Delivery of Galanin-Like Peptide to the Brain: Targeting with Intranasal Delivery and Cyclodextrins

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ABSTRACT

Galanin-like peptide (GALP) shows potential as a therapeutic in the treatment of obesity and related conditions. In this study, we compared the uptake by brain regions and peripheral tissues of radioactively iodinated GALP (I-GALP) after intranasal (i.n.), i.v., and i.c.v. administration. I-GALP was stable in blood and brain during the 10-min study time regardless of route of administration, and similar levels were achieved in cerebrospinal fluid after i.v. and i.n. administration. However, levels in most brain regions were approximately 4 to 10 times higher and uptake by spleen, representative of peripheral tissues, approximately 10% as high after i.n. than i.v. administration. Thus, i.n. administration provided about a 40- to 100 fold improvement in targeting brain versus peripheral tissues compared with i.v. administration. Uptake of I-GALP by whole brain after i.n. administration was inhibited by approximately 50% by 1 µg/ mouse of unlabeled GALP, thus demonstrating a saturable component to uptake. Combining I-GALP with cyclodextrins increased brain uptake approximately 3-fold. Selectivity for brain region uptake was also seen with route of administration and with use of cyclodextrins. The hippocampus had the greatest uptake after i.c.v. administration, the cerebellum after i.v. administration, the hypothalamus with i.n. administration without cyclodextrins, the hypothalamus and olfactory bulb (OB) after i.n. administration with α-cyclodextrin, and the OB after i.n. administration with dimethyl-β-cyclodextrin. These studies show that intranasal administration is an effective route of administration for the delivery of GALP to the brain and that targeting among brain regions may be possible with the use of various cyclodextrins.
taken up by the OB (Baker and Spencer, 1986), but they can also enter into other areas of the brain by mechanisms that have been partially elucidated (Balin et al., 1986). For example, horseradish peroxidase leaks between the intercellular spaces of the nasal epithelium, and it uses extraneuronal pathways to reach deep areas of the brain in mouse (Balin et al., 1986), whereas uptake of an exendin analog is dependent on an energy-requiring process (Banks et al., 2004).

Intranasal administration holds some advantages for delivery of peptides, especially peptides with unfavorable pharmacokinetics after i.v. administration (Born et al., 2002; Frey, 2002). For example, insulin delivered to the brain by i.n. administration can avoid the hypoglycemia of i.v. administration (Kern et al., 1999) in amounts that promote learning in humans (Benedict et al., 2004). Glucagon-like peptide-1 is rapidly degraded in blood, but it or its antagonist can be given by i.n. administration in amounts that exert cognitive and neuroprotective effects (During et al., 2003; Banks et al., 2004). Other peptides shown to be taken up in significant amounts include leptin (Kastin and Pan, 2006) and vasoactive intestinal peptide (Dufes et al., 2003).

Although i.n. delivery shows great promise, little work has yet been done that compares the relative uptake or brain distribution after i.v., i.n., or i.c.v. administration. In addition, compounds are often given with cyclodextrins to enhance uptake. Cyclodextrins are cyclic glucans that can form inclusion complexes with many substances, thus increasing solubility and absorption. Three general categories are α-, β-, and γ-cyclodextrins that consist of six, seven, or eight glucopyranose units, respectively. In vitro models of the BBB suggest that there are no differences in permeability among these three classes of cyclodextrins (Monnaert et al., 2004). However, no work has yet compared the relative effects of these various cyclodextrins on uptake of peptides administered by the i.n. route.

Here, we investigated the distribution of I-GALP into whole brain, olfactory bulb, and other selected brain regions, blood, cervical lymph nodes, and spleen after i.n., i.v., and i.c.v. administration. We also compared the effects of selected cyclodextrins on the uptake and distribution within brain of GALP administered by the i.n. route.

Materials and Methods

Radioactive Labeling of GALP. GALP (Peninsula Laborato ries, San Carlos, CA) was radioactively labeled with 131I by the chloramine-T method. In brief, 5 μg of GALP was added to 2 mCi of 131I (PerkinElmer Life and Analytical Sciences, Shelton, CT) and 10 μl of chloramine-T, and then the sample was vigorously mixed and incubated for 1 min. Labeled GALP (I-GALP) was purified on a G-10 column (Sephadex; Sigma Chemical Co., St. Louis, MO).

