretin infusion of 0.4 μg/kg (Horvath et al., 1998). Controlled trials have found no improvement 3 weeks (Dunn-Geier et al., 2000) and 4 weeks (Sandler et al., 1999; Chez et al., 2000) after a single infusion of secretin. A double-blind, placebo-controlled trial in which multiple doses of secretin were given has detected an improvement in the social interactions of 3- to 4-year-old autistic children (M. Goulet, J. R. Rushe, R. Boismenu, unpublished observations).

The putative effects of secretin on autism could be mediated through actions on peripheral tissues or by affecting brain function. Peripherally circulating hormones can affect brain function indirectly through several mechanisms, such as affecting afferent nerve transmission or releasing other centrally active substances. Little or no secretin is made in the brain (Kopin et al., 1990; Yung et al., 2001). This suggests that brain secretin is of peripheral origin, but this would require secretin to cross the blood-brain barrier (BBB). Other members of the secretin family have effects on the CNS (Kastin et al., 2001) and can cross the BBB. PACAP, for example, crosses the BBB by way of a saturable transport system termed peptide transport system-6 (Banks et al., 1993). About 0.11% of an intravenous dose of PACAP crosses the BBB by way of peptide transport system-6 to enter each gram of brain. This is an amount sufficient to reverse hippocampal neuronal loss caused by four-vessel occlusion when PACAP is given systemically within 24 h after the ischemia (Uchida et al., 1996). Furthermore, peripherally administered secretin can induce Fos expression in distinct brain regions in the rat (Goulet et al., 2001).

The ability of related peptides to cross the BBB raises the possibility that secretin analog (SA) might also be able to cross. This would provide a mechanism by which the peptide could exert effects on the brain. Herein, we examined the ability of an SA to cross the BBB of mice.

Materials and Methods

Synthesis and Radioactive Labeling

The analog [Tyr[10]]secretin-27 (SA) was synthesized by solid-phase methods and purified to homogeneity by HPLC (tyrosine substituted for leucine). SA was radioactively labeled with the method previously used to characterize its receptor binding activity (Dong et al., 1999). In brief, iodo-bead (Pierce Chemical, Rockford, IL) was incubated with Na[131]I for 10 s, and the mixture was purified on a C18 column with reverse-phase HPLC. Specific activity of the radioactively labeled SA (I-SA) was 165 Ci/mmol, assuming a 20% counting efficiency. Amounts of radioactivity injected below are not corrected for degradation of I-SA that occurred ex vivo (processing controls), 100 μl of I-SA in lactated Ringer’s and BSA solution was placed on the surface of a nonradioactive mouse brain or in a tube used to obtain carotid blood and the samples processed as described above. The percentage of radioactivity precipitated by acid was calculated as the percentage of total counts per minute (supernatant + pellet counts per minute) found in the pellet. Results for the brain and blood samples obtained after i.v. injection of I-SA were expressed as a percentage of the results for the processing controls. Brain and serum levels of radioactivity used in pharmacokinetic analyses were corrected to reflect only the portion that could be precipitated by acid.

Multiple Time Regression Analysis. Mice were anesthetized and the right carotid artery and left jugular vein isolated and exposed. Lactated Ringer’s (0.2 ml) containing 106 cpm of I-SA was injected into the jugular vein. To test for saturation, some mice had 10 μg of unlabeled SA included in the injection. Brain and serum samples were obtained 1 to 10 min later, as described above, and counted in a gamma counter, and the results were corrected to reflect only that portion precipitated by acid. The unidirectional influx rate (K1, expressed in units of micromolars per gram-minute) and the apparent volume of distribution at time 0 (Vc, expressed in units of micromolars per gram) were determined from the following equation.

\[
\frac{\text{Am/Cpt}}{\text{Inj/ml}} = K_1 \left[ \int_0^t \frac{\text{Cp(r)d}r}{(\text{Cpt})^2} \right] + V_c
\]

where Am is counts per minute per gram of brain, Cpt is counts per minute per milliliter of arterial serum at time t, and exposure time (Expt, expressed in minutes) is measured by the term \[\int_0^t \frac{\text{Cp(r)d}r}{(\text{Cpt})^2}\]. Only the linear portion of the relation between Am/Cpt and Expt was used to calculate K1 and Vc.

