Human and porcine immunoreactive gastric inhibitory polypeptides (IR-GIP) are not identical

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Immunoreactive gastric inhibitory polypeptide (IR-GIP) from human and porcine intestine was quantified by radioimmunoassay and the molecular forms characterised by gel permeation and reverse-phase high pressure liquid chromatography (HPLC). Gel filtration revealed two major immunoreactive peaks corresponding to the previously described 5-kDa and 8-kDa molecular forms, which appeared similar in both species. Isocratic reverse-phase HPLC revealed that the major immunoreactive GIP peak (5-kDa) in the human tissue eluted earlier than the corresponding porcine molecular form, indicating the latter to be less hydrophobic. These findings suggest significant species differences between human and porcine GIP.

Gastric inhibitory polypeptide *Glucose-dependent insulinotropic peptide*
Immunoreactive GIP *Molecular form* *Species difference* *Gel filtration* *Reverse-phase HPLC*

1. INTRODUCTION

GIP is a 42 amino acid polypeptide hormone which has been known as both gastric acid inhibitory polypeptide [1] and glucose-dependent insulinotropic polypeptide [2,3]. The polypeptide was initially isolated from porcine upper small intestine [4] where it has been localised to a discrete endocrine cell type called the K cell [5]. This and other studies [6,7] have demonstrated the existence of two immunoreactive peaks of GIP eluting from gel columns. The major immunoreactive peak elutes later and in the position of the pure porcine GIP standard (5 kDa); the early peak of a larger molecular form, as yet not isolated, has an estimated molecular mass of 8 kDa. Reverse-phase high pressure liquid chromatography (HPLC) of partially purified porcine GIP [8] has revealed 3 peaks denoted as I, II and III. Peak III is the major peak and is the 5-kDa molecular form. One of the other peaks has been speculated to be des-Tyr^1-Ala^2-GIP [8].

IR-GIP is regularly measured by radioimmunoassay using porcine GIP as standard [6,9–11]. Although it is assumed that human GIP is very similar to porcine GIP, the human form is as yet uncharacterised. Concentrations of IR-GIP in human subjects vary widely depending on which laboratory performs the radioimmunoassay [6,12]. This study was undertaken to determine whether porcine and human IR-GIP are identical using gel permeation and reverse-phase HPLC techniques.

2. EXPERIMENTAL

Fresh porcine duodenum was removed from 4 healthy pigs (mean weight 36 ± 4 kg) immediately after death. The mucosal layer was dissected from the underlying muscle and extracted. The tissue was diced and then plunged into boiling 0.5 M acetic acid for 10 min [3]. The extracts were stored at −20°C and briefly thawed and centrifuged prior to radioimmunoassay and chromatography.

Human duodenum was obtained at post mortem and IR-GIP extracted in an identical manner to the porcine tissue and stored at −20°C.

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Tissue concentrations of IR-GIP were determined using the radioimmunoassay in [6]. Briefly, the antibody appears to require the entire sequence for complete cross-reaction as neither large C- nor N-terminal fragments react. The antibody does not cross-react with any other known peptide hormone other than natural pancreatic glucagon (1% cross-reaction) and can detect changes of 0.8 fmol/tube with 95% confidence. Gel permeation was performed on a 100 × 1.5 cm column of Sephadex G-50 superfine (Pharmacia) eluted with 0.06 M phosphate buffer (pH 7.4) containing 0.15 mmol/l bovine serum albumin and 0.2 M sodium chloride at a flow rate of 12.3 ml/h. Fractions of 2.2 ml were collected and assayed for IR-GIP. All gel chromatography was carried out at 4°C. Elution constants (K_{av}) were calculated as in [14]. Isocratic reverse-phase HPLC was performed on the gel-filtered peaks of 5-kDa IR-GIP on an octadecylsilicyl-silica column (5 μm Techsil C_{18}, HPLC Technology Ltd, Cheshire) using 32.5% acetonitrile/water with 0.2% trifluoroacetic acid as eluent. The flow rate was 1 ml/min and 1-ml fractions were collected and assayed for IR-GIP. Prior to HPLC gel-filtered extracts and standards were prepared on activated micro-Bondapak C_{18} cartridges (Sep-pak, Waters) eluted with 50% acetonitrile/water with 0.2% trifluoroacetic acid. After centrifugation (12000 × g for 5 min) this was further diluted to 25% acetonitrile before injection. Oxidation of the pure porcine standard (10 pmol) was performed by adding 10 μl of 0.3 M hydrogen peroxide and allowing to react for 45 min. The reaction was terminated by the addition of 700 μl of 2 M acetic acid. After preparation the sample was columned on the HPLC system.