Measurement of BBB Permeability to i.v. I-GALP. Male ICR mice (2 months old; 35–45 g) from our in-house colony were anesthetized with an i.p. injection of 0.2 ml of urethane (40% solution), and the right carotid artery was exposed. Mice were given an i.n. administration of 2 μl of 250,000 cpm/μl I-GALP alone, I-GALP mixed with dimethyl-β-cyclo dextrin (I-GALP + B-CD) (Sigma-Aldrich, St. Louis, MO), I-GALP mixed with α-cyclodextrin (I-GALP + A-CD) (Sigma-Aldrich), or I-GALP with 1 μg/mouse of unlabeled GALP. A total of 2 μl was delivered to the cribriform plate by pushing a small cannula attached to a 10-μl syringe through the right nares to the depth of the crib riform plate. The I-GALP + cyclo dextrin (CD) solutions were prepared by mixing equal volumes of I-GALP with a 20% CD solution. Blood was collected from the right carotid artery, and the whole brain was removed at 1, 2, 3, 4, 5, 7.5, or 10 min after i.n. administration. The mouse was decapitated, and the cervical lymph nodes were identified, removed, and weighed. The brain was dissected on ice into OB, ant brain, hippocampus, hypothalamus, cerebellum, and brain stem, and each region was weighed. The whole blood was centrifuged at 5400g for 15 min at 4°C, and the level of radioactivity was measured in 50 μl of the resulting serum. The levels of radioactive iodine in the serum and tissue samples were determined by counting in a gamma counter for 30 min. The percentage of the injected dose taken up per gram of brain tissue (%Inj/g) was calculated at each time with the following formula:

\[
\%\text{Inj/g} = \left(\frac{\text{Am}/\text{Cpt} - 10(\%\text{Inj/ml})}{100}\right)
\]

where the value 10 approximates the vascular space in units of microliters per gram, and %Inj/ml represents the percentage of administered I-GALP in a milliliter of serum. For cervical lymph nodes and spleen, the %Inj/g was calculated by dividing the level of radioactivity in a gram of tissue by the dose of administered I-GALP and then multiplying by 100.

Intranasal Administration. Male ICR mice (2 months old; 35–45 g) from our in-house colony were anesthetized with an i.p. injection of 0.2 ml of urethane (40% solution), and the right carotid artery was exposed. Mice were given an i.n. administration of 2 μl of 250,000 cpm/μl I-GALP alone, I-GALP mixed with dimethyl-β-cyclo dextrin (I-GALP + B-CD) (Sigma-Aldrich, St. Louis, MO), I-GALP mixed with α-cyclodextrin (I-GALP + A-CD) (Sigma-Aldrich), or I-GALP with 1 μg/mouse of unlabeled GALP. A total of 2 μl was delivered to the cribriform plate by pushing a small cannula attached to a 10-μl syringe through the right nares to the depth of the crib riform plate. The I-GALP + cyclo dextrin (CD) solutions were prepared by mixing equal volumes of I-GALP with a 20% CD solution. Blood was collected from the right carotid artery, and the whole brain was removed at 1, 2, 3, 4, 5, 7.5, or 10 min after i.n. administration. The mouse was decapitated, and the cervical lymph nodes were identified, removed, and weighed. The brain was dissected on ice into OB, ant brain, hippocampus, hypothalamus, cerebellum, and brain stem, and each region was weighed. The whole blood was centrifuged at 5400g for 15 min at 4°C, and the level of radioactivity was measured in 50 μl of the resulting serum. The level of radioactive iodine in the serum and tissue samples was determined by counting in a gamma counter for 30 min. Results are expressed as %Inj/g.

