Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6762–6766, September 1990
Medical Sciences

Permeability of the murine blood–brain barrier to some octapeptide analogs of somatostatin

(transport/cancer/central nervous system/peptides/carrier-mediated)

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ABSTRACT **Analogs of somatostatin are being investigated clinically for the treatment of various malignancies, including brain tumors. We studied the ability of three therapeutically promising radioactively labeled somatostatin octapeptide analogs, RC-160, RC-121, and RC-161, to cross the blood–brain barrier (BBB) after peripheral or central injection. After i.v. injection, intact RC-160 was recovered from the blood and the brain. The entry rates were different for each compound but were generally low. By contrast, entry across the intact BBB increased 220 times when RC-160 was given in a serum-free perfusate. This suggests that some serum-related factor, probably the previously described protein binding or an aggregation-promoting factor, is the main determinant in limiting the blood-to-brain passage of somatostatin analogs. Entry into the brain was not inhibited by the addition of unlabeled analog to the perfusate, showing that passage was probably by diffusion across the membranes that comprise the BBB rather than by saturable transport. By contrast, a saturable system was found to transport peptide out of the central nervous system (CNS). The clearance from the CNS of RC-160 and RC-121, but not RC-161, was faster than could be accounted for by reabsorption of cerebrospinal fluid. Transport of radioactively labeled RC-160 out of the CNS was inhibited by unlabeled RC-160 or somatostatin but was not affected by some other peptides known to cross the BBB by their own transport systems. More than 80% of the radioactivity recovered from the blood after intracerebroventricular injection of RC-160 was eluted by HPLC at the position of the labeled analog, showing that the peptide had crossed the BBB in intact form. Our results indicate the presence of a saturable transport system in one direction across the BBB for some superactive analogs of somatostatin.**

Analogs of the peptide somatostatin hold promise in the treatment of several medical disorders. Two analogs already are currently used in human beings for the treatment of carcinoid and vasoactive intestinal peptide-secreting tumors. Clinical studies and experimental work suggest that somatostatin analogs may find other uses, such as in the treatment of acromegaly, diabetes mellitus (1, 2), and peptic ulcer disease (3). Perhaps the greatest potential for the application of somatostatin analogs is in the treatment of cancer (4, 5). Analogs of somatostatin have been shown to be effective against pancreatic (6), breast (7), bone (8, 9), and prostate (10, 11) cancers. Various brain tumors (meningiomas, astrocytomas, oligodendrogliomas, ganglioneuroblastomas, medulloblastomas) have been shown to have somatostatin receptors (12–16), raising the possibility that somatostatin analogs could be used in the localization and treatment of these tumors.

Several problems impede the therapeutic use of naturally occurring peptides, including poor absorption by the gastrointestinal tract, a short half-life in the circulation, and multiple actions. For use in the central nervous system (CNS), limited passage across the blood–brain barrier (BBB) may also be a factor. Most of these problems have been solved in the case of somatostatin by the development of analogs. Circulating half-life has been increased by the development of enzymatically resistant analogs. In addition, the administration of the analogs in sustained-delivery systems (microcapsules) permits the maintenance of high therapeutic blood levels (6, 10, 17). Many analogs show selectivity in their activities. For example, RC-121 is about 100 times more potent than somatostatin-(1–14) tetradecapeptide in the inhibition of growth hormone release but <5 times more potent in the inhibition of gastric acid release (17, 18).

In contrast, essentially no work has been done to investigate the ability of somatostatin analogs to cross the BBB, although it is known that radioactively iodinated Tyr-somatostatin-(1–14) pentadecapeptide can cross (19). This relationship to the BBB has therapeutic potential both for the development of compounds that cross the BBB to possibly treat some brain tumors and for the development of peripherally active compounds that cross poorly so as to exert minimal side effects on CNS function. Therefore, we investigated three somatostatin analogs that show high binding to somatostatin receptors in rat brain and human brain tumors for their ability to both enter and exit the CNS.