Clearance from Blood. The percentage of the intravenously injected dose of I-SA in a milliliter of arterial serum (%Inj/ml) was calculated from the following equation:

\[
\frac{\text{Inj/ml}}{100} = \frac{\text{Inj/mL}}{\text{Inj/Cpt}}
\]

where Inj is the total counts per minute per gram of brain. The log of %Inj/ml was plotted against time and the half-time disappearance calculated from the slope, and the volume of distribution was calculated from the intercept of the resulting line.

Percentage of Injected Dose Taken Up by Brain. The percentage of the intravenously injected dose of I-SA taken up by each gram of brain (%Inj/g) was then calculated from the following equation:

\[
\frac{\text{Inj/g}}{10} = \text{Am/Cpt} - V_c^{10} \times \% \text{Inj/ml}
\]

where Vc corrects the brain/serum ratio for the amount of I-SA in the vascular space.

Uptake by Various Tissues. Mice were anesthetized as described above, the left jugular vein and right carotid artery exposed, and 0.2 ml of lactated Ringer’s solution containing 106 cpm of I-SA...
and of T-Alb injected into the jugular vein. Five minutes later, arterial blood was collected from the carotid artery and the mouse immediately decapitated. The kidney, spleen, testes, right and left ventricle of the heart, stomach, duodenum, jejunum, ileum, colon, adrenal gland, pancreas, and whole brain (free of pituitary and pineal), and pieces of lung, liver, and thigh muscle were collected and weighed. The gastrointestinal tract organs were opened lengthwise, their luminal contents washed out, and the tissues padded dry before weighing. The level of radioactivity in the serum and tissue samples was determined in a gamma counter. The results are expressed as (counts per minute per gram of tissue)/(counts per minute per microliter of serum) = microliter per gram. Acid precipitation was performed on the adenral, kidney, and colon and on processing controls for those tissues.

Capillary Depletion

In other mice (n = 4), the relative distribution of I-SA between the cerebral cortex and capillaries was assessed by the method of Triguero et al. (1990) as modified for mice by Gutierrez et al. (1993). After anesthesia, mice received an injection into the jugular vein of 0.2 ml of lactated Ringer’s solution containing 1% bovine serum albumin containing 1 × 10⁶ cpm of I-SA and 1 × 10⁶ cpm of T-Alb. Five minutes later, the abdomen was opened, and blood was collected from the abdominal aorta. The thorax was opened, the thoracic descending aorta clamped, the left and right jugular veins severed, and the brain flushed of its intravascular contents by injecting 20 ml of lactated Ringer’s solution over 1 min into the left ventricle of the heart. The mouse was decapitated and the brain harvested. The cerebral cortex was isolated and weighed and placed in ice-cold physiological buffer (10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl₂, 1 mM MgSO₄, 1 mM Na₂HPO₄, and 10 mM D-glucose adjusted to pH 7.4). The cortex was then homogenized using a glass tissue grinder (10 strokes) in 0.8 ml of physiological buffer. Dextran solution, 1.6 ml of a 26% solution in physiological buffer, was added to the homogenate, mixed vigorously, and homogenized (three strokes). The homogenate was centrifuged at 5400 g for 15 min at 4°C in a swing bucket rotor. The pellet, which contains the brain vasculature, and the supernatant, which contains the brain parenchyma, were carefully separated and the radioactivity of each component determined using a gamma counter. The parenchyma/supernatant ratio was calculated by the following equation:

\[ \text{Ratio} = \frac{\text{cpm Fr}}{w} \frac{\text{cpm/μl serum}}{\text{cpm/μl serum}} \]  

where cpm Fr is the counts per minute in either the parenchyma or supernatant fraction, w is the weight of the cortex, and cpm/μl serum is the level counts per minute in a microliter of serum.

Brain Perfusion

Mice were anesthetized, the thorax opened, and the heart exposed. Both jugulars were severed and the descending thoracic aorta was clamped. A 26-gauge butterfly needle was inserted into the left ventricle of the heart and Zlokovic’s buffer (7.19 g/l NaCl, 0.3 g/l KCl, 0.28 g/l CaCl₂, 2.1 g/l NaHCO₃, 0.16 g/l KH₂PO₄, 0.17 g/l anhydrous MgCl₂, 0.99 g/l D-glucose, and 10 g/l bovine serum albumin added the day of perfusion) containing 10⁶ cpm/ml was infused at a rate of 2 ml/min for 1 to 5 min (Zlokovic et al., 1988). This rate of perfusion quickly fills the brain’s vascular space without disrupting the BBB (Shayo et al., 1996). An injection check of 10 μl of the buffer solution was taken before and after perfusion, so exact concentration of radioactivity could be calculated. After perfusion, the needle was removed, and the mouse was decapitated. Acid precipitation was performed on the brains and the counts per minute per whole brain corrected accordingly. Brain/perfusion ratios were calculated by dividing the counts per minute per brain by the weight in grams of the brain and by the counts per minute in a microliter of perfusion fluid to yield units of microliter per gram. Influx rate (Kᵢ) was determined from the slope of the relation between brain/perfusion ratios and time in minutes.

