Elevated plasma peptide YY in human neonates versus elevated plasma glucagon-like peptide-1 in adults

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ABSTRACT

Development of specific radioimmunoassays of human plasma levels of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) enables us to measure plasma levels of these peptides in umbilical cord blood, the levels in 1-5 and 14-38 days of postnatal life, and in adults. The mean value of plasma peptide level in cord blood is regarded as the prenatal level. The plasma IR-PYY level in the prenatal stage was 22 fmol/ml. The level in 1-5 days of postnatal life elevated to 60 fmol/ml, and then decreased to 30 fmol/ml in 14-38 days and to 20 fmol/ml in adults. In contrast, the plasma IR-GLP-1 level showed gradual and insignificant increase in prenatal to infant stages, but was markedly elevated in adults to approximately three times that in the prenatal stage. The difference in dynamic changes of these peptides was discussed in relation to adaptation to enteric feeding and to the entero-insular axis.

During perinatal development, the human gastrointestinal tract undergoes important physiologic changes (4, 8, 9). In the life, humans meet two prominent periods of taking nutrition at birth and weaning. During these periods, functional and morphological development takes place in the gastrointestinal system, in which various gastrointestinal hormones play important roles to regulate nutritive adaptation (12). The present study was carried out to show temporal sequence of changes in plasma levels of peptide YY (PYY) (18, 19) and glucagon-like peptide-1 (GLP-1) (7, 13) in prenatal, infant and mature stages; PYY is known to be secreted by feeding and to inhibit gastric secretion and gastrointestinal motility (16), and GLP-1 is known to stimulate insulin secretion in the presence of higher glucose level after feeding (6, 14, 17). The study was accomplished by the development of sensitive and specific radioimmunoassay (RIA) of human PYY and GLP-1, which enabled us to determine intact plasma levels of these pep-

tide without any pretreatment and extraction.

Plasma was obtained from umbilical cords, neonates and infants at the Children's Hospital of Shizuoka Prefecture. Plasma of neonates and infants were divided into two groups according to their ages; 1 to 5 days and 14 to 38 days of postnatal life. Plasma was collected immediately before feeding in a day. Parental informed consent was obtained in all the cases. Human adult plasma was also obtained from healthy adult volunteers after overnight fasting. Plasma was stored at -30°C until the use. The plasma was directly used for RIA without any pretreatment and extraction. The recovery tests were performed by the addition of synthetic PYY and GLP-1(7-36) amide to human plasma; no degradation of standard antigens occurred during incubation in the RIAs used in the present study.

Immunoreactive (IR) human PYY was measured using antiserum RY37, which was raised in a Japanese white female rabbit with polyvinylpyrrolidone-adsorbed synthetic human PYY in the Yanaihara Institute Inc. (Fujinomiya, Japan). The antiserum showed about 46.8% cross-reactivity with rat PYY, but no cross-reactivity with human

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Table 1 Cross-Reactivity of Anti-Human PYY Serum RY37 (a) and Anti-GLP-1(7–36) NH₂ Serum RY35 (b)

a		b	
Peptide	Cross-reactivity (%)	Peptide	Cross-reactivity (%)
Human PYY	100.0	Human GLP-1(7–36) NH ₂	100.0
Human PYY(1–19)	0	Human GLP-1(7–37)	0.5
Human PYY(19–36)	0.1	Human GLP-1(1–37)	1.0
Human PYY(26–36)	0.1	Human GLP-1(1–35)	< 0.1
Rat PYY	46.8	Human GLP-1(19–36) NH ₂	35.7
Rat PYY(13–36)	1.5	Human GLP-1(7–22)	0
Human NPY	0.8	Human glucagon	0
Human PP	0	Human GIP	0
		Rat secretin	0
		Human VIP	0
		Human PACAP27	0
		Human PHI	0

NPY and human PP (Table 1a), indicating that the RIA developed with antiserum RY37 was highly specific to human PYY. Human PYY was iodinated with ¹²⁵I-NaI (Amersham, Buckinghamshire, England) by slightly modified chloramine-T method (5). After 30 s, the reaction was stopped by the addition of ascorbic acid. ¹²⁵I-human PYY was separated by HPLC on a μ Bondasphare 5 μ C18–100Å column (Waters, Millipore, MS, U.S.A.) with a linear gradient of acetonitrile from 25% to 40% in 0.01 N HCl over 30 min at a flow rate of 1 ml/min. Fractions of 1 ml each were collected and determined for radioactivity.

RIA was performed in duplicate in a total volume of 0.7 ml of 10 mM sodium phosphate buffer, pH 7.4, containing 140 mM NaCl, 25 mM ethylenediaminetetraacetic acid, 0.5% (w/v) bovine serum albumin (Sigma Chemicals, St. Louis, MO, U.S.A.) and 0.02% (w/v) sodium azide. Antiserum RY37 was used at a final dilution of 1:700,000. B/F separation was carried out by double antibody method with goat anti-rabbit IgG serum and polyethylene glycol 6000. The minimal detection was about 1 fmol/assay.

