Radioimmunoassay study of hepatic clearance and disappearance half-time of somatostatin and vasoactive intestinal peptide in dogs*

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SUMMARY The effects of hepatic transit on concentrations of synthetic cyclic (ovine) somatostatin and of highly purified (porcine) vasoactive intestinal peptide were studied in five conscious dogs prepared with indwelling portal catheters. The peptides were infused *via* peripheral vein catheters or the portal catheters for 40 minutes at actual integrated doses of 2.8 pmol/kg/min for vasoactive intestinal peptide and 8.2 pmol/kg/min for somatostatin. Specific radioimmunoassays were used for the measurement of the circulating peptides. As estimated from the plateau peptide concentrations achieved in a hindleg vein during the portal and the peripheral infusions, the transhepatic loss of immunoreactive vasoactive intestinal peptide was $72.9 \pm 2.1\%$, against $27.5 \pm 12.5\%$ for somatostatin. For the given doses of peptides infused systemically, the half-life, metabolic clearance rate, and distribution volume were, respectively, 1.80 ± 0.1 minutes, 39.3 ± 5.2 ml/kg/min, and 103.7 ± 14.8 ml/kg for vasoactive intestinal peptide, and 1.0 ± 0.2 minutes, 95.2 ± 12.5 ml/kg/min, and 114.7 ± 6.0 ml/kg for somatostatin. These results indicate that (ovine) somatostatin and (porcine) vasoactive intestinal peptide are both readily cleared from plasma in dogs. In the present experimental conditions, vasoactive intestinal peptide, but not somatostatin, was rapidly altered through hepatic transit so that it escaped recognition by the assay system.

Somatostatin, a tetradecapeptide first isolated from ovine hypothalamus,¹ and vasoactive intestinal peptide (VIP), an octacosapeptide characterised from porcine upper intestine,² are two of the peptides common to the central nervous system and the digestive tract. Both are widely distributed in the gastrointestinal tract and the pancreas,^{3, 4} and exogenous preparations exhibit wide spectra of biological effects.^{5, 6} Physiological mechanisms of release and actions, however, are unclear for both.

The two peptides are considered local transmitters, either paracrine or neurocrine, although substances displaying somatostatin- and VIP-immunoreactivity have been detected in portal and

peripheral plasma.^{7, 8} The physiological importance of these circulating components is not known.

The effect of hepatic transit on VIP originating from the digestive tract remains controversial. Physiological studies have shown that the biological activity of the peptide is reduced when it is infused into the portal vein instead of the peripheral circulation,^{9, 10} but Strunz and colleagues¹¹ failed to confirm this apparent hepatic inactivation. The hepatic inactivation of somatostatin has not been investigated.

The present work was undertaken to study comparatively the effects of hepatic transit on (ovine) somatostatin and (porcine) VIP in conscious dogs. Results derived from peripheral infusions were used to evaluate kinetics of uptake and distribution of the two peptides in this species.

Methods

Synthetic cyclic somatostatin (Serono Pharm Präp GmbH, Freiburg/Breisgau, West Germany) and highly purified porcine VIP (a gift from Professor V

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Mutt, Karolinska Institutet, Stockholm) were dissolved in 0.1 M ammonium acetate pH 5.5 (somatostatin) and 0.05 M hydrochloric acid -0.1 M sodium chloride (VIP), and stored at -80°C . Before each experiment, an aliquot of the solution was diluted appropriately with 0.15 M sodium chloride and kept at 4°C until injected.

Five mongrel dogs (20–28 kg) that had been previously prepared with a Thomas gastric cannula, a Gregory cannula (positioned in the second part of the duodenum), and an indwelling portal catheter were used in the study. The silastic catheter in the portal lumen was inserted *via* a splenic tributary and was connected to a polyethylene catheter which was brought through the abdominal wall and pulled into the subcutaneous tissue of the back. Tests were not begun until 10 days after surgery. In no dog was there any sign of impaired gastric emptying. All portal catheters were patent for blood sampling at the time of the experiments, which were performed after an 18 hour fast without water restriction.

On the day of the test, the portal catheter was brought out through a small incision made under local anaesthesia and was connected to a no. 16 Intracath attached to a three-way stopcock. Another no. 16 Intracath was placed in a foreleg vein and connected to intravenous tubing identical with the one in the portal vein, so as to infuse the peptides *via* similar lines. Both lines were used for constant administration of isotonic saline or of the peptide solution, as appropriate, at a rate of 45 ml per hour with an infusion pump (Harvard Apparatus Co, Inc, Millis, Mass). A no. 16 Intracath was placed in a hindleg vein for blood sampling and was kept patent with a slow saline infusion.