Intracerebroventricular Injection. Male ICR mice (2 months old; 35–45 g) from our in-house colony were anesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). For each mouse, the skin was removed from over the skull, and a hole made into the lateral ventricle of the brain (1.0 mm lateral and 0.5 mm posterior to the bregma) with a 26-gauge needle. Tubing covered all but the skin was removed from over the skull, and a hole made into the lateral ventricle of the brain (1.0 mm lateral and 0.5 mm posterior to the bregma) with a 26-gauge needle. Tubing covered all but the

\[\text{Am}/\text{Cpt}(t) = K_i \cdot \left[ \int_0^t \frac{\text{Cpt}(t)}{\text{Cpt}(t) + V_i} \right] + V_i, \]

where Am is cpm per gram of brain, Cpt is cpm per microliter of arterial serum, and exposure time (Expt) is measured by the term \[\frac{\text{Cpt}^2}{\text{Cpt}(t) + \text{Cpt}(t)} / \text{Cpt}.\] The linear portion of the relation between Am/Cpt (whole brain/serum in units of microliters per gram) ratio versus exposure times (Expt in units of minutes) was used to calculate \(K_i\) (in units of microliters per gram-minute) and \(V_i\) (in units of microliters per gram). \(K_i\) is reported with its error term. In other mice, the cervical lymph nodes and spleen were identified, removed, and weighed. The brain was dissected on ice into the OB, anterior one third of the brain (ant brain), hippocampus, hypothalamus, cerebellum, and brain stem, and each region was weighed. The whole blood was centrifuged at 5400g for 15 min at 4°C, and the level of radioactivity was measured in 50 μl of the resulting serum. The levels of radioactive iodine in the serum and tissue samples were determined by counting in a gamma counter for 30 min. The percentage of the injected dose taken up per gram of brain tissue (%Inj/g) was calculated at each time with the following formula:

\[\%\text{Inj/g} = \left(\frac{\text{Am}/\text{Cpt} - 10(\%\text{Inj/ml})}{100}\right)
\]

where the value 10 approximates the vascular space in units of microliters per gram, and %Inj/ml represents the percentage of administered I-GALP in a milliliter of serum.
decapitated, and the cervical lymph nodes and spleen were identified, removed, and weighed. The brain was dissected on ice in OB, ant brain, hippocampus, hypothalamus, cerebellum, and brain stem, and the regions were weighed. The whole blood was centrifuged at 5400g for 15 min at 4°C, and the level of radioactivity measured in 50 μl of resulting serum. The level of radioactive iodine in the serum and tissue samples was determined by counting in a gamma counter for 30 min. The results are expressed as %Inj/g.

Cerebrospinal Fluid Collection. Male ICR mice (2 months old; 35–45 g) from our in-house colony were anesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). The left jugular vein and right carotid artery were exposed. The mice were given an i.v. injection into the left jugular vein of 0.2 ml of 1% BSA in LR containing 300,000 cpm of I-GALP. Other mice were given an i.n. administration of 2 μl of 250,000 cpm/μl of I-GALP + B-CD. A total of 2 μl was delivered to the cibriform plate by pushing a small cannula attached to a 10-μl syringe through the right nare to the depth of the cibriform plate. Five minutes later, the skin overlying the posterior fossa was removed, and a 20-gauge needle connected to polyethylene-20 tubing was pushed into the posterior fossa. CSF was allowed to passively fill the tubing, and the amount of CSF collected was determined by measuring the length in centimeters of the polyethylene-20 tubing filled with CSF and multiplying by 1.134 (the volume of the tubing being 1.134 μl/cm). Only CSF that was absolutely clear was analyzed. Blood was collected from the right carotid artery, and the whole brain was removed and weighed after i.n. or i.v. administration. The mouse was decapitated, and then the OB and other brain regions were dissected on ice from the whole brain, and each region was weighed. The whole blood was centrifuged at 5400g for 15 min at 4°C, and the level of radioactivity was measured in 50 μl of resulting serum. The level of radioactive iodine in the serum and tissue samples was determined by counting in a gamma counter for 30 min. Values for whole brain were calculated by adding the regional weights and regional levels of radioactivity. The %Inj/ml was calculated with the following formula:

\[
\text{%Inj/ml} = 100\left(\frac{\text{Cpt}}{\text{Inj}}\right)
\]

(3)

where Inj is the dose administered and Cpt is the level of radioactivity in a milliliter of serum at time t. The %Inj/g of OB and WBr was calculated at each time with the following formula:

\[
\text{%Inj/g} = \left(\frac{\text{Am/Cpt} - 10}{\text{%Inj/ml}}\right) \times 100
\]

(4)

