Ontogeny of circulating gastrin and pancreatic polypeptide in the foetal sheep

Arthur Shulkes and Kenneth J. Hardy

Department of Surgery, University of Melbourne, Austin Hospital, Melbourne, Victoria, Australia

Abstract. Hypoacidity and hypergastrinaemia are reported in the newborn human. To investigate the ontogeny of gastrin, plasma gastrin concentrations were measured in chronically cannulated foetal sheep from 100 days gestation to term (145 days) and up to 16 days following delivery. Plasma pancreatic polypeptide (PP) concentrations were measured as a marker of vagal activity. Compared with adult values, foetal plasma gastrin concentration was low at 101–105 days, being 7 ± 1 pmol/l (mean ± SEM) but was greater than adult values from 130 days gestation and increased to 47 ± 5 at 141–145 days, and 90 ± 13 at 1–5 days post-partum. Maternal plasma gastrin levels during these periods ranged between 21 and 29 pmol/l and was not related to gestational age. Similarly, maternal plasma PP levels, which varied between 220 and 400 pmol/l, did not correlate with gestational age and did not differ significantly from non-pregnant sheep. Foetal plasma PP was low, 20 ± 3 at 101–105 days, rose to 92 ± 17 at 141–145 days, and increased to adult levels in the first week post-partum. Basal foetal and maternal plasma PP were inhibited by iv atropine injection. The increase in plasma PP may represent a maturity of vagal influence. Gastrin and PP have a trophic action on the gastro-intestinal tract, so the observation of significant levels of circulating gastrin and PP in the foetus suggests that they may be involved in maturation of the gastro-intestinal tract.

The adaptation of the foetus from parenteral to enteric feeding requires a dramatic change in the absorptive, secretory and motility characteristics of the gastro-intestinal tract (Grand et al. 1976). In the adult, the gastro-intestinal peptides have effects on secretion, absorption, motility and on the growth of the gastro-intestinal tract: their role in foetal life is unknown. We have utilised the chronically cannulated ovine foetus to examine the ontogeny of two gastro-intestinal peptides: gastrin and pancreatic polypeptide (PP).

The concentration of plasma gastrin has been reported to be elevated in the newborn human (Euler et al. 1977; Rodgers et al. 1978; Lucas et al. 1980a) at a time when acid secretion has apparently not commenced (Euler et al. 1977). However, the circulating levels prior to birth are not known. In addition, gastrin has been proposed to be a trophic factor on the gastro-intestinal tract (Johnson 1976) so the relationship between plasma gastrin levels and foetal development is of particular interest.

PP is a peptide located in specific cells of the pancreas (Larsson et al. 1976). In the adult, it is released by a meal and is under vagal cholinergic influence (Schwartz et al. 1976; Adrian et al. 1977). PP has been detected in the foetal pancreas (Paulin & Dubois 1978) but there is no information on circulating levels in utero. In the adult, plasma PP has been proposed as a marker of abdominal vagal activity (Schwartz et al. 1979). The levels of plasma PP during gestation could be a marker of the development of foetal abdominal vagal influence.

The purpose of this study was to determine the levels of plasma gastrin and PP in the foetus by repeated blood sampling during the last third of gestation and the first 2 weeks post-partum, and to determine the molecular forms of gastrin in the gastric antrum and PP in the pancreas at similar gestational times to the blood samples.
Material and Methods

Foetal plasma gastrin and PP were measured by sampling from arterial and venous lines in the chronically cannulated sheep foetus from 101 days gestation and followed up to 2 weeks after delivery. The peptides were determined by radioimmunoassay and their response to the cholinergic inhibitor, atropine, measured. Tissue samples from separate foetuses were collected and the peptides' molecular form determined by gel chromatography.