After 30 minutes of recovery and a 20 minute basal period, 10.2 pmol/kg/min somatostatin or 5.0 pmol/kg/min VIP was infused for 40 minutes *via* the portal or the peripheral line. The initial peptide and route of infusion were randomised. The second test, performed on the same day after one hour of recovery, used the other peptide infused *via* the other line. Two days later, each dog was given the peptides in the same order, but *via* the reverse routes of administration.

Blood from the hindleg catheter was collected in tubes with Trasylol (800 U/ml) and heparin (8 U/ml) every 10 minutes before the peptide infusion, at 10, 20, 30, 35, and 40 minutes during it, and at one, two, three, four, six, eight, 10, 12, 14 and 16 minutes after it. The fraction of somatostatin or VIP lost in the infusion line was estimated in a parallel experiment in which the two peptides, prepared along the same protocol as for *in vivo* studies, were perfused through five unused lines each. The aliquots of concentrated somatostatin and VIP used for these

controls were taken from the same batch as those used for both the standard curves and the *in vivo* experiments. The effluent from each line was collected on ice during the 40 minute infusion, vortexed, and assayed immediately for the given peptide. The average figures then obtained were taken as the integrated doses actually delivered to the dogs, and were used for calculations. No attempt was made to study the adsorption kinetics of somatostatin and VIP on plastic tubing. With the present infusion sets, the integrated loss of peptide on tubing was $18.5\% \pm 11.0\%$ (SEM) for somatostatin and $44.0\% \pm 4.4\%$ for VIP.

Half-life, metabolic clearance rate (MCR), and distribution volume (DV) were estimated from the data of systemic infusion, using as incremental plateau value the mean incremental plasma peptide concentrations at 35 and 40 minutes during infusion. The half-life was calculated by the relation $T_{50} = 0.693/k$, where k is the slope of the regression line between the natural logarithm of incremental plasma peptide concentration, expressed as percentage of plateau value, and time in minutes. The MCR, in ml/kg/min, was equivalent to D/P , where D is the dose of peptide in pmol/kg/min and P the incremental peptide concentration at plateau value in pmol/ml. The DV, in ml/kg, was equal to D/Pk .

The relation $P_s - P_p/P_s$, where P_s and P_p are the mean incremental plasma peptide concentrations achieved during systemic (P_s) and portal (P_p) infusion, was used as an index of the fraction of peptide lost through a single hepatic transit.

The plasma somatostatin concentration was measured with a radioimmunoassay previously described,¹² which was improved for determination of somatostatin in plasma by using antiserum 56D.¹³ This antiserum, raised against synthetic cyclic somatostatin conjugated with bovine albumin through carbodiimide condensation¹² recognises primarily the 8–11 portion of the tetradecapeptide, as checked with analogues resulting from alanine substitution in positions 4, 6, 8, and 11 (a gift from Dr J Rivier and Professor R Guillemin), which have relative potencies (ID₅₀) of 1.0, 0.04, 0.008 and <0.0004 , respectively, as compared with the unaltered molecule. 1-tyrosyl somatostatin (Serono) was labelled to a specific activity of 150–220 $\mu\text{Ci}/\mu\text{g}$ with ^{125}I (Amersham) using lactoperoxidase. It was purified by chromatography on a 1×10 cm column of carboxymethylcellulose CM 52, equilibrated and loaded in 0.05 M ammonium acetate pH 5.5 and developed with 0.5 M ammonium acetate pH 6.0. The sensitivity was 0.9–1.8 pmol/l of incubate. The within-assay (35 pairs) and between-assay (53 pairs) variations were 7.1% and 12.3%, respectively (coefficient of variation). The

recovery of 2, 4, 8, and 16 pg somatostatin per tube in plasma varied from 80.4% to 98.1% (seven determinations per dose).

VIP was measured with a radioimmunoassay recently reported¹³ using antiserum 76A, which shows only little reactivity towards the C-terminal portion of the porcine peptide (ID 50:0.1) and no reactivity towards the N-terminal part of the molecule (<0.0001 , a gift from Professor M Bodanszky), as compared with the whole molecule ($=1.0$). Highly purified porcine VIP was labelled to a specific activity of 200–300 $\mu\text{Ci}/\mu\text{g}$ with ^{125}I using lactoperoxidase, and was purified on a 1×10 column of SP C25 Sephadex equilibrated and loaded in 0.2 M ammonium acetate (pH 5.0) and developed with 0.8 M ammonium acetate (pH 5.6) with 5% human plasma. The sensitivity was 0.5–1.0 pmol/l. The estimates for within-assay, between-assay variation, and recovery, tested as for somatostatin, were 7.0%, 7.2%, and 79.7% to 100.4%, respectively.

The two systems appeared to be specific against the other gastrointestinal peptides and brain peptides¹² tested at concentrations of 100 ng per tube.

