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Characterization of Blood-Brain Barrier Permeability to PYY_{3-36} in the Mouse

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List of non-standard abbreviations: blood-brain barrier, BBB; cocaine-and-amphetamine-regulated transcript, CART; intracerebroventricular, icv; neuropeptide Y, NPY; unlabeled Peptide YY_{3-36}, unlabeled PYY; ^{131}I-Peptide YY_{3-36}, I-PYY; ^{99m}Tc-Albumin, T-Alb; high pressure liquid chromatography, HPLC; unidirectional influx rate, Ki; volume distribution, Vi.

Abstract

Peptide YY_{3-36} (PYY) has emerged as an important signal in the gut-brain axis, with peripherally administered PYY affecting feeding and brain function. For these effects to be direct, PYY would have to cross the blood-brain barrier (BBB). Here, we determined the permeability of the BBB to PYY radioactively labeled with ^{131}I (I-PYY). Multiple-time regression analysis showed the unidirectional influx rate (Ki) from blood-to-brain for I-PYY to be 0.49 ± 0.19 μl/g-min, a rate similar to that previously measured for leptin. Influx was not inhibited by 1 μg/mouse of unlabeled PYY, suggesting PYY crosses the BBB by transmembrane diffusion. About 0.176 % of the iv injected dose of I-PYY was taken up by brain, an amount similar to that for other peptides important in gut-brain communication. Capillary depletion showed that 69% of I-PYY crossed the BBB to enter the parenchymal space of the brain, and HPLC demonstrated that the radioactivity in this space represented intact I-PYY. After intracerebroventricular injection, I-PYY crossed from brain-to-blood by the mechanism of bulk flow. We conclude that PYY crosses in both the blood-to-brain and brain-to-blood directions by non saturable mechanisms. Passage across the BBB provides a mechanism by which blood-borne PYY can affect appetite and brain function.

Peptide YY has emerged as a major component of the gut-brain axis regulation of feeding, body weight, and nutritional status. It is a member of the neuropeptide Y family that includes neuropeptide Y (NPY), pancreatic polypeptide, and two forms of peptide YY, a 36 amino acid form and the 3-36 form (PYY). Each of these forms acts through the NPY receptors (Larhammar, 1996). For example, PYY_{1-36} is an agonist at the Y1/Y2 receptors and promotes feeding, whereas PYY_{3-36} is an antagonist at the Y2 receptor and inhibits feeding (Grandt et al., 1994b). PYY is abundant in human blood (Grandt et al., 1994a) and is released from endocrine cells found throughout the small intestine in proportion to the caloric content of a meal (Ekblad and Sundler, 2002). Blood-borne PYY can affect appetite and influence neuronal activity at the arcuate nucleus (Batterham et al., 2002).

The arcuate nucleus in the adult is clearly separated from the peripheral circulation and median eminence by the endothelial and ependymal arms of the blood-brain-barrier (BBB) (Peruzzo et al., 2000;Rethelyi, 1984). Therefore, PYY would have to negotiate the BBB to reach the receptors in the arcuate nucleus. The BBB controls the exchange of peptides and regulatory proteins between the CNS and blood (Kastin et al., 1990;Banks and Kastin, 1996;Banks and Kastin, 1993;Banks and Kastin, 1985;Brownlees and Williams, 1993;Begley, 1992) and has emerged as a major regulator of communication between the central nervous system and the peripheral tissues (Banks and Kastin, 1990). One area where this regulation is particularly clear is in the area of feeding hormones. Leptin (Banks et al., 1996), ghrelin (Banks et al., 2002c), MSH (Wilson et al., 1984), cocaine-and -amphetamine-regulated transcript (CART) (Kastin and Akerstrom, 1999a), and insulin (Baura et al., 1993; Banks and Kastin, 1998; Banks et al., 1997) have all been shown to cross the BBB by saturable and non-

saturable mechanisms to a substantial extent, as have cytokines which can also influence feeding behavior (Banks et al., 2001a; Banks et al., 1995a). Other members of the NPY family, including pancreatic polypeptide (Yokel, 1983) and NPY (Kastin and Akerstrom, 1999d) have been shown to cross the BBB.

Here, we determined whether PYY_{3-36} can cross the BBB of the mouse. We investigated both blood-to-brain and brain-to-blood pathway using radioactively labeled PYY.

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Methods

Radioactive labeling of PYY and albumin: PYY is a small peptide of 4049.71 MW with a tyrosine at the amino-terminal which is available for iodination. PYY purchased from Phoenix Pharmaceuticals Inc. (Belmont, CA) was radioactively labelled with ^{131}I (Perkin Elmer, Boston, MA) by the lactoperoxidase method. Briefly, 5 μg of PYY was mixed with 30 μl of 0.4 M sodium acetate (pH 5.6), 10 μl of lactoperoxidase (10 μg/ml), and 2 mCi of ^{131}I. The reaction was started by adding 0.02 ng of H_2O_2 in a volume of 10 μl. Ten min later, an additional 0.02 ng of H_2O_2 was added. At the end of this second 10 min period, radioactively labelled PYY (I-PYY) was purified on a Sephadex G-10 column. Albumin was labeled with ^{99m}Tc (T-Alb) by mixing with stannous tartrate, adjusting to pH 2.0-3.0 with 0.2 M HCl, and incubating for 20 min.