Radioimmunoassays

Plasma gastrin was measured using antibody 1296 donated by Dr. J. Walsh, Department of Medicine, UCLA (Walsh 1974). This antibody recognizes the C-terminal end of gastrins larger than the pentapeptides and was used at a final concentration of 1:600 000. Sulphated and non-sulphated forms are measured equally. Gastrin-34 and gastrin-14 were approximately 2/3 as potent as the heptadecapeptides (Dockray & Walsh 1975). Plasma PP was measured using bovine PP as standard and antibody 615-R-110-146-10 raised against bovine PP. The PP standard and antibody were generous donations of Dr. R. Chance of Eli Lilly & Co. The methods used in our laboratory have been reported previously (Shulkes et al. 1982).

Foetal preparation

Healthy pregnant Merino-Dorset Horn cross ewes of known gestational age 90–110 days (term is 145 ± 5 days) had food and water withheld for 24 h before surgery. The ewes were anaesthesitized by induction with 10 ml of 5% pentobarbitone and maintained with a 3% halothane-oxygen mixture. The anaesthetic regime was varied to maintain the partial pressure of oxygen of maternal arterial blood greater than 100 mmHg and partial pressure of carbon dioxide between 25 and 40 mmHg. Following a vertical lower abdominal incision, the instruments were discarded, the surgical team regowned and the incision isolated by a plastic ring drape. The foetus was delivered through a transverse uterine incision and breathing prevented by a wet pack over the head. Silastic cannulae (0.76 mm i.d., 1.65 mm o.d., 1.5 ml long) were inserted into the left carotid artery and right internal jugular vein and filled with heparin-saline. The foetal and uterine incisions were closed, and the cannulae brought out via a flank incision in the ewe. The ends of the cannulae were stoppered, wrapped in sterile gauze and placed in a plastic bag attached to the skin of the ewe. Sterile conditions were maintained throughout.

Experimental procedure

After a 3 day post-operative recovery period, maternal and foetal blood samples were taken every 1 to 3 days. A 4 ml foetal blood sample was obtained from the foetal carotid or jugular cannula. All sampling was under sterile conditions and the cannulae were kept patent by refilling them with heparin (1000 U/ml) at the tip, followed by heparinized saline (200 U/ml). The blood was taken into a lithium heparin tube containing 2000 U of Trasylol (Bayer), centrifuged, and the plasma stored at −20°C until assayed.

Effect of atropine

Three experiments were performed on 3 chronically cannulated foetuses of 125–135 days gestation. Each had been cannulated at least 10 days earlier. After 2 basal foetal blood samples, 100 μg/kg estimated foetal body weight atropine was injected over 1 min into the foetal jugular vein cannula. Blood samples were taken via the foetal carotid artery every 20 min for a further 60 min. Using the same protocol, the effect of atropine on basal PP and gastrin was also tested in 9 non-pregnant sheep.

Preparation of tissue extracts

Four pregnant sheep with foetuses aged 90, 100, 136, 139 days and a newborn lamb were used. The ewes were anaesthetized with thiopentone, the foetus removed rapidly, and the foetal distal abomasum (antrum) and pancreas dissected quickly, weighed and snap frozen on dry ice. At a later date, the tissues were extracted. Five parts of distilled water were added to one part tissue and boiled for 10 min in a boiling water bath. The extract was centrifuged for 15 min at 3000 r.p.m. and the supernatant (water extract) stored at −20°C. The extract was then homogenized (Polytron, Beckman), boiled in 5 parts 3% acetic acid, centrifuged and the supernatant (acid extract) stored at −20°C.

Chromatography

One ml samples were applied to a Sephadex G-50 superfine column 1 × 100 cm and eluted at 4°C with 0.02 M barbitol buffer containing 0.1% bovine serum albumin, 0.005% sodium azide, with a flow rate of 8 ml/h. [125I]-albumin, [22Na] and [125I]gastrin-17 or BPP were used for internal calibration. The column was previously calibrated with [125I]albumin, [22Na], gastrin-17-1, gastrin-34-1 and bovine PP. One ml fractions were collected. To compare the molecular forms in the adult with those in the foetus, the antrum and pancreas from an adult non-pregnant ewe were processed and chromatographed in a similar way to the foetal tissues.