Both assay systems were used simultaneously to measure VIP and somatostatin in the same sample. A standard curve in charcoal-treated plasma was generated for each dog studied. Unknown samples and peptide-free plasma were acidified to pH 5.5–6.0 with 0.4 M acetic acid before incubation. Incubation lasted for five days at 4°C in 0.05 M potassium phosphate–0.1 M sodium chloride pH 5.5, with addition of tracer after 24 hours and of second antibody (used for separation of bound and free peptide) added on the fourth day. Results were expressed as pmol/l and converted into pmol/ml for calculations. The sensitivity limit of the individual assay was taken as the actual value for samples with concentrations below the limits of detection.

Results

Systemic infusion of VIP at 5.0 pmol/kg/min (actual value as measured: 2.8 pmol/kg/min) induced an increase in peripheral plasma VIP from 4.0 ± 0.8 pmol/l to a mean plateau value of 81.0 ± 10.1 pmol/l. An increase from 4.5 ± 0.8 to 25.6 ± 3.4 pmol/l was found when the same dose was delivered into the portal vein (Fig. 1). The estimated loss of peptide on hepatic transit was $72.9 \pm 2.1\%$. After the infusion was stopped, the plasma VIP concentration decreased to baseline in 14 minutes after systemic administration, and in eight minutes after portal infusion. Estimated from the results of systemic infusion, the half-life (Fig. 2), metabolic clearance rate, and apparent volume of distribution of porcine

Fig. 1 Mean $\pm$ SE peripheral plasma VIP concentration in five conscious dogs given 5.0 pmol/kg/min (actual dose 2.8 pmol/kg/min) highly purified porcine VIP intraportally or systemically.

Fig. 2 Regression lines of incremental VIP and somatostatin concentrations in peripheral plasma (expressed as percentage of plateau value) with time after stopping an infusion of actual 2.8 pmol/kg/min VIP or 8.2 pmol/kg/min somatostatin delivered during 40 minutes. Mean incremental plateau value taken as 100% at end of infusion (zero time).

VIP were calculated to be 1.80 ± 0.1 minutes, 39.3 ± 5.2 ml/kg/min, and 103.7 ± 14.8 ml/kg, respectively.

Systemic infusion of 10.2 pmol/kg/min (actual dose as measured: 8.2 pmol/kg/min) somatostatin caused plasma somatostatin to increase from 15.2 ± 6.8 to a mean plateau value of 108.6 ± 14.0 pmol/l, as compared with an increase with portal infusion from 18.4 ± 6.5 at basal to 83.8 ± 16.8 pmol/l (Fig. 3). The apparent loss of somatostatin on hepatic transit was $27.5 \pm 12.5\%$. At the end of the infusion, the curves of plasma somatostatin after portal and systemic infusions were superimposable, and the mean values for both returned to baseline in four minutes (Fig. 3). Estimated from the data of systemic infusion, the half-life (Fig. 2) was calculated by extrapolation to be 1.0 ± 0.2 minutes. The metabolic clearance rate was 95.2 ± 12.5 ml/kg/min and the apparent volume of distribution, 114.7 ± 6.0 ml/kg.

Fig. 3 Mean $\pm$ SE peripheral plasma somatostatin concentrations in five conscious dogs given 10.2 pmol/kg/min (actual dose 8.2 pmol/kg/min) synthetic cyclic somatostatin intraportally or systemically.

Discussion

The present study was undertaken to evaluate comparatively the effects of hepatic transit on ovine somatostatin and porcine VIP administered to conscious dogs at single doses known to induce significant biological effects. Ovine somatostatin delivered intravenously at a theoretical rate of 1 μ g/kg/h (10.2 pmol/kg/min) has been shown to inhibit the postprandial release of gastrin and acid response in man,¹⁴ as well as the release of insulin and glucagon in dogs.¹⁵ Porcine VIP, delivered at the theoretical dose of 1 μ g/kg/h (5.0 pmol/kg/min), significantly inhibited the meal-induced acid response and release of gastrin in dogs.¹⁶ As the

adsorption of secretin, which is structurally related to VIP, on plastic tubing, amounts to nearly 50% of doses which were similar to those used here for VIP and somatostatin,¹⁷ the adsorption of these preparations on the infusion sets was checked in parallel control experiments. Somatostatin was only slightly adsorbed, while the integrated loss of VIP during a 40 minute infusion almost reached 50% of the initial dose, a figure rather similar to that reported in the single study in which this estimation was reported.¹⁸ We did not attempt to delineate the adsorption kinetics of somatostatin and VIP on plastic tubing, because the infusion lines were strictly replicated for the portal and the peripheral infusions. However, the fact that the values of plasma somatostatin and of plasma VIP at 35 and 40 minutes under peripheral or portal infusion did not vary from the corresponding 20 and 30 minute estimates by more than 10%, does suggest that the delivery of each peptide was fairly constant during the second 20 minute period.