Measurement of the unidirectional influx rate: Male ICR mice (2 months old) were anaesthetized with an intraperitoneal (i.p.) injection of 0.2 ml of urethane (40% solution). The left jugular vein and right carotid artery were then exposed and the mice given an injection into the left jugular vein of 0.2 ml of lactated Ringer's solution (LR) containing 250,000 cpm of I-PYY. Blood was collected from the right carotid artery and the whole brain was removed and weighed at 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 min after the iv injection. The whole blood was centrifuged at 5,400 x g for 10 min at 4 °C and the level of radioactivity measured in 50 μl of the serum. The levels of radioactivity in serum and in whole brain were counted in a gamma counter for 3 min. The brain/serum ratios for whole brain were calculated and the unidirectional influx rate (Ki, in units of μl/g-min) and the initial volume of distribution in brain at time zero (Vi, in units of μl/g) was determined by multiple-time regression analysis with the

formula:

$$Am/Cpt = Ki[\int_0^t Cp(\tau)d\tau]/Cpt + Vi \quad (1),$$

where Am is cpm/g of brain, Cpt is cpm/μl of serum, and exposure time (Expt) is measured by the term $[\int_0^t Cp(\tau)d\tau]/Cpt$. The linear portion of the relation between Am/Cpt ratios vs exposure times (Expt) was used to calculate Ki and Vi. Ki is reported with its error term.
To test for saturation of blood to brain passage, other mice were given an injection into the left jugular vein of 0.2 ml of LR containing 250,000 cpm of I-PYY plus 1 μg/mouse of unlabeled PYY.

Capillary depletion: Male ICR mice (2 months old) were anaesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). The left and right jugular veins were exposed. Mice received iv injections of I-PYY and Tc-Alb. Five minutes after the iv injection, the abdomen was opened and arterial blood collected from the abdominal aorta. The thorax was then opened, the descending aorta clamped, both jugular veins severed, and 20 ml of LR perfused over 1 min into the left ventricle of the heart. After that, the mouse was decapitated and the whole brain removed, weighed, and placed in an ice-cold glass homogenizer. The brain was homogenized by 10 vertical strokes in 0.8 ml of physiological buffer (10 mM HEPES, 141 mM NaCl, 4 mM KCl, 2.8 mM $CaCl_2$, 1 mM $MgSO_4$, 1 mM NaH_2PO_4, and 10 mM D-Glucose, pH 7.4). Dextran solution (1.6 ml of a 26% solution) was added to the homogenate, mixed, and homogenized with an additional 3 vertical strokes. The homogenate was centrifuged at 5400 x g for 15 min at 4 °C. The resulting supernatant (brain parenchymal fraction) and pellet

(capillary fraction) were separated. The levels of radioactivity in the capillary and brain parenchymal fractions were counted in a gamma counter for 3 min.

Measurement of the amount of the iv dose taken up by brain: The percent of the injected dose present in a ml of serum (%Inj/ml) was calculated with the formula:

$$\%Inj/ml = 100(Cpt)/Inj \quad (2),$$

where Inj is the dose administered. The percent of the injected dose taken up per gram of brain (%Inj/g) was calculated at each time with the formula:

$$\%Inj/g = [(Am/Cpt-Vi)\%Inj/ml]1000 \quad (3).$$

Stability of I-PYY in serum and brain: Male ICR mice (2 months old) were anaesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). The left jugular vein and right carotid artery were then exposed. The mice were given an injection into the left jugular vein of 0.2 ml of LR containing 200,000 cpm of I-PYY. Blood was collected from the right carotid artery and the whole brain was removed at 1, 5, and 10 min after iv injection. The whole blood was centrifuged at 5,000 x g for 10 min at 4 ˚C. Of the resulting serum, 50 µl was added to 250 µl of 1 % bovine serum albumin in 0.25 M sodium phosphate buffer, then precipitated with 250 µl of 30% trichloroacetic acid (TCA). The whole brain was homogenized in 3 ml of LR for 10 sec and 2 ml of brain homogenate was centrifuged at 5,400 x g for 30 min at 4 ˚C. A volume of 250 µl of 30% TCA was added to 250 µl of the brain

supernatant and mixed. The acidified solutions of serum and brain were centrifuged at 5,400 x g for 10 min at 4 ˚C. The levels of radioactivity in the acidified serum and brain supernatants and pellets were counted in a gamma counter for 3 min. The percent of precipitate radioactivity in serum and brain was calculated by the formula:

$$100[(\text{Pellet cpm})/(\text{Pellet cpm}+\text{Supernatant cpm})] \qquad (4).$$

HPLC: Male ICR mice (2 months old) were anaesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). The left jugular vein and right carotid artery were then exposed and then 10,000,000 cpm of I-PYY were injected into the left jugular vein. Blood was collected from the right carotid artery and the whole brain was removed 5 min after iv injection. The whole blood was centrifuged at 5,400 x g for 10 min at 4 ˚C and the serum added to 4 ml of 1% trifluoroacetic acid (TFA) in H_2O, mixed, and then centrifuged at 5,400 x g for 30 min at 4 ˚C. The resulting supernatant was lyophilized. The whole brain was collected in 4 ml of 0.1 M acetic acid on ice and boiled in a water bath for 10 min. After cooling on ice, the whole brain was homogenized for 60 sec with a Polytron homogenizer and than centrifuged at 5,400 x g for 30 min at 4 ˚C and the resulting supernatant was lyophilized. For the serum processing control, about 1,000,000 cpm were added to the bottom of a tube before the collection of arterial blood from a mouse, and for the brain processing control, about 1,000,000 cpm was added to the surface of a normal brain from a mouse. The blood and brain samples for processing controls were then processed as described above. The brain and serum samples were reconstituted in 2 ml of 0.1% TFA in HPLC water, vigorously mixed, and centrifuged at 5,400 x g for 30 min at 4

°C. Of the resulting supernatants, 0.1 ml was injected onto a C-18 column for HPLC analysis and eluted with a gradient that linearly progressed from 100% of solution A (0.1% TFA in H_2O) to 100% of solution B (0.1% TFA in acetonitrile) in 30 min, with fractions collected every minute.