Stability of I-SA

HPLC Analysis. Mice were anesthetized and I-SA (3 × 10⁶ cpm/mouse) injected into the left jugular vein in a volume of 0.2 ml of lactated Ringer’s solution. Serum from the carotid artery and the whole brain were obtained 2, 5, or 8 min later. Each whole brain was mechanically homogenized in 2 ml of water containing 0.25 mM each of EDTA, l-thyroxine, N-ethylmaleimide, and 1,10-phenanthroline, and the homogenate centrifuged at 5000 g for 10 min at 4°C. The samples were stored at −70°C until assay. Samples were separated by reversed-phase HPLC on a C₈ column. Mobile phase (acetonitrile with 0.1% trifluoroacetic acid) was against water with 0.1% trifluoroacetic acid and increased from 0 to 60% in 60 min. Fractions were collected every minute for 60 min and counted on the gamma counter. Processing controls were also analyzed and used to correct results as described above for acid precipitation.

Octanol/Buffer Partition Coefficient. The lipid solubility of I-SA was measured by determining its octanol/buffer partition coefficient. I-SA (10⁶ cpm) was added to 0.5 ml of 0.25 M chloride-free phosphate-buffered solution and 0.5 ml of octanol. This was vigorously mixed for 1 min and the two phases separated by centrifugation at 4500 g for 10 min. Aliquots of 100 μl were taken in triplicate from each phase and counted. The mean partition coefficient was expressed as the log of the ratio of counts per minute (octanol phase) to counts per minute (phosphate-buffered saline phase).

Entry into Cerebrospinal Fluid. Mice were anesthetized, prepared as described above, and given an injection into the jugular vein of 10⁶ cpm of I-SA with or without 10 μg/mouse of unlabeled SA. Five minutes later, the scalp was removed from the posterior aspect of the head, exposing the muscles overlying the posterior fossa. A 30-gauge needle connected to a length of PE-10 tubing was inserted into the posterior fossa with the head in a dependent position. CSF was collected into the PE tubing by capillary action. After collecting about 10 μl of CSF, the tubing was removed, arterial blood collected from the previously exposed carotid artery, the mouse decapitated, and the whole brain removed. The exact amount in microliters of CSF collected was determined by measuring the length in centimeters of PE tubing filled with CSF and multiplying by 0.668. Only CSF that was absolutely clear was analyzed. The CSF, brain, and serum were counted in a gamma counter. Acid precipitation was performed on CSF, brain, and serum and each sample’s level of radioactivity (I) were corrected. The results were expressed as brain/serum (microliters per gram), CSF/serum (microliters per milliliter), and brain/CSF (milliliters per gram) ratios.

Uptake into Brain Regions. Mice were anesthetized, prepared as described above, and given an i.v. injection of 10⁶ cpm of I-SA. Five minutes later, arterial serum was obtained from the carotid artery, and the brain was removed and dissected into 11 regions after the manner of Glowinski and Iversen (1966). Each region was weighed, and its level of radioactivity was determined in a gamma counter. The results were expressed as (counts per minute per brain region)/(regional wt in grams) (counts per minute per microliter of serum) = microliter per gram of brain region.

Statistics

Means are reported with their standard errors and the number (n) per group. Two group comparisons were performed using Student’s two-tailed, unpaired t test with comparisons considered significant at p < 0.05 level. When more than two means were compared, analysis of variance was performed followed by Newman-Keuls post test. Regression lines were calculated by the least-squares method with the Prism 3.0 program (GraphPad Software, San Diego, CA). The slope (Kᵢ) with its error term, the intercept (Y₀) with its error term, the regression coefficient (r), the n value, and the p value are...
reported. Regression lines were compared for statistically significant differences with the Prism 3.0 program, which first compares slopes, and if there is no statistically significant differences ($p > 0.05$), then compares intercepts.