Acid Precipitation. Male ICR mice (2 months old; 35–45 g) were anesthetized, prepared, and administered I-GALP by the i.n., i.c.v., or i.v. route. For i.n. administration, I-GALP was given with B-CD. Blood was collected from the right carotid artery, and the OB and WBr were removed at 5 and 10 min (n = 3–6 mice/time) after i.n., i.c.v., or i.v. administration of I-GALP. The OB and WBr were dissected from brain on ice, and 150 μl of 0.25 M phosphate-buffered solution and 150 μl of 10 mM enzymatic cocktail (19.82 mg/ml 100 mM 1,10-phenanthroline, 12.51 mg/ml 100 mM N-ethylmaleimide, 37.22 mg/ml 100 mM EDTA, and 88.89 mg/ml 100 mM L-thyroxine in distilled water, Sigma-Aldrich) were added to the OB or WBr. The samples were homogenized on ice with a Tissue Tearor (Biospec Products, Inc., Racine, WI) for 20 s at a setting of 20. The homogenate was centrifuged at 5400g for 15 min. A portion of the supernatant (150 μl) was transferred to a second tube, and 150 μl of 30% trichloroacetic acid (TCA) (Sigma-Aldrich) was added to precipitate the I-GALP. Whole blood was centrifuged at 5400g for 15 min at 4°C, and the level of radioactivity was measured in 50 μl of the resulting serum. Ten microliters of serum was added to a tube containing 500 μl of 1% BSA in LR, the contents were mixed, and 500 μl of 30% TCA was added. This combination was then mixed and centrifuged. To determine the amount of degradation that occurred during this processing, 500,000 cpm of I-GALP was added in vitro to serum, OB, or WBr obtained from a mouse that had not received an i.n., i.c.v., or i.v. administration (n = 2 mice). Samples were then processed, homogenized, and centrifuged as described above. After addition of TCA, the serum, brain, and processing controls were centrifuged, and the supernatant was separated from the pellet. The percentage of radioactivity precipitated was calculated by the following formula:

\[
100\left(\frac{\text{pellet cpm}}{\text{pellet cpm + supernatant cpm}}\right)
\]

(5)

The percentage precipitated from samples was divided by that which precipitated in the processing controls, and this value was multiplied by 100 to give an index of intact I-GALP.

Statistical Analysis. The Prism 4.0 program (GraphPad Software Inc., San Diego, CA) was used in statistical analysis. Regression lines were computed by the least-squares method in Prism. Means are reported with their n and S.E., and they were compared by analysis of variance (ANOVA) followed by Newman-Keuls range test.

Results

Table 1 shows the results for acid precipitation (percentages) of radioactivity extracted from OB, WBr, and serum at 5 and 10 min after i.n., i.c.v., or i.v. administration of I-GALP + B-CD or I-GALP (n = 3–6 mice/region). The amount of radioactive iodine that precipitated with acid varied little between the 5- and 10-min values, but a two-way ANOVA did show variations among administration routes [F(5,76) = 5.19; p < 0.001], regions [F(2,76) = 14.7; p < 0.001], and interactions between routes and regions [F(10,76) = 5.83; p < 0.001]. Newman-Keuls tests showed the following differences at p < 0.05: WBr INA at 5 min was less than i.e.v. or i.v. WBr at 5 min and i.v. WBr at 10 min was higher than i.e.v. or i.n. WBr at 10 min.

We measured the rate of unidirectional influx (Kᵢ) from blood-to-brain for I-GALP administered by i.v. injection. Figure 1 shows the relation between whole brain-serum ratio (microliters per gram) and exposure time (minutes). Kᵢ for I-GALP into brain was 0.90 ± 0.35 μl/g-min (r = 0.58, p < 0.05, n = 15 mice). Equilibrium between brain and blood was reached approximately 5 min after injection. The Vi was
11.0 ± 1.4 μl/g, a space about equal to the vascular volume of the brain.

Uptake into cerebrospinal fluid was measured after i.n. administration of I-GALP + CD and after i.v. injection of I-GALP into each of three mice (Fig. 2). Whole brain, OB, and CSF were collected 5 min later. CSF had the highest level of I-GALP of any region after i.v. administration, but olfactory bulb had the highest level after i.n. administration. A two-way ANOVA was significant for route \([F(1,12) = 15.0; p < 0.005]\) and interaction \([F(2,12) = 4.29; p < 0.05]\), but not for region. Newman-Keuls analysis showed that I-GALP was higher in the OB after i.n. administration than in the OB or WBr after i.v. administration. CSF values did not differ between the i.n. and the i.v. routes.