Brain perfusion of I-PYY and I-PYY + unlabeled PYY: Male ICR mice (2 months old) were anaesthetized with an i.p. injection of urethane 40 % solution (0.2 ml). I-PYY was diluted in Zlokovic's buffer (pH 7.4; 7.19 g/l NaCl, 0.3 g/l KCl, 0.28 g/l $CaCl_2$, 2.1 g/l $NaHCO_3$, 0.16 g/l KH_2PO_4, 0.17 g/l anhydrous $MgCl_2$, 0.99 g/l D- glucose, and 10 g/l BSA added on the day of perfusion). The heart was exposed by opening the thorax. The descending thoracic aorta was clamped and the right and left jugular veins severed. A 21-gauge butterfly needle was inserted into the left ventricle of the heart, and the buffer containing I-PYY (250,000 cpm/ml) was infused at a rate of 2 ml/min for 1, 2, 3, or 4 min. In other mice, unlabeled PYY (1 µl/ml) was included in the I-PYY. After perfusion, the butterfly needle was removed, the mouse was decapitated, and the brain removed. The brain was weighed and counted in a gamma counter for 3 min. The brain/perfusate ratio (µl/g) was calculated by the formula:

Brain/Perfusate ratio = (cpm/g-brain)/[(cpm/µl-serum)(g-brain)].

Intracerebroventricular (icv) injection for measurement of brain-to-blood passage: Male ICR mice (2 months old) were anaesthetized with an i.p. injection of 0.2 ml of urethane (40% solution). For each mouse, the skin was removed from 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 terminal 3.0 - 3.5 mm of the needle. The mice were given an injection of 1.0 μl LR containing 10,000 cpm of I-PYY into the lateral ventricle of the brain. The amount of radioactivity in the brain at t = 0 was estimated in mice which had been overdosed with urethane. The whole brain was removed at 0, 2, 5, 10, and 20 min after the injection and three mice were studied at each time interval. The levels of radioactivity for whole brain was counted in a gamma counter for 3 min and the mean of the three mice at each time interval was used in subsequent calculations. The experiment was repeated so that 2 determinations for each of the five time points (each determination the mean of three mice) was calculated. The log of the mean residual radioactivity per whole brain was plotted against time and the slope used to calculate the half-time disappearance rate. In other mice, unlabeled PYY (1 μg/mouse) was included in the injection and the brain removed 10 min after the icv injection. The level of radioactivity in whole brain was determined with a gamma counter and divided by the cpm injected to yield the percent injected icv that was retained by whole brain (%Inj/brain).

Statistics: Statistical analysis was performed with the use of the Prism 3.0 program (GraphPad Software, Inc, San Diego, CA). Regression lines were calculated by the least squares method and are reported with their correlation coefficient, r, n, and p value. Means are reported with their standard error terms and n. Two means were compared by t-test analysis.

Results

We first measured the rate of unidirectional influx (Ki) from blood to brain for I-PYY. Figure1 shows the relation between brain/serum ratios (μl/g) and exposure time (min). The Ki for I-PYY into brain was 0.49 ± 0.19 μl/g-min, r = 0.682, n = 10, $p < 0.05$). We next determined whether the mechanism by which I-PYY crossed the BBB was saturable. We did this by injecting I-PYY with or without 1 μg/mouse of non-radioactive PYY. The brain/serum ratios of I-PYY (11.02 ± 0.16 μl/g) and of I-PYY + unlabeled PYY (12.4 ± 0.31 μl/g) 5 min after iv injection (n = 14 mice/group) were statistically significant ($p<0.05$; Figure 1, inset).

To determine whether I-PYY crossed the BBB to enter brain parenchyma, we performed capillary depletion. Results are reported for experiments that used vascular washout and after correction for Tc-Alb 5 min after iv injection. The brain parenchyma/serum ratio (μl/g) was measured to be 2.47 ± 0.07 μl/g (n = 4 mice) and was significantly higher than the capillary/serum ratio of 1.10 ± 0.18 μl/g (n = 5 mice; P = 0.0015). These results show that the majority of material taken up by brain had crossed the BBB to enter the parenchyma by 5 min after injection.

We then determined the amount of the intravenously injected dose that entered the brain. To do this, we first calculated the percent of the injected dose present in one ml of serum (%Inj/ml). Fig. 2A shows the log (%Inj/ml) of I-PYY at various times after iv injection. A statistically significant relation existed between log (%Inj/ml) and time (r = 0.71, $p < 0.001$, n =

5 mice/time). The half-time disappearance rate from serum was calculated from the slope of this relation to be 13.1 min. Fig. 2B shows the percent of the injected dose taken up per gram of brain (%Inj/g) 1 - 10 min after iv injection. The peak value for %Inj/g was at about 2 min after iv injection and was 0.176 ± 0.035, n = 5 mice/time.

Table 1 shows results for acid precipitation at 1, 5, and 10 min after iv injection. The amount of radioactivity that precipitated with acid had decreased little during the 10 min study time. These results show that essentially all of the radioactivity in brain and serum remained attached to peptide during the course of the study.

I-PYY was found to cross the BBB at a rate of 2.34 ± 0.74 μl/g (n = 18) when assessed by brain perfusion (Fig. 3). This is a rate faster than after iv injection.

Figure 4 shows the relation between % total cpm and HPLC fraction number for processing controls and for samples taken 5 min after iv injection. For the brain procdssing control, 42 % eluted in the same position as I-PYY, and 69 % of the serum processing control eluted as intact I-PYY. Of the radioactivity recovered 5 min after iv injection of I-PYY, 37 % eluted as intact I-PYY for brain and 66 % for serum (Fig . 4 lower panels show the mean of 2 experiments). Correcting for degradation during processing, we calculated that 88 % of radioactivity from brain and 96 % of radioactivity from serum eluted as intact I-PYY 5 min after iv injection.