METHODS

Peptides. The somatostatin analogs RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2), RC-121 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2), and RC-161 (Ac-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2) were synthesized by solid-phase methods in our laboratory and were purified by HPLC (17). The analogs were radioiodinated by the chloramine-T method. Peptide (5 μg in 5 μl of 0.01 M acetic acid) was mixed with 40 μl of 0.5 M sodium phosphate buffer and 1 mCi (1 Ci = 37 GBq) of ^{125}I. Chloramine T was added, and 30 sec later the reaction was stopped by adding cystine and diluting the reaction mixture with 0.5 ml of 0.5 M phosphate buffer. The labeled peptide was purified by HPLC with a C_{18}

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Abbreviations: BBB, blood brain barrier; k_i, unidirectional influx rate constant; V_i, functional distribution volume of brain in rapid reversible equilibrium with plasma; $t_{1/2}$, half-time disappearance of analog from the brain; CNS, central nervous system; i.c.v., intracerebroventricular; Tc-Alb, albumin labeled with technitium-99m.

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column (W-Porex 5C18, Rancho Palos Verdes, CA). Albumin was labeled with ^{99m}Tc (Tc-Alb) with kits purchased from Medi-Physics (Paramus, NJ).

Passage from Blood to Brain. A graphical method as reported by Blasberg *et al.* (20) and Patlak *et al.* (21) was used. Male CD-1 mice (20–25 g) purchased from Charles River Breeding Laboratories were anesthetized by i.p. injection of urethane (2 g/kg of body weight), and the right carotid artery and left jugular vein were exposed. The radioiodinated somatostatin analog RC-160, RC-121, or RC-161 was injected as a bolus into the jugular vein in a volume of 0.2 ml of lactated Ringer's solution containing 1% bovine serum albumin. Arterial blood was collected from a cut in the carotid artery at various times between 1 and 20 min after i.v. injection. The whole blood was centrifuged for 10 min at 2300 × *g* at 4°C, and 0.05 ml of serum was assayed for radioactivity in a γ counter (cpm/ml). Mice were immediately decapitated after collection of the arterial blood, and the whole brain (with the pineal and pituitary glands removed) was also assayed in a γ counter (cpm/g). The brain-to-serum ratio of analog concentrations expressed in units of ml/g at time *t* was plotted on the ordinate against the respective exposure time. Exposure time is the ratio of the integration of the arterial serum concentration to a given time *t* divided by the arterial serum concentration at time *t* (21). The slope of the linear portion of the curve relating the brain–blood ratio and exposure time is equal to the k_i (ml/g per min), the unidirectional influx rate constant, and the intercept is V_i, the portion of the measured distribution volume that rapidly and reversibly equilibrates with the plasma (21).

Blood-to-brain passage was also measured with a modified version of the brain perfusion method described by Takasato *et al.* (22). Male Sprague–Dawley rats (Harlan–Sprague–Dawley) weighing 300–350 g were anesthetized with i.p. injection of sodium pentobarbital. The right pterygopalatine artery and the right external carotid artery were ligated. The right common carotid artery was cannulated and ligated proximally. The right half of the brain was then perfused for 60 sec through the common carotid artery at a rate of 4.65 ml/min. As recommended by Takasato *et al.* (22), an additional 8 sec of perfusion time was allowed for the perfusate to reach the brain and fill the vascular bed, so that total perfusion time was 68 sec. The infusion consisted of 123 mM NaCl, 25 mM $NaHCO_3$, 5.5 mM D-glucose, 4.0 mM KCl, 2.5 mM $CaCl_2$, 1.8 mM $MgCl_2$, 1.2 mM KH_2PO_4, 0.68 mM 71-kDa dextran (23), radioiodinated RC-160, and the vascular marker Tc-Alb. Some rats also received an infusion that contained 0.40 mM unlabeled RC-160. Rats were immediately decapitated at the end of the infusion, and the anterior half of the right cerebral cortex was removed, weighed, and assayed in a γ counter set to differentially detect technetium and radioactive iodine. The rate of entry into the brain (PA for capillary permeability × capillary surface area) was determined with the equation:

$$PA = -F \ln[1 - (q/C_{pf}Ft)],$$

where *F* was the flow rate (in ml/min per g) of the infusion, *q* was the amount of RC-160 that had entered the brain (corrected for vascular contamination as assessed with Tc-Alb), C_{pf} was the concentration of RC-160 in the infusate, and *t* was the perfusion time. PA is in units of ml/g per min.

Passage from CNS to Blood. The clearance from the CNS of radioiodinated RC-160, RC-161, or RC-121 was assessed in mice anesthetized with i.p. urethane by injection of lactated Ringer's solution containing 25,000 cpm of peptide and 1% bovine serum albumin into the lateral cerebral ventricle (i.c.v.) as described (24). Brains were removed 2, 5, 10, and 20 min later. The value *A*, the amount of material available for transport at time 0, was estimated in mice given an overdose of urethane. The half-time disappearance of the analog from the brain ($t_{1/2}$) was calculated from the relationship between the logarithm of the cpm per whole brain and time. Transport rate, *T*, in nmol of analog per g of brain per min was calculated with the equation:

$$T = (A - M)C/itw,$$

where *A* is cpm available for transport, *M* is the cpm remaining in the brain of individual mice at the time of decapitation, *C/i* is the nmol/cpm of originally injected material, *t* is the time elapsed between injection and decapitation, and *w* is brain weight in g.

Self inhibition was tested by inclusion of 10 nmol of unlabeled RC-160 in the i.c.v. injection. Other possible inhibitors tested at this dose were somatostatin, arginine vasopressin ([Arg^8]vasopressin), oxytocin, Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), tyrosine, iodotyrosine, and leucine.

Recovery and Identification of Radioactivity. After either i.v. or i.c.v. injection, the radioactivity appearing in the blood and brain was recovered and characterized by HPLC. Serum was collected as described above and mixed with 5 times its volume of 2 M HCl/absolute ethanol, 1:7 (vol/vol). Brains were also placed in this solution (5 ml/1 g) and then homogenized in a Polytron (Brinkmann) at a setting of 6 for 30 sec. These samples were allowed to sit at room temperature for 1–2 hr for further extraction and then were centrifuged at 50,000 × *g* for 30 min. The supernatants were lyophilized, reconstituted in methanol, filtered, and subjected to HPLC. Control samples consisted of brain or blood samples to which RC-160 labeled with ^{125}I was added *in vitro*. These samples were subjected to the extraction procedure and HPLC. The results are expressed relative to these controls.

The iodinated peptides and their radioactive derivatives were separated by HPLC (Waters M6000A) on a C_{18} column. A multislope linear elution was used with solvent mixtures of 0.1% CF_3COOH in water (solvent A) and 0.1% CF_3COOH in 70% aqueous acetonitrile (solvent B). Fractions were collected every 30 sec, and their levels of radioactivity (with the background subtracted) were measured in a γ counter and plotted against time. Since the stock solution of ^{125}I-labeled RC-160 showed elution of a small and a large peak of radioiodinated peptide at about fractions 39 and 43, these fractions were added together for subsequent calculations. These peaks accounted for about 87% of the radioactivity of the standard, and so 0.87 was used as a correction factor in computing the percent of eluted intact peptide in experimental columns.

Miscellaneous Procedures. The effect of aluminum on passage of RC-160 was tested in mice given an i.p. injection of either aluminum chloride (100 mg of elemental aluminum per kg of body weight in a volume of 0.2 ml per mouse) or an equal volume of 0.9% NaCl acidified to match the pH of the aluminum solution. One hour later, the CNS-to-blood passage was determined as described above, and the blood-to-brain passage was assessed by the graphical method.