Results

Plasma gastrin

The longitudinal data in 8 chronically catheterized foetal preparations which went successfully through to term are shown in Fig. 1. Foetal plasma gastrin concentration was 7 ± 1 pmol/l (mean ± SEM) at 101 days, and then increased as gestation progressed. In the week
Foetal plasma gastrin concentrations (mean ± SEM) in 8 foetal preparations which went successfully through to term. The days of gestation have been grouped into 5 day blocks. The figure above each bar indicates the number of estimations. Analysis of the simultaneously obtained foetal and maternal blood levels by the two tailed paired t-test was as follows: 101–110 days, \( P < 0.001 \); 111–120 days, \( P < 0.01 \); 121–130 days, NS; 131–135 days, \( P < 0.05 \); 136–140 days, \( P < 0.01 \); 141–145 days, \( P < 0.001 \); +1–+5 days, \( P < 0.001 \); +6–+10 days, NS; +11–+15 days, NS.

Prior to birth, foetal plasma gastrin was 47 ± 5 and in the first week after birth it was 90 ± 13 pmol/l. By 11–15 days, values were similar to adult sheep. Maternal plasma gastrin of these sheep ranged between 20 and 30 pmol/l and was not related to the stage of gestation. Average basal plasma gastrin in 10 non-pregnant ewes was 21 ± 5 pmol/l.

The foetal to maternal ratio of plasma gastrin is plotted on a semi-log scale in Fig. 2 and was calculated from paired samples collected simultaneously from the foetus and mother. There was a linear correlation with the stage of gestation. Foetal plasma gastrin was 25% of adult values at 100 days, but was higher than maternal values from 130 days until 2 weeks post-partum.

**Plasma PP**

The foetal plasma PP levels for the same 8 foetuses are shown in Fig. 3 and the foetal to maternal ratio of plasma PP is plotted in Fig. 4. At 101 days, foetal...
Foetal plasma PP concentrations (mean ± SEM) in the same 8 foetal preparations described in Fig. 1. Analysis of the simultaneously obtained foetal and maternal blood levels by the two tailed paired t-test was as follows: 101–110 days, 
\( P < 0.001; \) 111–120 days, 
\( P < 0.001; \) 121–130 days, 
\( P < 0.001; \) 131–135 days, 
\( P < 0.001; \) 136–140 days, 
\( P < 0.001; \) 141–145 days, 
\( P < 0.001; \) +1–+5 days, NS; +6–+10 days, NS; +11–+15 days, NS.

**Effect of atropine**

Atropine injection to 3 foetuses decreased the plasma PP by 27% from 36 ± 8 to 26 ± 3 pmol/l at 45 min (Fig. 5). A decrease was seen in all 3 foetuses. Atropine injection to the non-pregnant sheep decreased plasma PP by 52% (Fig. 5). There was no change in plasma gastrin in either group.

**Chromatography**

**Antrum.** Nearly all the gastrin was present in the water extract. Gel chromatography of the water extract of foetal, neonatal and adult antrum showed that there were 2 peaks of immunoreactive gastrin which eluted in the position of gastrin-17 and gastrin-34. The proportion of gastrin-17 ranged between 80 and 90% and was not related to the stage of development. A typical chromatogram is shown in Fig. 6. When a large amount of foetal antral extract (150 pmol) was chromatographed and assayed at several dilutions, small amounts of other forms of gastrin were detected. The relative amounts of big, big gastrin, component I and component IV were 0.5, 0.6 and 2%, respectively. The big, big gastrin in the void volume was probably non-specific interference due to the large amount of protein in the extract (Rehfeld et al. 1977). No PP was detected in any of the antral extracts.

**Pancreas.** PP was extracted in equal amounts into the water and acid extract. Gel chromatography of both the water and acid extract was performed. At all ages, only one form of PP was present in the acid extract and this eluted in the position of authentic PP. There were 3 forms of PP in the water extract. Approximately 95% coeluted with authentic PP while the remainder eluted as 2 earlier peaks.
**Fig. 4.**
Ratio of foetal plasma PP to simultaneously obtained maternal values in the 8 foetal preparations.