Brain-to-blood efflux of I-PYY was measured after icv injection. Figure 5 shows the relation between log (brain cpm) and time was statistically significant, r = 0.65, n = 10 mice, $p < 0.001$), demonstrating efflux from brain. The half-time disappearance rate calculated from

the slope of this relation was 37.8 min. The %Inj/brain for I-PYY and of I-PYY + unlabeled PYY were calculated 10 min after icv injection (n = 16 mice/group). The inclusion of unlabeled PYY did not have a statistically significant effect on brain retention of I-PYY [26.56 ± 1.70 % (I-PYY); 28.29 ± 1.82 % (I-PYY + unlabeled PYY)], suggesting that a saturable component to efflux does not exit.

Discussion

PYY has joined a growing list of important peptides secreted by the GI tract which influence feeding behavior by acting within the central nervous system (CNS). PYY is released in response to feeding in proportion to the calorie load of the meal, inhibiting further food intake. A limited, but diverse number of pathways are available by which a blood-borne substance can influence the CNS. Direct passage of a substance across the BBB is one of the them. Passage across the BBB has been described for several peptides and regulatory proteins with effects on feeding, including leptin (Banks et al., 1996; Banks, 2001; Banks and Lebel, 2002; Banks et al., 2002b), pituitary adenylate cyclase-activating polypeptide (PACAP) (Banks et al., 1993; Nonaka et al., 2002), tumor necrosis factor-α (Banks et al., 1995a; Banks et al., 2001b), interleukin-6 (Banks et al., 1994; Banks et al., 1995a; Banks et al., 2001a), insulin (Baura et al., 1993; Banks et al., 1997; Banks and Kastin, 1998; Banks et al., 1999; Banks et al., 2000), ghrelin (Banks et al., 2002c), MSH (Wilson et al., 1984), and CART (Kastin and Akerstrom, 1999a). Here, we found that PYY crosses in both the blood-to-brain and brain-to-blood directions by non-saturable mechanisms.

We first determined whether intravenous I-PYY could cross the BBB, and measured its unidirectional influx rate (Ki). It's Ki of 0.49 ± 0.19 μl/g-min is similar to that of similar sized peptides shown to cross the BBB by non-saturable mechanisms (Banks et al., 1995b;Kastin and Akerstrom, 1999a;Kastin and Akerstrom, 1999c;Kastin and Akerstrom, 1999b;Banks et al., 2002a;Banks et al., 2002c). To determine whether the mechanism of entry had a saturable

component, we included 1 μg of non-radioactive PYY in the injection, an amount shown to substantially inhibit transport systems for peptides and regulatory proteins such as leptin (Banks, et al., 1996; Banks, 2001; Banks and Lebel, 2002; Banks et al., 2002b), PACAP (Banks et al., 1993; Nonaka et al., 2002), tumor necrosis factor-α (Banks et al., 1995a; Banks et al., 2001b), interleukin-6 (Banks et al., 1994; Banks et al., 1995a; Banks et al., 2001a), and insulin (Baura et al.,1993; Banks et al., 1997; Banks and Kastin, 1998; Banks et al., 1999; Banks et al., 2000). Inclusion of unlabeled PYY did not inhibit uptake of I-PYY, showing that passage across the BBB was most likely by transmembrane diffusion. However, there was a small, statistically significant increase with inclusion of unlabeled PYY. Such a paradoxical increase is sometimes caused by saturable brain to blood transport or blood-borne binding factors. These possibilities were considered in subsequent experiments.

Capillary depletion studies confirmed that 69% of the total radioactivity taken up by brain after intravenous injection crossed the BBB to enter the parenchymal space of the brain. HPLC confirmed the stability of the labeled PYY in brain and blood. Both acid precipitation and HPLC demonstrated that the radioactivity entering the brain represented intact I-PYY.

The percent of the intravenously injected dose taken up per ml of brain is a function of the rate of entry and presentation to the brain via the circulation. As shown in figure 2A, the half-time disappearance rate from blood was 13.1 min. The %Inj/g is important in determining an effective intravenous dose and is a function of BBB permeability and blood concentration. As shown in figure 2B, the peak value for %Inj/g occurred about 2 min after iv injection and was

0.176 ± 0.035 %Inj/g. This is similar to the uptake of other peptides and proteins, such as [Tyr^{10}]secretin-27 (Banks et al., 2002a) and ghrelin (Banks et al., 2002c), that are known to effect brain function by virtue of their abilities to cross the BBB. Secretin has been shown to have a peak %Inj/g of 0.180 while human ghrelin was 0.063.

Brain perfusion was also used to examine whether I-PYY could cross the BBB. Results showed that I-PYY crossed the BBB by a non-saturable mechanism and so agreed with the iv results. However, the entry rate was 4-5 times faster as measured by brain perfusion. One mechanism that might explain these results is the presence of a blood-borne binding factor. Since brain perfusion replaces blood with buffer, the effects of binding factors is negated. This could also explain the paradoxical increase seen when unlabeled PYY was injected intravenously.

We found that PYY could also cross from brain to blood. The relation between log (brain cpm) and time after the icv injection of I-PYY was statistically significant, and the half-time disappearance rate was 37.8 min. This rate and the finding that there was not a saturable component shows that brain-to-blood passage is likely to occur with the reabsorption of cerebrospinal fluid into the blood. Brain-to-blood passage either by saturable or non-saturable mechanisms can contribute significantly to blood levels and affect the functioning of peripheral tissues (Martins et al., 1997;Banks and Kastin, 1997;Clark et al., 1983;Chen and Reichlin, 1998;Chen et al., 1997;Yao et al., 1993). The lack of a saturable component makes it unlikely that efflux contributes to the paradoxical increase in I-PYY uptake when unlabeled PYY was co-injected intravenously.