3. RESULTS

IR-GIP concentrations in human and porcine duodenal mucosa were 108.4 ± 16.1 and 80.8 ± 21.2 pmol/g, respectively. The gel filtration profiles obtained are shown in fig.1. Porcine IR-GIP eluted in two major peaks – an early peak with a K_{av} of 0.31 ± 0.01 (n = 4) and a subsequent much larger peak with a K_{av} of 0.53 ± 0.02 (n = 4). The early peak comprised 8% of total IR-GIP. The profile of human duodenal IR-GIP was very similar to the porcine one and the K_{av} values were almost identical (0.33 ± 0.01 and 0.55 ± 0.02, respectively). The early peak comprised 13% of total IR-GIP. Pure porcine standard eluted with a K_{av} of 0.54 ± 0.01. (n = 4).

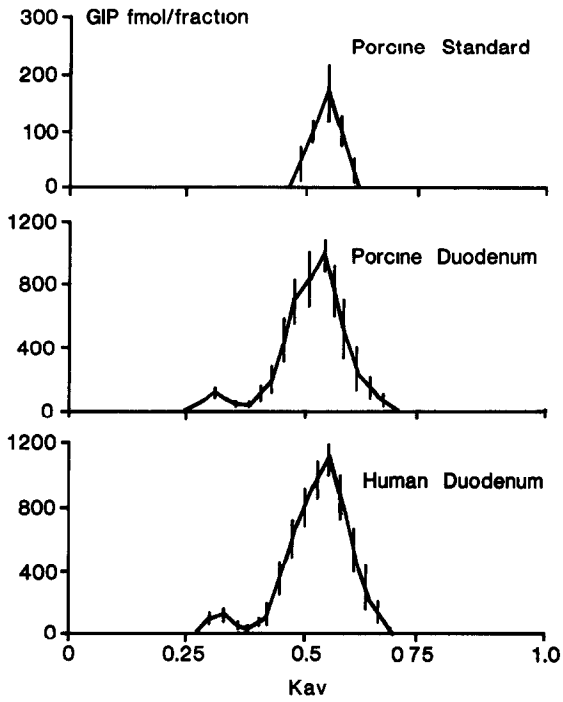

Fig.1. Sephadex G-50 gel filtration of GIP-immunoreactive material from porcine and human duodenal mucosa. Samples for chromatography were centrifuged and the supernatant applied to a 1 m × 100 cm column (Pharmacia) of G-50 superfine (Pharmacia) and equilibrated with 0.06 M phosphate buffer (pH 7.4) containing 0.15 M BSA (Miles). The column was eluted with the same buffer. Fractions (2.2 ml) were collected at a flow rate of 12.3 ml/h and assayed by radioimmunoassay for IR-GIP. The column was previously calibrated with Dextran blue and ^{125}I. The profile obtained with the pure porcine GIP (courtesy of J.C. Brown; M 5100) is included.

On isocratic reverse-phase HPLC, gel-filtered porcine duodenal mucosa IR-GIP (of K_{av} 0.53) was resolved into 3 components (fig.2): two small peaks eluting at 11 and 15 min and the major peak eluting last (21 min) and coinciding with the elution of the pure porcine standard under identical conditions. The profile of the elution of the human duodenal IR-GIP from a gel column (K_{av} 0.55) also revealed 3 peaks – as with the porcine tissue 2 small peaks at 9 min and 13 min retention time preceded the elution of the major immunoreactive