**Results**

Stability of I-SA in brain and serum after i.v. injection was first determined. The portion of radioactivity in serum and brain precipitated by acid decreased with time (Fig. 1). These values were not different in mice that received unlabeled SA (data not shown). These results were used to correct the brain and serum values of the other experiments. HPLCs of radioactivity recovered from processing controls and from brain and serum 5 min after i.v. injection of I-SA are shown in Fig. 2. Table 1 shows the summary of HPLC results for brain and serum over time. In general, acid precipitation overestimated degradation of I-SA, especially in brain.

The top panels of Fig. 3 show the results of multiple-time regression analysis for I-SA. The top left panel shows an experiment measuring the unidirectional influx rate of I-SA at $0.949 \pm 0.0388 \mu l/g$-mm. The top right panel shows that unlabeled SA ($10 \mu g/mouse$) did not inhibit the influx of I-SA; the influx rate for I-SA was $1.53 \pm 0.221 \mu l/g$-mm and for I-SA + SA was $1.50 \pm 0.046 \mu l/g$-mm. Therefore, the dominant mechanism of passage across the vascular BBB is likely the nonsaturable process of transmembrane diffusion. The log of the octanol/buffer partition coefficient was $-2.27$. Capillary depletion showed that over 90% of the I-SA taken up by brain had entered the parenchymal space (Table 2).

Unlabeled SA did saturate the clearance of I-SA from blood, decreasing the volume of whole body distribution from 8.4 to 1.97 ml (Fig. 3, bottom left). The half-time disappearance of I-SA was 3.58 min. The increase in serum levels resulting from the injection of SA means that more of the injected dose is presented to the brain. As a result, brain uptake increased (Fig. 3, bottom right). The results for %Inj/g were fitted to a one-site binding model (hyperbola). This model gave a maximum uptake of 0.118%Inj/g in the absence of SA and a maximum uptake of 0.295%Inj/g in its presence;

![Fig. 1. Relation between time and the log of the percentage of radioactivity extracted from brain or serum that could be precipitated with acid. The amount of radioactivity that could be precipitated decreased with time in both serum and brain.](image1)

![Fig. 2. Representative HPLCs for serum and brain. Results for radioactivity extracted 5 min after i.v. injection of I-SA and for processing controls is similar.](image2)

### Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Serum</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>86.5 ± 0.3 (2)</td>
<td>70.1 ± 4.3 (2)</td>
</tr>
<tr>
<td>5</td>
<td>50.4 ± 16.6 (2)</td>
<td>107 ± 49.0 (2)</td>
</tr>
<tr>
<td>8</td>
<td>68.6 (1)</td>
<td>80.8 ± 15.9 (2)</td>
</tr>
</tbody>
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half of this maximum value was reached 1.8 to 1.9 min after i.v. injection.

The unidirectional influx rate measured during brain perfusion was $2.73 \pm 0.69 \mu l/g-mm$ (Fig. 4, top). Acid precipitation showed that about 70% of the radioactivity from blood was precipitated by acid with no change in the amount of material degraded over time. HPLC, however, found less degradation on average than with processing controls. A representative HPLC for radioactivity extracted from brain after 5 min of perfusion is shown in the bottom panel of Fig. 4.

Figure 5, top, shows that I-SA entered the CSF. All results were corrected for acid precipitation, including those for I-SA in CSF and for T-Alb in brain, serum, and CSF. All CSF samples were clear without evidence of blood. Acid precipitation of I-SA gave results similar to those described above. For CSF, about 90% of the $^{131}$I radioactivity precipitated with acid, indicating that it was largely I-SA. Similarly, about 90% of the Tc radioactivity found in brain and serum was precipitated by acid, but only 49% of the Tc radioactivity in CSF precipitated. The CSF/serum ratio was much higher for I-SA than for T-Alb, demonstrating an uptake not accounted for by leakage, extracellular pathways, or traumatic tap. For comparison, the brain/serum ratios from the same mice are shown. The CSF/brain ratio after subtracting the T-Alb values from both compartments was 16.0, indicating that I-SA enters the CSF much more rapidly than the parenchyma.