In conclusion, we found that PYY crosses the BBB bi-directionally by non-saturable

processes. The amount of PYY entering the brain after CNS injection is an amount that has been found for similar sized substances to induce effects on the CNS. A brain-to-blood efflux of PYY suggests that PYY produced in brain could contribute to blood levels, thus influencing peripheral function. We propose that passage across the BBB may be an important mechanism by which blood-borne PYY mediates its effects on the CNS.

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Footnotes:

Supported by VA merit Review (WAB), R01 NS41863 (WAB) and R01AA12743 (WAB).

Legends for Figures

Figure 1. Uptake of I-PYY by brain after iv injection. The slope of the line measures Ki, the unidirectional influx rate into brain. Ki (slope) = 0.49 ± 0.19 µl/g-min, Vi (intercept) = 11.81 ± 1.57 µl/g-min, r = 0.682, $P < 0.05$, n = 10 mice. The brain/serum ratio (µl/g) of I-PYY, and I-PYY + unlabeled PYY 5 min after iv injection is shown in the inset. The brain/serum ratio increase with unlabeled PYY was statistically significant. n = 14 mice/group.

Figure 2A. Percent of the injected dose of I-PYY per ml of serum (%Inj/ml) as a function time. The half-time disappearance rate was 13.1 min, r = 0.71, $P < 0.001$, n = 5 mice/time.

Figure 2B. Percent of the injected dose of I-PYY found per g of brain (%Inj/g). The peak value for %Inj/g was at about 2 min after iv injection and was measured to be 0.176 ± 0.035.

r = 0.04, n = 5 mice/time.

Figure 3. The transport of I-PYY with or without unlabeled PYY (1 µg/ml) when studied by the brain perfusion method. The transport rates were 2.34 ± 0.74 µl/g-min (I-PYY, n = 18) and 2.11 ± 0.82 µl/g-min (+ unlabeled PYY, n = 10). There was no statistically significant difference between the two groups.

Figure 4. HPLC of I-PYY data. Upper panels are processing controls and show degradation of I-PYY which occurred ex vivo (during processing) in brain and serum. For brain control, 42 %

eluted as intact I-PYY and for serum 69 % eluted as intact I-PYY. Bottom panels show elution of radioactivity recovered from brain and serum 5 min after iv injection of I-PYY. Values shown are the means of 2 experiments. For brains, a mean of 37 % eluted as intact I-PYY and 66 % for serum. After correction for ex vivo degradation, 88 % (37/42 = 0.88) of radioactivity from brain and 96 % (66/69 = 0.96) of radioactivity eluted as I-PYY.

Figure 5. log (Brain CPM) of I-PYY at 0, 2, 5,10, and 20 min after icv injection. The half-time disappearance rate was 37.8 min, $r = 0.653$, $P < 0.001$, $n = 10$ mice/time. The inset shows %Inj/Brain of I-PYY, and I-PYY + unlabeled PYY 10 min after icv. injection. There was no statistical difference between these two groups ($n = 16$ mice/group).