Somatostatin was apparently affected only moderately by hepatic transit (uptake or alteration of $27.5 \pm 12.5\%$ of peptide infused through the portal system). Whether the fraction of peptide resisting hepatic transit was biologically active was not tested in the present study. However, antiserum 56D recognises a portion of the tetradecapeptide which appears to be essential for biological activity, especially the phenylalanine residue in position 8.¹⁹ Our data do not exclude a transhepatic alteration of the N-terminal portion of the tetradecapeptide, but provide circumstantial evidence for possible effects of the somatostatin originating from the digestive system on post-hepatic target organs.

From the present results, it appears that somatostatin (at least the ovine tetradecapeptide) is readily cleared from plasma in dogs, with a half-life of approximately one minute. This figure is roughly comparable with that (1.82 minutes) derived from studies in dogs by Schusdziarra and colleagues.¹⁵ These authors recorded an increase of plasma somatostatin of about 300% above basal for an apparent dose of approximately 10.0 – 12.0 pmol/kg/min, as compared with the 600% increase obtained with 10.2 pmol/kg/min in the present study. The discrepancy appears to result from different estimates for basal plasma somatostatin concentrations in dogs, probably because of different antiserum specificities or assay methodologies. Half-life values of 1.1 – 3.0 minutes were recently reported in man,²⁰ with MCR values of 15 – 45 ml/kg/min. The figure obtained here and that given by Schusdziarra and colleagues¹⁵ in dogs suggest that somatostatin may be cleared more rapidly from plasma in dogs than in man.

In experimental conditions identical with those applied for somatostatin, the half-life of porcine VIP was close to two minutes. This figure is intermediate between those reported by Domschke and colleagues¹⁸ in man and by Modlin and colleagues²¹ in anaesthetised pigs, both close to one minute, and that (about three minutes) reported by Strunz and colleagues¹¹ in dogs with portacaval transposition. As to other aspects of VIP kinetics, the present figures are different from those obtained by Modlin and colleagues²¹ and Domschke and colleagues.¹⁸ Using the same VIP assay for the two studies, these groups recorded a distribution volume of VIP of less than 2% of the body weight both in man and pigs, as compared with a figure of about 10% in the present experiment. As the doses of peptides actually infused were controlled in all these studies, and as at least some of these doses were comparable, the most likely reason for this discrepancy is the difference between the plasma VIP levels achieved under infusion, which were lower in our study than in theirs. The same batch of pure VIP was used here for the *in vivo* infusions, and *in vitro* adsorption controls, and for setting up the standard curves, so that it is unlikely that the comparatively low plasma VIP levels achieved here reflected simply the administration of a spuriously low amount of peptide. Though the metabolism of porcine VIP may be different in dogs and in other species, as suggested by the fact that the half-life in dogs¹¹ was found to be three times as high as those in man¹⁸ and in pigs²¹ in the same assay system, an additional explanation may be that different components with VIP immunoreactivity, circulating in plasma during a prolonged infusion of the peptide, are recognised to different extents by the various antisera. This hypothesis would require collaborative studies investigating the molecular patterns of the immunoreactive components detected in the different systems.

From studies performed in conscious dogs with portacaval transposition, Strunz *et al.*¹¹ reported that neither the biological activity nor the immunoreactivity of porcine VIP was affected by hepatic transit. These results were clearly at variance with previous evidence of biological inactivation of VIP in anaesthetised dogs⁹ and cats¹⁰ and immunological alteration of the peptide in anaesthetised pigs.²¹ As anaesthesia may well have interfered with the hepatic metabolism of VIP, the present study was undertaken to compare the fate of VIP with that of somatostatin in conscious dogs. The results clearly support previous evidence for rapid alteration of VIP on single hepatic transit in dogs. As the present VIP assay recognises only slightly the C-terminal portion and not at all the N-terminal portion of the molecule, the sharp difference between the plateau

levels achieved under portal versus peripheral infusion may have resulted either from complete uptake of the molecule or, alternatively, from its cleavage into fragments poorly recognised by our antiserum. Both alternatives, however, appear likely to result in reduction of biological potency of the same order as that depicted by Kitamura and colleagues⁹ and of Konturek and colleagues.^{10 22 23}

Somatostatin and VIP are two of the peptides shared by the nervous system and the digestive tract. The physiological status of each peptide remains to be elucidated, though the current line of evidence favours somatostatin as a paracrine substance and VIP as a neurotransmitter. Although they were obtained after infusing single test doses of non-canine sequences, the present results suggest that a possible physiological role of digestive somatostatin beyond the liver should not be overlooked, while the rapid hepatic clearance of immunoreactive porcine VIP, along with the presence of VIP receptors on the hepatocytes²⁴ and the fact that the exogenous peptide induces glycogenolysis,²⁵ suggests a possible gut-liver axis for the peptide released from gastrointestinal stores.

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