Means are reported with their standard errors. Statistical comparisons were made by analysis of variance followed by Duncan's multiple range test if there were more than two groups. Regression coefficients were computed by the least-squares method. Statistical comparisons were made between regression lines with the BMDP P1R computer program (BMDP Statistical Software, Los Angeles). Theoretical lines for the disappearance of radioactivity from blood after i.v. injection were generated by computer.

RESULTS

Passage from Blood to Brain. The disappearance of the ^{125}I-labeled somatostatin analogs from blood after i.v. injec-

tion was consistent with distribution in two compartments. The $t_{1/2}$ of the fast phase was 1.05 min for RC-160, 4.6 min for RC-121, and 1.7 min for RC-161, whereas the $t_{1/2}$ for the slow phase was 10.8 min for RC-160, 27.1 min for RC-121, and 12.5 min for RC-161.

The relationship between exposure time and the brain/serum ratio was linear for RC-160 and RC-121 until about 30 min and for RC-161 until about 60 min. These portions of the curves were then used as previously described to compute entry rates (20, 21). The entry rate constant (k_i) of RC-160 was 9.23×10^{-5} ml/g per min with a V_i of 13.7 μl/g; $r = 0.809$ (Fig. 1). The k_i and V_i for RC-121 were 4.01×10^{-5} ml/g per min and 13.6 μl/g ($r = 0.631$), respectively, and for RC-161 they were 23.2×10^{-5} ml/g per min and 16.4 μl/g ($r = 0.921$).

Pretreatment of mice with aluminum injected i.p. increased the k_i for RC-160 by 7.3% and decreased the V_i by 10.6% [no aluminum: $Y = (13.4 \times 10^{-5})x + 0.0161$, $n = 6$, $r = 0.817$; with aluminum: $Y = (14.7 \times 10^{-5})x + 0.0136$, $n = 6$, $r = 0.814$, where Y is the brain–blood ratio in ml/g, x is the exposure time in min, k_i is the slope in ml/g per min, and V_i is the intercept in ml/g]. Comparison of the regression lines showed a tendency towards a statistical difference: $F(2,8) = 4.174$, $P = 0.057$.

By HPLC, 94.2% of the radioactivity recovered from blood 10 min after i.v. injection of ^{125}I-labeled RC-160 was eluted as intact peptide. After 60 min, however, only 5.2% was eluted at the position of ^{125}I-labeled RC-160. For brain tissue uncorrected for vascular contamination, 41.6% of recovered radioactivity was eluted as iodinated RC-160 at 10 min and 36.9% at 60 min.

With brain perfusion, RC-160 was found to have a PA of $(2.03 \pm 0.46) \times 10^{-2}$ ml/g per min [at these levels of entry, PA is equivalent to k_i, see Blasberg *et al.* (20)]. Inclusion of 0.4 mM of unlabeled RC-160 in the infusate did not alter PA significantly: $(2.49 \pm 1.21) \times 10^{-2}$ ml/g per min; $F(1,3) = 0.137$. In brains that were perfused for 8 sec (enough time to fill only the vascular space of the brain with perfusate), only 11% of recovered radioactivity was eluted as intact iodinated RC-160, but after 68 sec of perfusion, 53% of the radioactivity was eluted as intact peptide. After 68 sec of perfusion, 48% of the measured radioactivity would have been derived from the intravascular compartment as assessed by the simultaneously infused Tc-Alb. Correction for intravascular contamination as previously described (23) showed that 91.8% of the material entering the brain was intact ^{125}I-labeled RC-160.