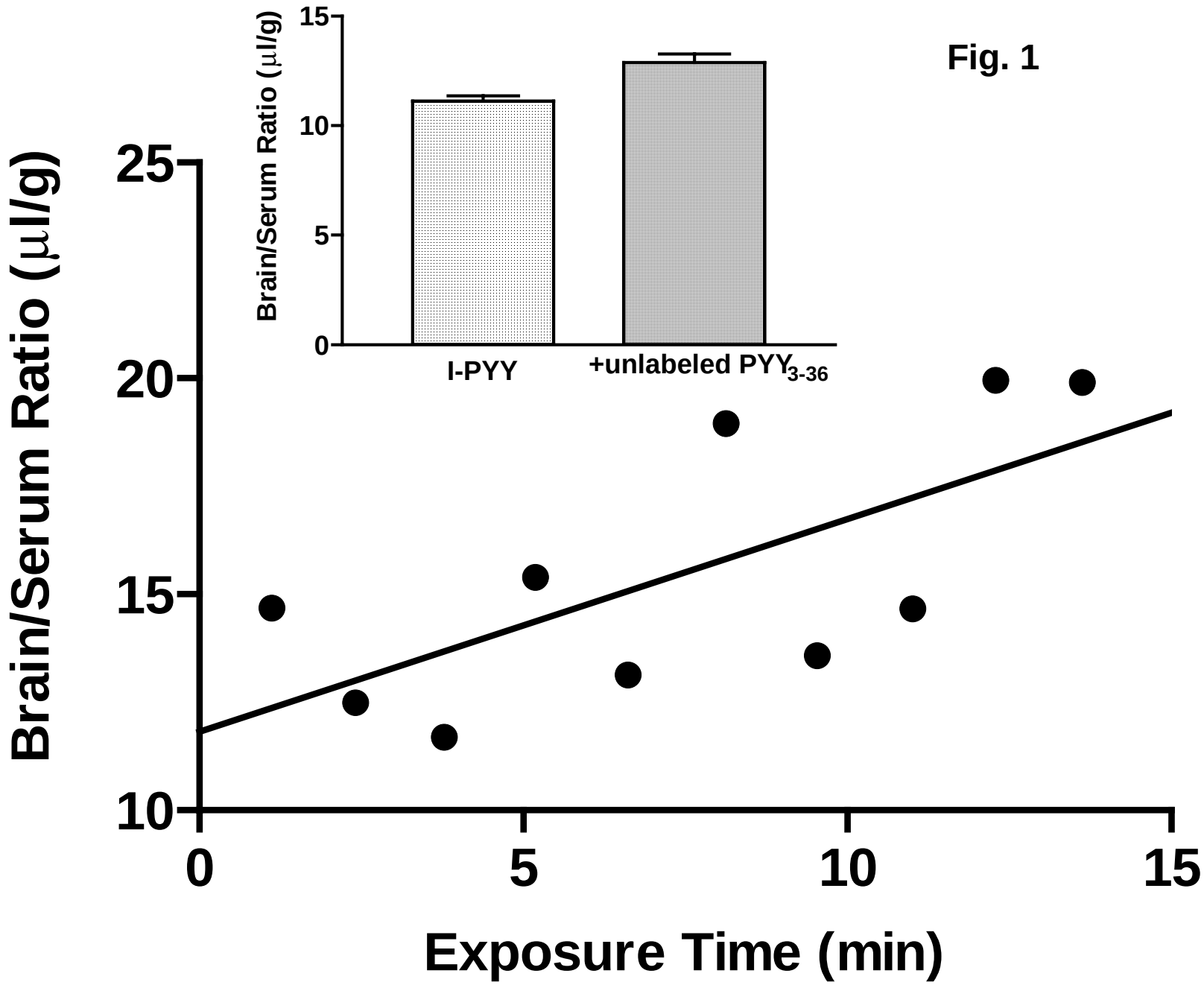


Fig. 1

Fig. 2

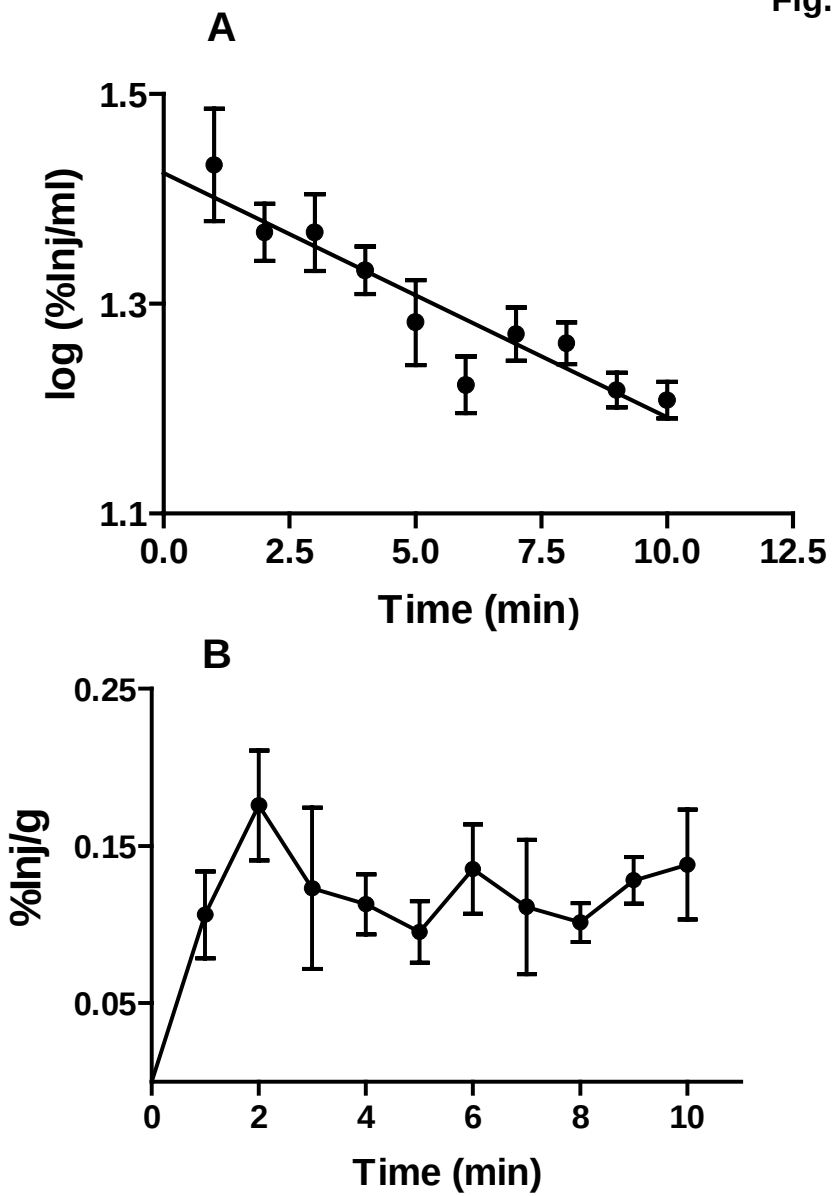


Fig. 3

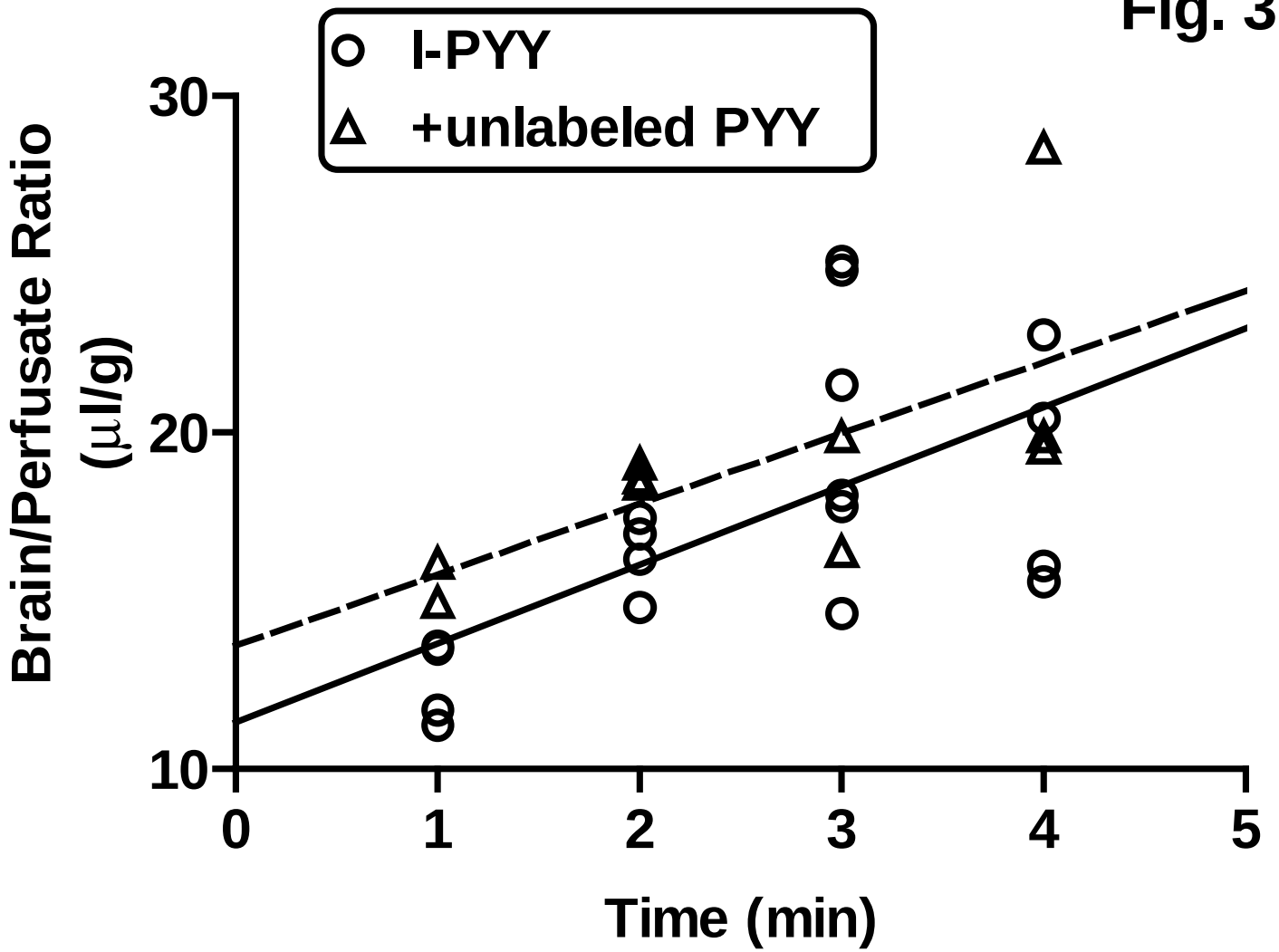