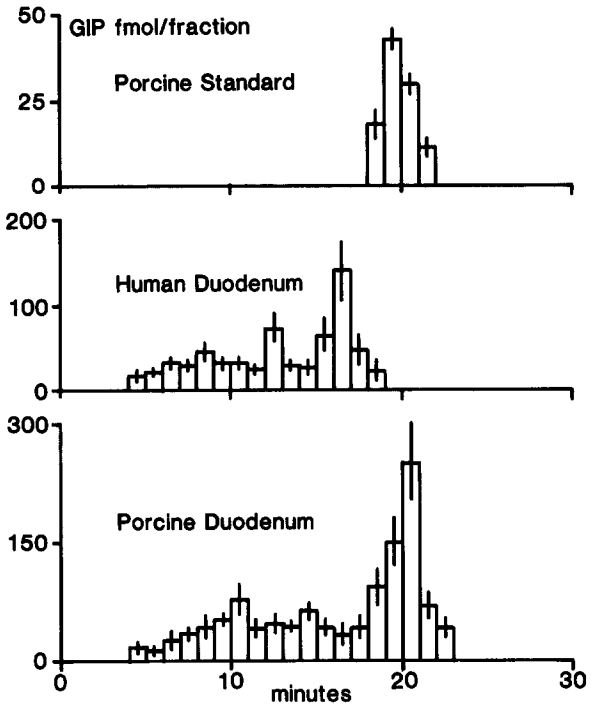

Fig.2. Isocratic reverse-phase HPLC of IR-GIP of the major peak (K_{av} 0.54) obtained by Sephadex G-50 gel filtration of human and porcine duodenal mucosa. Samples were prepared (see text) and applied to a 5 μm Techsil C_{15} column (HPLC Technology Ltd) equilibrated in 32.5% acetonitrile/water with 0.2% trifluoroacetic acid. The column was eluted with this buffer at a flow rate of 1 ml/min and 1-ml fractions collected. These were assayed for IR-GIP. The column was washed with 50% acetonitrile, 0.2% trifluoroacetic acid with water at the end of each run and then re-equilibrated with the eluting buffer.

peak (17 min). The major peak in the human tissue eluted before the corresponding porcine IR-GIP peak.

Oxidation of the pure porcine standard yielded 3 peaks: a major peak coinciding with the elution of the natural, unoxidised porcine standard and two small peaks coinciding with the elution of peaks at 11 and 15 min on the porcine duodenal extract HPLC profile. Further HPLC runs with 50% acetonitrile/0.2% trifluoroacetic acid failed to elute any other major immunoreactive peaks (<1% of total IR-GIP) of either human or porcine GIP. Recoveries from both G-50 and HPLC columns were between 82 and 104%.

4. DISCUSSION

The elution constants (K_{av}) of the major molecular forms of IR-GIP obtained from gel filtration studies agree with data in [4,6]. These molecular forms have been estimated to be 8 kDa (K_{av} = 0.32) and 5 kDa (K_{av} = 0.55). There is no apparent difference between porcine and human IR-GIP molecular forms from gel chromatography. However the HPLC profiles clearly differentiate between the major molecular form (5 kDa) of human and porcine duodenal IR-GIP. The human form elutes before the porcine IR-GIP of similar size indicating it is less hydrophobic. There are two other peaks of IR-GIP in both gel-filtered human and pig extracts which are of similar molecular size. These peaks were also generated by oxidation of the porcine standard. One is likely to represent the sulphoxide of the single methionine residue of porcine GIP; the other has been suggested to be des-Tyr^1-Ala^1-GIP [8].

Reverse-phase HPLC studies of partially purified GIP of porcine origin have revealed 3 peaks of IR-GIP [8]. The latter study employed much greater concentrations of peptide and a different solvent system and though recovery was <10% the profile appears very similar to that obtained here.

In conclusion, the findings show a species difference between human and porcine IR-GIP, the former being a less hydrophobic moiety. This difference in hydrophobicity is also evident in the oxidation/fragmentation products of the 5-kDa IR-GIP.

There is considerable controversy as to whether GIP plays an important role in the entero-insular axis [15]. Infusion of porcine GIP into man reproducing plasma concentrations seen after a meal fails to enhance significantly insulin release to IV glucose [16].

The difference between porcine and human IR-GIP however may lead to unequal cross-reaction of the two forms with various antibodies, which may partly explain the non-agreement in human IR-GIP concentrations as measured by different porcine GIP antibodies in various laboratories [6,12,17]. It is therefore possible that porcine GIP reagents underestimate the post-prandial rise of GIP in man (though it is remarkably similar in

magnitude to that seen in the pig). Thus infusion experiments to test the entero-insular axis in man may have employed insufficient GIP. Furthermore, the different molecular forms could possess different biological activities, so that porcine GIP infusions in man may be less active than the endogenous peptide. While endogenous GIP release by fat in man also failed to enhance significantly insulin release to IV glucose full exploration of the entero-insular axis in man must now await purification of human GIP.

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