**Fig. 5.**
Effect of atropine (100 μg/kg body weight) to adult and foetal sheep on plasma PP. The atropine dose to the 3 foetuses aged between 125 and 135 days was based on estimated foetal body weight.

**Fig. 6.**
Elution pattern for gastrin on a G-50 Sephadex column of water extract from antrum of a 139 day foetus.

**Fig. 7.**
Elution pattern for PP on a G-50 Sephadex column of water extract from pancreas of a 139 day foetus.
There was no difference in the proportions of PP at the different gestational ages and in the adult. Fig. 7 shows the profile of the extract from the pancreas of a 139 day old foetus. Foetal pancreatic extracts contained small amounts of gastrin 1.7 ± 0.3 pmol/g, n = 8 compared with just detectable amounts in the adult pancreas 0–0.5 pmol/g.

Discussion

These studies have utilized the chronically cannulated ovine foetus to determine the levels of circulating gastrin and PP during gestation and in the immediate post-partum period. The use of the chronically cannulated sheep foetus has allowed the longitudinal pattern of development to be studied for up to 50 days. The complications of stress and anaesthesia and individual variations which occur with acute preparations and single point samplings have been avoided. The major findings were that gastrin and PP circulate in the foetal sheep with a progressive increase from 100 days gestation and a sharp increase in the week before birth. The major differences between the 2 peptides was that plasma gastrin exceeded maternal levels from 130 days gestation while PP was always lower than maternal values.

Hypergastrinaemia in the newborn human, dog and pig has been documented (Lucas et al. 1980a; Malloy et al. 1979; Moazam et al. 1980), although the aetiology remains unclear. Increased vagal stimulation or epinephrine release associated with parturition and the onset of suckling has been proposed (Euler et al. 1977). However, the present investigation, by measuring the circulating levels before birth, demonstrates for the first time that the hypergastrinaemia commences 15–20 days before parturition. There was a further sharp increase in the immediate post-partum period and this may be related to the parturition process and the subsequent commencement of feeding. Blood samples were taken at the same time each morning, but no attempt was made to isolate the lamb from the mother for any period prior to obtaining the blood sample. However, feeding studies in the newborn human (Rodgers et al. 1978; Lucas et al. 1980b) and pig (Moazam et al. 1980) failed to show any post-prandial increase in plasma gastrin.

As gastrin infused into the pregnant ewe does not cross the placenta (Shulkes et al. 1981), a plasma gastrin foetal/maternal ratio greater than 1 and a sustained increase following birth suggests that the gastrin is foetal in origin. Chromatography of foetal antrum showed that 80 to 90% of the tissue gastrin was in the form of gastrin-17, with the remainder as gastrin-34. Trace amounts of big, big gastrin, component I and component IV were also present. A similar ratio was present in the antrum of the adult sheep. The human foetal stomach in late gestation also has a gastrin-17 to gastrin-34 ratio comparable to the adult (Larsson et al. 1977; Track et al. 1979).

The foetal organ of origin for gastrin could not be determined from the present series of experiments. There were large amounts of gastrin in the antrum from 100 days gestation, ranging between 25 and 100% of adult values. However, others have shown that the distribution of gastrin in the foetus is different from the adult. For instance, in the human foetus, (Track et al. 1979) and in the neonatal rat (Larsson 1977), the concentration of gastrin in the duodenum is higher than in the antrum. The foetal sheep model will allow the performance of specific foetal organ excision with monitoring of foetal plasma gastrin until birth to determine the organ of origin.