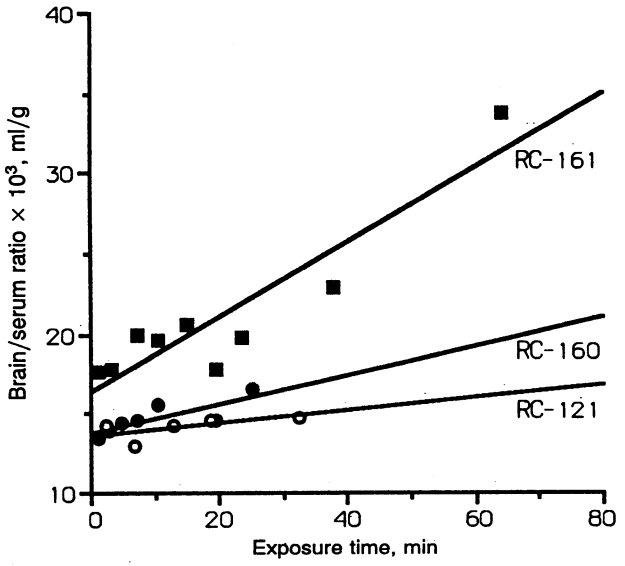

FIG. 1. Entry rates of somatostatin analogs into the brain after i.v. injection. Exposure time was calculated as the integration of the arterial serum concentration to a given time divided by the value of the arterial serum concentration at that time (20, 21). The slope for the linear region of the curves relating exposure time to the brain/serum ratio of analog concentrations at time t is the rate constant for the unidirectional influx (k_i) into the brain in ml/g per min. The k_i values were 4.01×10^{-5} ml/g per min for RC-121 (○), 9.23×10^{-5} for RC-160 (●), and 23.2×10^{-5} for RC-161 (■).

Passage from CNS to Blood. Fig. 2 shows the relationship between the logarithm of cpm remaining in brain after i.c.v. injection (Y) and the time after injection (t) for the three compounds. For RC-160, the equation was $Y = -0.0125t + 4.08$, $r = (-)0.885$, $P < 0.05$ with a $t_{1/2}$ from the brain of 24.0 min. For RC-121, the equation was $Y = -0.0209t + 4.167$, $r = (-)0.994$, $P < 0.01$ with a $t_{1/2}$ of 14.4 min. For RC-161, the equation was $Y = -0.00568t + 4.127$, $r = (-)0.927$, $P < 0.05$ with a $t_{1/2}$ of 53.0 min. A correlation existed between $t_{1/2}$ from the brain and k_i for the three analogs: $t_{1/2} = 2.025\ k_i + 5.87$, $n = 3$, $r = 0.9997$, $P < 0.05$.

Inclusion of 10 nmol of unlabeled RC-160 in the i.c.v. injectate decreased the transport of ^{125}I-labeled RC-160 from the brain to 7.9 ± 11.0% of the control level; $F(1,18) = 42.1$, $P < 0.001$. For the other seven compounds tested (somatostatin, arginine vasopressin, oxytocin, Tyr-MIF-1, tyrosine, iodotyrosine, and leucine), analysis of variance showed a statistically significant effect $F(7,51) = 6.52$, $P < 0.001$. This was due to the effect of somatostatin, which decreased the transport rate to 28.3 ± 22.4% ($P < 0.005$) of the control level and was the only one of the seven compounds that showed a statistically significant effect by Duncan's multiple range test. The transport rate in mice pretreated with aluminum decreased by 65.3 ± 7.2%; $F(1,28) = 13.0$, $P < 0.001$.

After i.c.v. injection, 71.0% of the radioactivity recovered from blood was eluted by HPLC at the position of ^{125}I-labeled RC-160 (Fig. 3), while 100% of the radioactivity recovered from brain was eluted at that position.