The bottom panel of Fig. 5 shows the effects of including 10 $\mu g$ of unlabeled SA in the injection. Unlabeled SA had no effect on the T-Alb brain/serum or CSF/serum ratios (data not shown), and these values have been subtracted from the I-SA ratios shown in the bottom panel of Fig. 5. A t test showed that the CSF/serum ratio for I-SA was significantly decreased by unlabeled SA ($p < 0.01$). There was no effect on the brain/serum ratio. Inclusion of unlabeled SA decreased the CSF/brain ratio to 0.2.

Figure 6 shows uptake into various brain regions. The
highest uptake was into the hypothalamus and the slowest was into the frontal cortex.

Figure 7, top, shows the distribution of I-SA among 15 tissues (n = 5). These results have been corrected for vascular space by subtraction of T-Alb. The bottom panel shows those tissues for which inclusion of unlabeled SA (n = 3) decreased the tissue/serum ratio for I-SA; they have also been corrected for vascular space. A trend (p < 0.10) toward a decrease was noted for heart, jejunum, pancreas, testis, and thigh muscle. Inclusion of unlabeled SA did not affect the T-Alb tissue/serum ratio for any tissue except kidney (p < 0.05), which decreased from 160 ± 15 (n = 5) to 97 ± 11 (n = 3). There were no trends toward a decrease for any of the other T-Alb tissue/serum ratios. The percentage of extracted radioactivity that was precipitated by acid was, after correction with processing controls, 97 ± 2% for the adrenals, 32 ± 3% for the colon, and 23 ± 2% for kidney (n = 2/tissue).

**Discussion**

These results show that I-SA completely crosses the BBB as an intact molecule at a modest rate. The amount of I-SA taken up by the brain after i.v. injection is in the range seen for other peptides and regulatory proteins that affect brain function by crossing the BBB. I-SA entered all regions of the CNS with uptake by the CSF, hypothalamus, hippocampus, and olfactory bulb being particularly high. Uptake into the CSF but not by brain was inhibited by 10 μg/mouse of unlabeled SA, strongly suggesting that this peptide crosses the choroid plexus by a saturable transport system and crosses the vascular BBB by transmembrane diffusion. Clearance from blood and uptake by some peripheral tissues was inhibited by the 10-μg dose of SA, resulting in increases in serum levels, which, in turn, increased the percentage of the injected dose entering the brain. These findings support the hypothesis that peripherally administered SA can affect CNS function. These findings and their implications are discussed in detail below.

I-SA injected intravenously crossed the BBB with a unidirectional influx rate (Kᵢ) of about 1 to 1.5 μl/g-mm. In all of these studies, we used mice that were 3 to 4 weeks of age. This precedes puberty in mice and so parallels the use of secretin in childhood autism. By 21 days of age, the BBB in mice and rats has largely matured and has a permeability to serum albumin similar to that of adult rodents (Davson and Segal, 1996). Brain and serum levels of radioactivity had to be corrected for accumulation of degradation products in

![Graph](image-url)

**Fig. 4.** Brain perfusion studies for I-SA. Top, Kᵢ as measured by brain perfusion was 2.73 ± 0.69 μl/g-mm. Bottom, HPLC of radioactivity extracted from brain at end of 5-mm perfusion. Proportion of radioactivity eluting as I-SA was higher than found for processing controls (compare with Fig. 2, top right). Overestimation of degradation products by processing controls can occur when degradation products are cleared in vivo from brain.

**Brain Perfusion**

![Graph](image-url)

**Fig. 5.** Top, uptake of I-SA into brain and CSF. T-Alb is included for comparison, which for CSF measures entry by extracellular pathways or from BBB disruption and for brain measures vascular space. The uptake rate into the CSF compartment was about 16 times faster than uptake by brain. Bottom, effect of including unlabeled SA in the i.v. injection of I-SA on CSF/serum and brain/serum ratios. The unlabeled SA inhibited uptake of I-SA into CSF but not into brain.
these compartments. Acid precipitation, which can be a reliable estimate of the amount of radioactivity representing intact peptide, was used to make these corrections. In this case, however, acid precipitation underestimated the amount of intact peptide, especially at later time points for brain. This may have led to an underestimation of $K_i$ by as much as 2-fold.