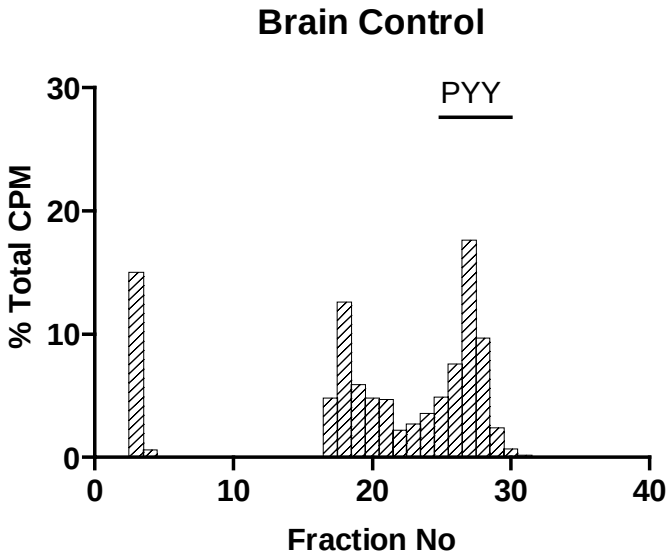
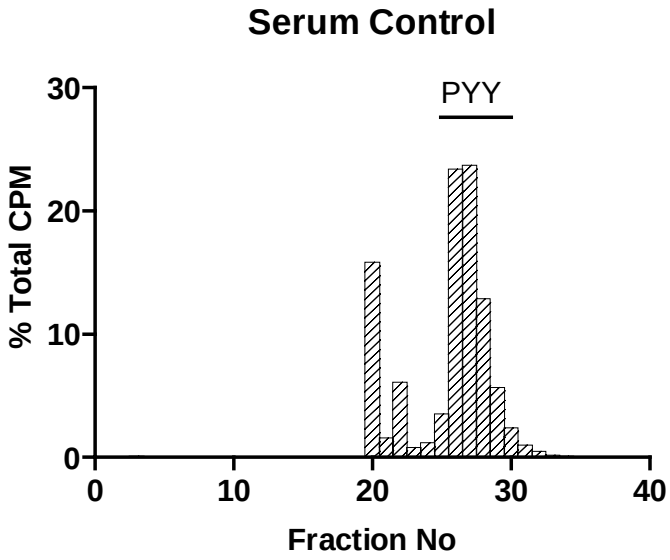
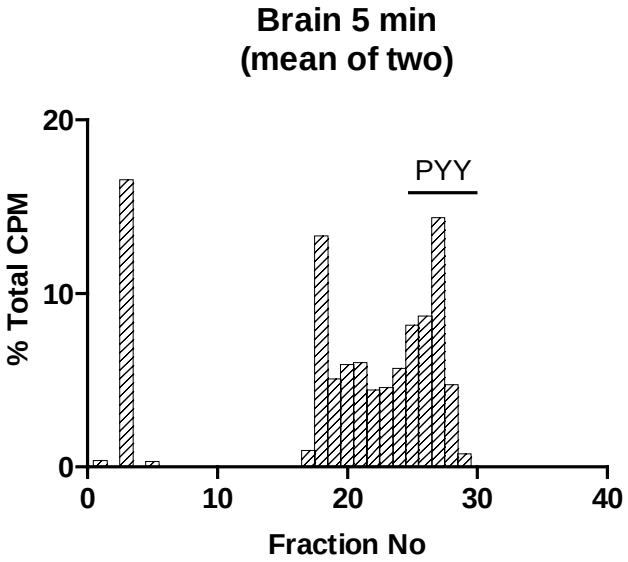