DISCUSSION

The unidirectional influx rates into the brain as assessed by the graphical method after i.v. injection were variable among the somatostatin analogs but low in general. The entry rate for RC-121, the analog that entered most slowly, was near the value generally found for albumin, a substance often used to measure the vascular space of the brain because of its low rate of entry. The entry rate for RC-161, the analog that

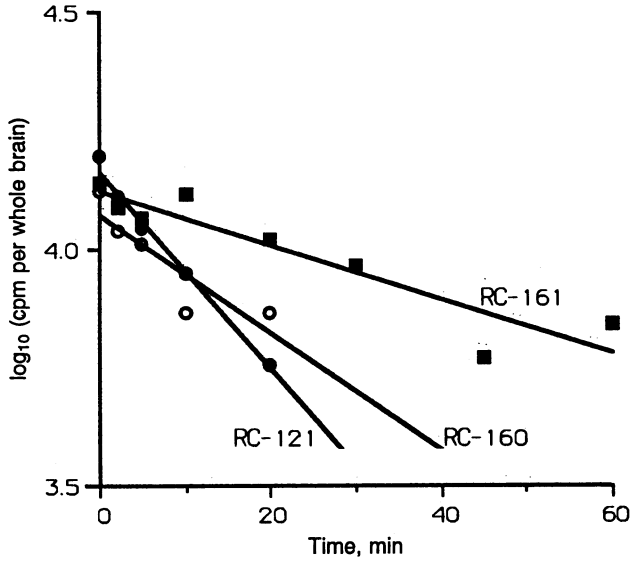

FIG. 2. Disappearance of somatostatin analogs from brain after i.c.v. injection. Rates for $t_{1/2}$ were 14.4 min for RC-121 (●), 24.0 min for RC-160 (○), and 53.0 min for RC-161 (■).

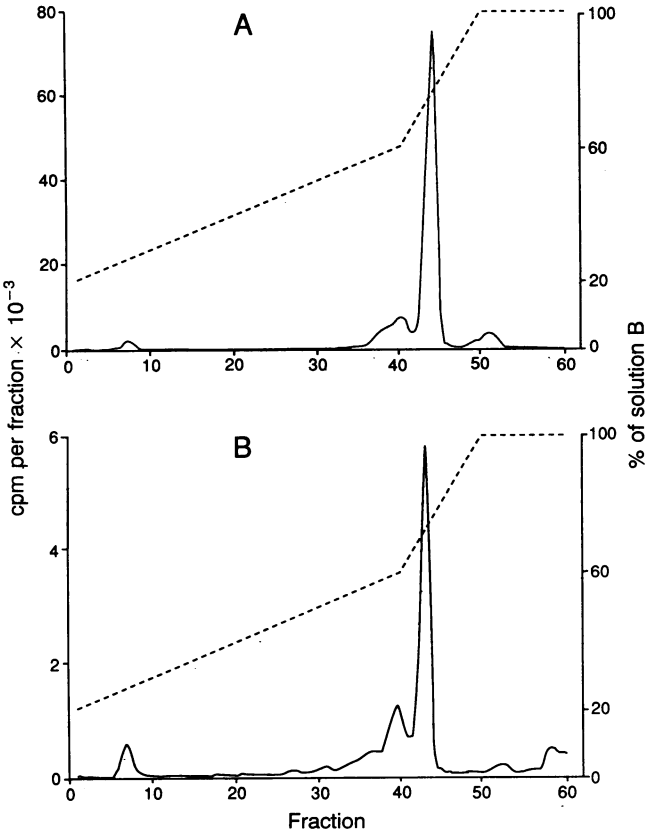

FIG. 3. HPLC of radioactivity recovered from blood after i.c.v. injection of radioiodinated RC-160. (*A*) Elution profile of the stock solution. (*B*) Elution profile of the blood.

entered most rapidly, was more than 5 times greater than that for RC-121, showing that some somatostatin analogs crossed the BBB better than others. RC-160 had a permeability intermediate to that of RC-161 and RC-121. Consequently, RC-160 was used for HPLC and brain perfusion as a compound representative of these octapeptide somatostatin analogs.