It is likely that different mechanisms are involved in the hypergastrinaemia before and after birth. The post-partum hypergastrinaemia, although not initiated by oral food intake, may be maintained by this, since plasma gastrin is diminished in infants not enterically fed (Lucas et al. 1980a) and in rats on total iv alimentation (Johnson et al. 1975). Plasma gastrin may be changing in late gestation due to a maturation of vagal stimulating influence, especially with our observation of the increasing PP levels in the foetus. The probable absence of basal gastric acid secretion in utero (Shulkes et al. 1981; Euler et al. 1977; Malloy et al. 1979) and the resultant lack of feedback acid inhibition could also result in an elevated gastrin. However, the temporal relationship between gastric acid and gastrin release in the foetus has not been studied. The increase in foetal plasma gastrin cannot be explained by a decrease in metabolic clearance rate as our initial studies have shown that gastrin metabolic clearance rate is, in fact, elevated in the foetus (Shulkes et al. 1981).

The presence of significant amounts of gastrin in the circulation of the foetal sheep and the atypical tissue distribution demonstrated in other species (Track et al. 1979) suggest that gastrin may have a different function in foetal life. One possibility
is a growth promoting role on the foetal gastrointestinal tract. A trophic action of 
gastrin has been shown conclusively using animal models and clinically with the demonstration of hypertrophy 
and hyperplasia of gastric mucosa in patients with gastrin secreting tumours (Johnson 1976). Gastrin 
has also been implicated in the development of control of lower oesophageal sphincter pressure in 
the neonate (Cohen 1974).

Until now, data on PP in the foetus has been based on tissue levels and immunohistochemistry 
(Paulin & Dubois 1978; Sundler et al. 1977). The neonatal pancreas has a high proportion of endo-
ocrine tissue (Grand et al. 1976) and our preliminary findings from the pancreases extracted for chro-
matography suggest that PP is present in higher concentrations in the sheep foetal pancreas than in 
the adult. An abundance of PP cells has also been reported in the human neonatal pancreas (Paulin 
& Dubois 1978; Sundler et al. 1977). Chromatography of the foetal pancreas showed that the great 
majority of pancreatic PP coeluted with authentic PP, although small amounts of apparently larger 
forms were also present.

Thus, the low levels of circulating PP appears to be due to a failure of secretion, rather than insuffi-
cient PP synthesis or a preponderance of precursor forms. Foetal plasma PP was only 4% of adult levels 
6 weeks prior to birth, but rose rapidly to 30% of adult levels in the week before birth. The increase 
in foetal PP was not associated with any change in maternal plasma PP, suggesting that the increase 
was of foetal origin. A sharp increase also occurred in the first week post-partum. It could not be 
determined whether the neonatal increase was associated with the commencement of feeding. 
However, Lucas et al. (1980c) have reported that in the human neonate, there was no change in PP 
following feeding. As in the sheep, plasma PP is low in the newborn human with an increase in the first 
week of life (Lucas et al. 1980c).

The increase of plasma PP during gestation may be due to an increase in vagal activity. It is relevant 
that the changes in PP match the progressive increase in vagal influence on foetal heart rate 
(Walker et al. 1978). The rapid increase at birth could be a result of the vagal stimuli associated with 
birth. Schwartz et al. (1979) have proposed that the magnitude of the inhibitory effect of atropine on 
plasma PP is a marker of vagal cholinergic influence. In the present report, atropine caused a 27% 
decrease in plasma PP in the foetus and a 52% decrease in non-pregnant ewes. Whilst a choliner-
gic influence on PP is present in the foetus, it is substantially less than in the adult sheep. Longitu-
dinal studies of atropine injections during gestation and in the neonatal period will be required to 
determine whether the increases in PP during gestation is associated with changes in vagal influence. 
Another possibility is foetal truncal vagotomy, and this is currently being explored.

The role of PP in foetal life remains to be determined. However, pharmacological doses of 
PP stimulate pancreatic DNA synthesis (Greenberg et al. 1977) and it is therefore possible that the 
changes in circulating and tissue PP are influencing pancreatic growth. The fact that very high levels 
are present in the pancreas at a time of minimal secretion gives the opportunity to examine the 
development of mechanisms controlling PP secretion and indirectly the development of vagal control. 
Since many of the gastro-intestinal peptides are found in the vagus (Uvnäs-Wallensten 1981), 
these studies also have significance in the development of neural-endocrine relationships.

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