Fig. 4







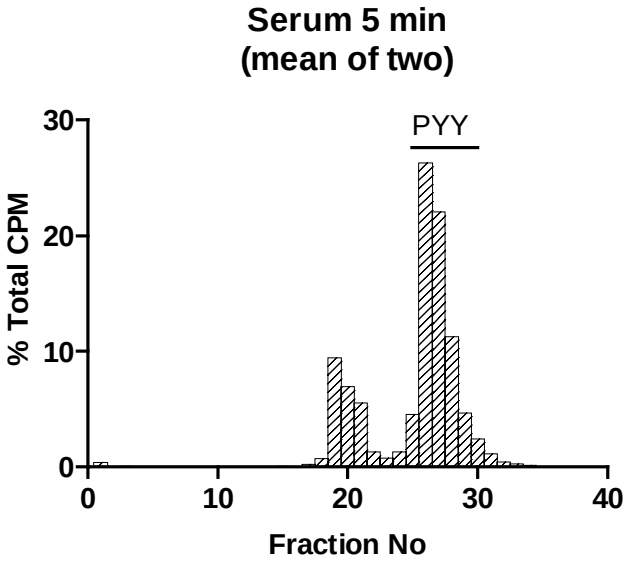


Figure 5

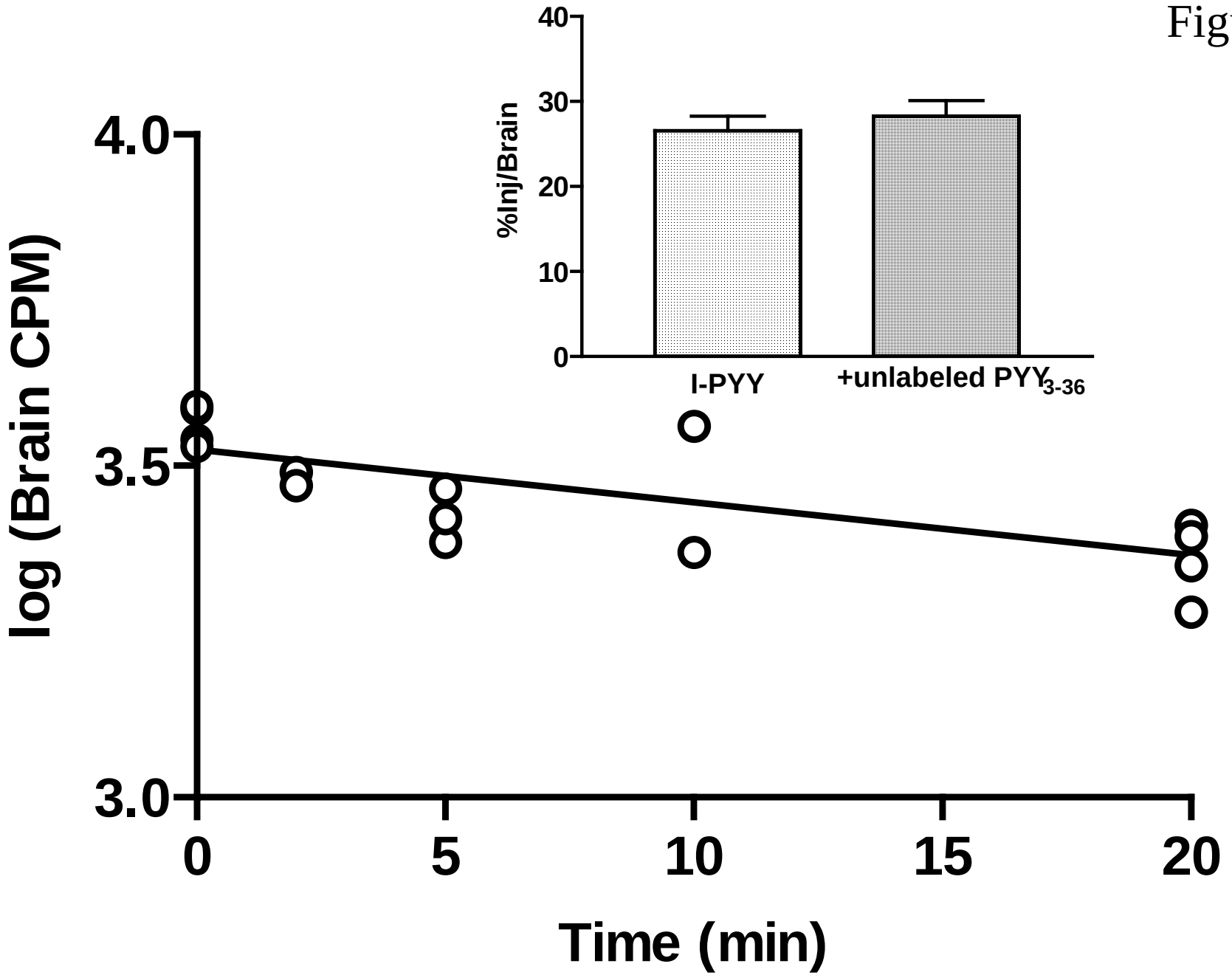


Table 1.
Acid Precipitation of I-PYY by brain and serum.

Time (min)	Brain	Serum
1	91.4 ± 0.7 (n=2)	104.7 ± 0.5 (n=4)
5	98.0 ± 3.2 (n=2)	102.3 ± 0.9 (n=4)
10	84.7 ± 6.0 (n=3)	96.9 ± 1.2 (n=4)