HPLC showed that 10 min after i.v. injection of ^{125}I-labeled RC-160, most of the circulating radioactivity represented intact peptide and that some of this intact RC-160 had entered the brain. Sixty minutes after injection, the percentage of radioactivity that represented intact RC-160 had decreased to about 5% in the blood and to 37% in the brain. Thus, somatostatin octapeptide analogs can enter the brain after i.v. injection, but the entry rates are low, and a significant portion of the material found in the brain represents degradation products.

By contrast, the entry rate into the brain for RC-160 was >200 times greater when assessed by the blood-free perfusion method than when assessed by the i.v. injection method. These two methods usually give nearly identical results (22) and, at these levels of entry, can be directly compared (20, 21). The enhanced entry with a blood-free perfusate suggests that some factor in blood greatly impedes the entry of somatostatin analogs into the brain. The entry was not inhibited by the addition of excess RC-160 to the perfusate, showing that entry did not occur by a saturable mechanism.

Disruption of the BBB also could not explain this enhanced entry. Perfusion of the brain by the method used here has been shown not to alter BBB integrity (22), and the inclusion of Tc-Alb in the perfusate as a vascular marker would measure and correct for any increased volume of distribution within the brain. Enzymatic degradation was not a factor because the material entering the brain was shown by HPLC to be intact peptide. The impeding substance in serum could be a binding protein, since somatostatin is known to circulate primarily in a bound form (25), or it could represent a factor in serum that promotes aggregation (26). Other factors known to influence the passage of peptides across the BBB include age, lighting conditions, stresses, strain/species, and neurotoxins (27, 28).

Most of the radioactivity entering the brain during serum-free perfusion was found by HPLC to be intact RC-160. By contrast, most of the radioactivity in the vascular space of the brain was degraded material. This suggests that the elimination of serum resulted in RC-160 crossing the BBB more readily than its degradation products, probably because of its high lipid solubility. The greater degradation of RC-160 in the vascular space during serum-free perfusion in comparison with that after i.v. injection suggests that serum may protect RC-160 from degradation as it flows through the capillary bed of the brain. Thus, the absence of serum enhances both the passage of RC-160 across the BBB and its degradation in the vascular compartment. Both of these findings could be explained by an increased availability of unbound or unaggregated peptide.

The results have several implications for the possible therapeutic uses of somatostatin analogs. The very low rate of entry into the brain of some analogs means that they should be nearly devoid of direct effects on the CNS, an advantage if peripheral actions are desired. It had been misstated for years that peptides could not cross the BBB, but this misconception was based on only a few studies that used insensitive methods. The much more sensitive graphical method used here has been used to measure entry rates for several peptides and centrally active proteins (29–31).

The negligible rate of entry must be overcome if these analogs are to be used in the treatment of those brain tumors with an intact BBB. The higher entry rate found for RC-161 suggests that this could be accomplished by modification of the structure of the peptide. Alternatively, prevention of interaction of the peptide with serum could also increase entry. Brain perfusions have been used in humans (32), but another approach might be the use of sustained delivery systems. Microcapsules permit the maintenance of therapeutic blood levels of somatostatin analogs for up to 4 weeks (6, 10, 17). By circumventing the rapid degradation that normally occurs, the use of microcapsules might enhance passage across the BBB. Pretreatment of animals with aluminum, which acutely enhances the permeability of the BBB to many peptides including somatostatin-(1–14) (19) had a minimal effect on the entry of RC-160.

In contrast to the nonsaturability of blood-to-brain transport, RC-160 was transported out of the CNS by a saturable system. The $t_{1/2}$ of RC-160 was 24 min, faster than the $t_{1/2}$ of 30–45 min predicted from nonsaturable processes such as the reabsorption of cerebrospinal fluid in mice (29, 33). The clearance of ^{125}I-labeled RC-160 could be inhibited by unlabeled RC-160 and by somatostatin-(1–14) tetradecapeptide.