Brain distribution was determined 10 min after administration of I-GALP + B-CD by i.n. administration (Fig. 3A). ANOVA showed a statistical difference among the brain regions \([F(5,11) = 20.52; p < 0.001]\). OB had much higher levels than any other tissue. Brain distribution was determined 10 min after administration of I-GALP by i.v. injection (Fig. 3B). ANOVA showed a statistical difference among the brain regions \([F(5,32) = 46.02; p < 0.001]\). Hippocampus had higher levels than any other tissue, although levels were also high in the hypothalamus. Brain distribution was determined 10 min after i.v. administration of I-GALP (Fig. 3C) showed no statistical difference among the brain regions. Cervical lymph node and spleen distribution were determined 10 min after i.n., i.c.v., or i.v. administration. ANOVA showed a statistical difference among the cervical lymph nodes and spleen \([F(5,18) = 46.86; p < 0.001]\). The i.v. route produced much higher levels in cervical lymph nodes of I-GALP than the i.n. or i.v. routes. The i.v. route produced much higher levels in spleen than the i.n. or i.v. routes (Fig. 3D).

The time-dependent uptake of I-GALP + B-CD by whole brain, olfactory bulb, cervical lymph nodes, and spleen was further explored (Fig. 4). Entry of I-GALP + B-CD into the olfactory bulb after i.n. administration (n = 3 mice/time point) was higher than into any of the other tissues (Fig. 4A). After i.v. injection, I-GALP uptake (n = 3 mice/time point) by spleen was higher than by any other tissue, and WBr had the lowest uptake (Fig. 4B). Figure 4C compares these results for the brain regions of WBr and OB after i.n. or i.v. administration. Entry into OB was higher than uptake by WBr after i.n. or i.v. injection, and i.n. injection produced higher levels than did i.v. for either OB or WBr uptake. Figure 4D compares these results for the cervical lymphatic nodes and the spleen after i.n. or i.v. administration. Entry of I-GALP into the spleen after i.n. administration was higher than into the cervical lymph nodes or spleen after i.n. administrations or into the cervical lymph nodes after i.v. administration. The i.v. injection produced higher levels in spleen than did the i.n. injection.

Brain distribution was determined 10 min after I-GALP without CD or I-GALP + A-CD by i.n. administration (n = 13–15 mice) (Fig. 5). Hypothalamus was again higher than any other tissue after i.n. administration of I-GALP (Fig. 5A). OB and hypothalamus were much higher than any other brain regions after i.n. administration of I-GALP + CD (Fig. 5B). Thus, A-CD results were distinct from those with either I-GALP alone or with B-CD. Every brain region after i.n. administration of I-GALP + A-CD was higher than after i.n. administration of I-GALP without CD.

Saturation of uptake was determined by obtaining carotid artery serum, olfactory bulb, or whole brain 10 min after administration of I-GALP with or without unlabeled GALP (1 μg/mouse). Carotid artery serum, olfactory bulb, and whole brain all showed a 40 to 50% decrease in uptake with the addition of unlabeled GALP, with a statistically significant decrease for serum \((t = 3.08, df = 32, p < 0.005, n = 17/group)\) and whole brain \((t = 2.82, df = 28, p < 0.01, n = 15–16/group)\), but not olfactory bulb (Fig. 6).

**Discussion**

This study compared the i.n., i.v., and i.c.v. routes of administration for their abilities to deliver I-GALP to various regions of the brain. We found the rate of uptake \((K)\) of I-GALP after i.v. administration to be 0.90 ± 0.35 \(μl/g\)-min) for I-GALP, a rate almost identical to that found by Kastin et al. (2001). Equilibrium between WBr and blood was reached approximately 5 min after i.v. injection. When expressed as %Inj/g, steady state was reached in WBr, OB, spleen, and...
Fig. 4. Time course data for uptake of I-GALP after i.n. (INA) or i.v. (IV) administration of I-GALP. For i.n. administration, I-GALP was given with dimethyl-β-cyclodextrin. Tissues were collected between 1 and 10 min after administration. A, comparison of uptake by WBr, OB, cervical lymph nodes (Ly), and spleen (Sp) after i.n. administration. B, comparison of these regions after i.v. administration. C, comparison of i.n. versus i.v. administration for just the brain regions. D, comparison of i.n. versus i.v. administration for just the peripheral tissues. n = 3/time point.