To negate the effects of degradation in the circulation, we measured $K_i$ by brain perfusion and found that uptake was indeed higher, with a value of $2.73 \mu g/\text{g-mm}$. Brain perfusion sometimes produces higher $K_i$ values when a transporter is partially saturated or when uptake by brain is retarded by binding to serum proteins. The $K_i$ from the brain perfusion experiment was also corrected based on acid precipitation, which indicated that about 70% of the radioactivity in brain was intact I-SA. HPLC showed little or no degradation in brain after correction by processing control, and so $K_i$ may have been underestimated by about 30%. The $K_i$ for I-SA, therefore, likely is between 2 and 3 $\mu g/\text{g-mm}$.

Capillary depletion and CSF sampling showed that I-SA completely crossed the BBB to enter the CNS. The CSF/serum ratio was about 16 times higher than the brain/serum ratio. Because the time between injection and sampling of brain and CSF was short, the results suggest that I-SA crossed at the choroid plexus more rapidly than at the brain vasculature.

I-SA uptake by brain was not inhibited by a dose of 10 $\mu g$/mouse, or about 500 $\mu g$/kg. It is possible that a higher dose may have unmasked saturable transport, but it exceeds the dose used in autism of about 0.4 $\mu g$/kg. The 10-$\mu g$/mouse dose was also sufficient to saturate clearance from blood and uptake by the colon, kidney, and liver. Therefore, it is probable that the major mechanism by which I-SA crosses the vascular BBB is by transmembrane diffusion. The log of the octanol-buffer partition coefficient for I-SA was $(-1.27)$, which is in the lower range for peptides (Banks and Kastin, 1985).

I-SA uptake by CSF exceeded that of T-Alb, demonstrating that entry into the CSF was not caused by residual leakage of serum proteins. Only absolutely clear CSF was used in these studies, and the large difference between CSF/serum ratios for I-SA and T-Alb confirms that the results are not due to traumatic taps. The CSF/serum ratio for I-SA was about 16 times greater than the brain/serum ratio, showing that uptake into CSF is much greater than uptake into brain. Unlabeled SA inhibited uptake into CSF but had no effect on brain uptake. Therefore, I-SA is transported into the CSF by a saturable transport system.

When $10^6$ cpm of I-SA was injected intravenously, about 0.118% of the injected dose entered the brain. Because $10^6$ cpm of I-SA was estimated to contain about 43 ng of SA, about 0.051 ng was taken up per gram of brain. Inhibition of clearance resulted in higher serum levels of I-SA; that is, a greater percentage of the injected dose remained in blood longer. This increased presentation to brain and, because blood-to-brain passage was not saturable, resulted in more I-SA entering brain. When 10 $\mu g$ of SA was included in the I-SA intravenous injection, about 0.295% of the injected dose entered the brain, or about 30 ng of SA. This exceeds the $K_D$ of 0.2 nM (0.6 ng/ml for SA with a molecular weight of 3089) for the brain secretin receptor (Fremeau et al., 1983). These
levels of uptake are similar to those for other peptides and regulatory proteins that affect brain function after crossing the BBB. For example, the effect of intravenously administered interleukin-1α on cognition is partially mediated by cytokine, which has crossed the BBB to act within the posterior division of the septum. The uptake also exceeds that for morphine, which is less than 0.02% In/g.

Uptake of I-SA was not uniform throughout the CNS but varied about 6-fold. The areas with the fastest uptake were the hypothalamus and hippocampus and the areas with the slowest were the frontal and parietal cortices. Abnormalities in both these regions, especially the hippocampus, have been reported in autism. The hippocampus and other limbic structures are proportionately smaller in autism (Aylward et al., 1999). Hippocampal neurons tend to be smaller, more tightly packed, and to have less branching of the dendrites (Raymond et al., 1995). The pattern of uptake differs from that of secretin receptor distribution, suggesting that initial passage across the BBB is independent of receptor location (Fremeau et al., 1983). The ability of secretin to stimulate cAMP is, however, highest for the hypothalamus and hippocampus and lowest for the frontal cortex (Karelon et al., 1995).

In conclusion, these studies show that an analog of secretin crosses the BBB to enter the CSF and the parenchymal space of the brain. Entry is at a modest rate and the amount of SA crossing the BBB is sufficient to affect brain function. Entry is most likely to be mediated by nonsaturable transmembrane diffusion at the vascular BBB but by a saturable transporter at the choroid plexus. I-SA entered every region of the brain but uptake was highest for hypothalamus and hippocampus. We conclude that the therapeutic effect of SA seen in autism could be due to its ability to cross the BBB and so act at sites within the CNS.

References