Pretreatment of mice with aluminum, which can selectively inhibit saturable transport systems in the BBB (33, 34), inhibited transport of RC-160 out of the CNS. Neither tyrosine nor iodotyrosine inhibited transport, showing that it was not these fragments being conveyed by this system. More than 80% of the radioactivity recovered from the blood after i.c.v. injection was eluted by HPLC as intact ^{125}I-labeled RC-160, thus confirming that it is the intact peptide that is being transported out of the brain. Arginine vasopressin, a peptide similar in size to RC-160 that also has a six-membered ring and is transported by its own system (35), did not inhibit the transport of RC-160. Neither Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), the first peptide shown to have a saturable brain-to-blood transport system (36), nor leucine, an allosteric regulator of the transport system for Tyr-MIF-1

Table 1. BBB transport, inhibition of growth hormone secretion, and binding to rat cerebral cortex of three somatostatin analogs

Analog	$k_i \times 10^5$, ml/(g·min)	$t_{1/2}$ brain to blood, min	Inhibition of growth hormone*	Binding to rat cerebral cortex*
RC-121	4.01	14.4	177	8.14
RC-160	9.23	24.0	113	0.13
RC-161	23.20	53.0	55	0.16

*Relative to somatostatin-(1–14) tetradecapeptide.

(37), affected the transport of RC-160. This demonstration of saturability, transport of intact peptide, specificity, and distinctiveness from previously described systems constitutes the description of an additional saturable transport system for peptides. By the previously proposed nomenclature (38), this would be peptide transport system (PTS) 5.

Brain-to-blood transport of RC-121 was even faster than that of RC-160 ($t_{1/2}$: 14.4 vs. 24.0 min), suggesting that its structure is more ideally suited to the binding site of the transport complex. The only difference between RC-160 and RC-121 is the presence of tryptophan at the C-terminal position of RC-160 as compared with threonine in RC-121. However, the $t_{1/2}$ of RC-161 was 53.0 min. The primary structure of RC-161 is identical to that of RC-121 except for the presence of an N-terminal acetyl group on RC-161, rendering RC-161 more lipophilic. Thus, changes at both the C- and N-terminal positions greatly affect transport by this saturable system.

The low rate of clearance from the CNS for RC-161 may have contributed to the longer period during which the unidirectional influx into the brain could be measured for this compound. The unidirectional rate of influx is determined with the graphical method from only the linear portion of the curve relating exposure time and brain–blood ratio. Nonlinearity of influx occurs later primarily because efflux from the CNS becomes significant relative to influx. Therefore, the longer period during which influx could be measured for RC-161 may be related to the longer $t_{1/2}$ from the brain. The correlation between $t_{1/2}$ from the brain and entry rate into the brain also suggests that these two parameters may be interrelated for these three somatostatin analogs. Such a relationship does not generally exist among unrelated compounds (30).

The k_i of the three somatostatin analogs was inversely related to their previously described (17) ability to inhibit growth hormone secretion (Table 1). The pituitary, which does not have a BBB and is the presumed site of action for inhibition of growth hormone secretion, was excluded from the brain samples used to determine the k_i. RC-121, the analog that entered the brain the least well and was also the one most quickly transported out of the CNS, is about equipotent to RC-160 in inhibiting glucagon, gastric acid, and insulin release (17). RC-121 is more powerful than RC-160 in displacing somatostatin from binding sites in the rat cerebral cortex and human meningioma cells (12). Thus, two factors important to the therapeutic use of these analogs, permeability to the BBB and affinity to binding sites, are not related.

Taken together, our studies show that biologically active analogs of somatostatin can cross the BBB and thus theoretically could be considered for the treatment of some brain tumors. A substance in serum, possibly a binding protein or an aggregation factor, may exert a major influence on the blood-to-brain passage of peptide. By contrast, brain-to-blood transport of these compounds is dominated by a previously unknown saturable transport system.

This work was supported by the Medical Research Service of the Veterans Affairs (W.A.B., A.V.S., and A.J.K.) and by the National Institutes of Health Grant CA 40077 (to A.V.S.).

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