Fig. 3. Comparisons of tissue and brain regional uptake after i.n. (INA), i.v. (IV), and i.c.v. administration of I-GALP. For i.n. administration, I-GALP was given with dimethyl-β-cyclodextrin. Tissues were collected 10 min after administration, and the results are expressed as %Inj/g. A, results for brain regions after i.n. administration. *, p < 0.05 compared with OB. B, results for brain regions after i.c.v. administration. *, p < 0.05 compared with hypothalamus; #, p < 0.05 compared with hippocampus. C, results for brain regions after i.v. administration; there were no statistically significant differences among brain regions. D, results for peripheral tissues after i.n., i.v., and i.c.v. administration. *, p < 0.05 compared with i.c.v. lymph nodes. Lymph Nodes, cervical lymph nodes.
cervical lymphatics within approximately 1 min of i.v. injection and within 1 to 5 min after i.n. administration. I-GALP was found by acid precipitation to be stable in blood, OB, and brain after i.v., i.n., or i.c.v. administration. I-GALP may have been somewhat less stable in WBr after i.n. administration compared with the other compartments and routes of administration.

In contrast to the similarity between i.n. and i.v. routes for time to equilibrium, stability in tissues, and also CSF uptake, the distributions in CNS and peripheral tissues differed markedly among the three routes of administration. In general, brain uptake was 5 to 10 times higher after i.n. than after i.v. administration of I-GALP. OB uptake was particularly high after i.n. administration, consistent with its entry into the CNS at the cribriform plate. High OB uptake is typical with i.n. administration, especially in rodents, which have very large OB. Otherwise, distribution throughout the rest of the brain was rather uniform after i.n. administration. In contrast, the cerebellum had the highest uptake after i.v. administration, with olfactory bulb having the second highest uptake. It is possible that the saturable transporter located at the BBB is most active in these brain regions.

Hippocampus, an area of the brain with receptors for GALP was an area of intermediate uptake after i.v. injection, but the area with the second highest uptake after i.c.v. administration. The high uptake for hippocampus and hypothalamus after i.c.v. injection may reflect the proximity these tissues have to the lateral ventricle, whereas after i.v. injection, it may be that receptor density is the main determinant.

The relative uptakes by the peripheral tissues of the cervical lymph nodes and the spleen are consistent with the access of these tissues according to the route of administration. After i.v. administration, I-GALP has equal access to the cervical nodes and spleen by way of the systemic circulation. Thus, splenic and cervical lymph node levels of I-GALP approximate each other. After i.c.v. administration, I-GALP would enter the circulation primarily with the reabsorption of cerebrospinal fluid into the cervical lymphatics (Widner et al., 1987; Boulton et al., 1999). This would give I-GALP immediate access to the (Yamada et al., 1991; Knopf

Fig. 5. Uptake of I-GALP by brain regions when administered without CD (A) or with α-CD (B). ** p < 0.001. n = 13 to 15.

Fig. 6. Inhibitory effect of unlabeled GALP on uptake of I-GALP after i.n. administration. I-GALP was given with or without 1 µg/mouse of unlabeled GALP. Top, statistically significant inhibition by unlabeled GALP for entry into carotid arterial serum of I-GALP (n = 17/group). Middle, an arithmetic, but not statistically significant, decrease in uptake by olfactory bulb (n = 16/group). Bottom, statistically significant decrease in uptake by whole brain. *, p < 0.01. n = 15 to 16/group.
uptake by the hypothalamus, not the OB. This could indicate dextrin. Both cyclodextrins tended to increase uptake of the I-GALP was coadministered with 1 whole brain of I-GALP was reduced approximately 50% when saturable component. In these experiments, no cyclodextrin may be possible by varying the cyclodextrin used. Indeed, uptake by brain regions and distribution among peripheral tissues is very different for the i.n., i.c.v., and i.v. routes of administration. This, in turn, suggests that the relative mix of CNS and peripheral effects will differ drastically with these different routes of administration. Finally, the results suggest that targeting of various brain regions may be manipulated by the use of various cyclodextrins.

References