IR-GLP-1 level was determined by RIA specific GLP-1 developed using antiserum RY35 (Yanai-hara Institute Inc., Fujinomiya, Japan) raised in a Japanese white female rabbit with polyvinylpyrrolidone-adsorbed synthetic human GLP-1(7–36) amide. Antiserum RY35 showed about 35.7% cross-reactivity with GLP-1(19–36) amide among the GLP-1 related peptides such as GLP-1(7–37), (1–37), (1–35) and (7–22), and did not cross-react with human glucagon, gastric inhibitory polypep-

tide, vasoactive intestinal polypeptide, pituitary adenylate cyclase activating polypeptide 27, peptide histidine isoleucine and rat secretin (Table 1b). Human GLP-1(7–36) amide was iodinated and separated as described for human PYY. RIA was performed similarly to human PYY described above. Antiserum RY35 was used at a final dilution of 1:70,000. The minimal detection was about 1.3 fmol/assay.

The RIAs of human PYY and GLP-1 developed in the present study were satisfactory in sensitivity, stability and specificity. Variances in interassay and intraassay were less than 12% and 11% for PYY and GLP-1, respectively. The RIA of specific for human PYY could recognize only PYY among the same family of peptides including NPY and PP. Moreover, both RIAs can be used for direct determination of plasma levels without any pretreatment and extraction, which was proved by recovery tests.

The results were expressed as the mean $\pm$ SEM. The data were analyzed by one-way analysis of variance and statistical significance was assessed with the Schffe's test. Probability values less than 5% were considered to be significant.

Mean values of plasma IR-PYY levels in umbilical cords, neonates, infants and adults are shown in Fig. 1a and Table 2. At 1–5 days of life, plasma IR-PYY attained the level about three times (59.1 ± 10.6 fmol/ml) higher than that in cord blood (21.6 ± 3.8 fmol/ml), which was regarded as the prenatal level. IR-plasma PYY level at 14–38 days of life decreased to 30.4 ± 9.7 fmol/ml, which was still 1.5 times as high as that in cord blood. Plasma

Fig. 1 PYY (1a) and GLP-1 (1b) concentrations in umbilical cord blood ($n = 7$), 1–5 days of life ($n = 10$), 14–38 days of life ($n = 5$) and adult ($n = 19$). Plasma PYY and GLP-1 levels were determined directly with RIA using antiserum PY37 and PY35. Values are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ vs cord blood

IR-PYY level in adults decreased to that in cord blood. The results indicate that plasma IR-PYY levels increased within seven days after birth and then decreased gradually to the adult levels.

Plasma GLP-1 levels tended to increase gradually from prenatal stage to 14–38 days of life, but the level at 14–38 days was not significantly higher than the prenatal level (Fig. 2a). Plasma IR-GLP-1 level in adults (100.0 ± 4.1 fmol/ml) was more than three times as high as that in cord blood (29.3 ± 8.3 fmol/ml). The results show that plasma IR-GLP-1 remains at almost the same level from the prenatal to infant stage, but increases to the highest level in adults.

In summary, the plasma PYY level in humans at 1–5 days of postnatal life was three times higher than that at the prenatal stage, gradually decreased at 14–38 days, and reached the prenatal level again in adults; in contrast, the plasma IR-GLP-1 level was elevated insignificantly by 14–38 days of life, but significantly increased in adults, the value being more than three times higher than that at the prenatal stage.

Table 2 PYY and GLP-1 Concentrations in Umbilical Cord Blood, 1–5 Days of Life, 14–38 Days of Life and Adult

	IR-PYY	IR-GLP-1
Umbilical cord blood	21.6 $\pm$ 3.8	29.3 $\pm$ 8.3
1–5 days	59.1 $\pm$ 10.6*	37.0 $\pm$ 9.7
14–38 days	30.4 $\pm$ 9.7	42.6 $\pm$ 7.9
Adult	19.1 $\pm$ 1.7	100.0 $\pm$ 4.1**

fmol/ml, mean $\pm$ SEM; * $P < 0.05$, ** $P < 0.01$ vs each cord blood

The elevation of plasma IR-PYY was already reported in human neonates and infants by Adrian *et al.* (2), who showed that the PYY levels were high in cord blood, rose postnatally in the premature infants, and fell at 18 days of life. They have suggested that the postnatal stage of plasma PYY is a feature of early adaptation to extrauterine life. It has also been reported that rat ileal and pancreatic PYY immunoreactivity and mRNA are higher in postnatal period (around 7 to 19 days of life) than in adults (3, 10, 11). Gomez *et al.* (3) have shown that expression of intestinal PYY mRNA and PYY are coordinated and developmentally regulated: the changes in the profile of PYY gene expression take place during the initiation of enteral feeding after birth and with the switch of diet at the time of weaning. On these results, they have proposed that PYY possesses trophic effects on the intestine, and that PYY plays an important role in the processes of intestinal development and dietary adaptation. Their view is compatible with the results in the present study. A potential inducer of PYY secretion might be ingested fat in colostrum, as the content of fat is higher in colostrum than in common milk. Fat is surely a potent secretagogue for PYY (1, 16).

GLP-1 may play a major hormonal role in the entero-insular axis (5), which stimulates insulin secretion in the environment of elevated plasma glucose after feeding. In human neonates and infants, the present study showed gradual but insignificant increase in plasma GLP-1 levels, indicating that feeding can not immediately be a secretagogue of GLP-1. In rat neonates, however, the contents of GLP-1 in the pancreas and the intestine increased at 3 to 8 days and 36 days of life parallel with developmental pattern of PYY, followed by the maximal peak of insulin content in the pancreas at 150 days of life (11). Thus, developmental patterns of GLP-1 are quite different between plas-

ma levels in human neonates and tissue levels in rat neonates. It has been reported that fetal pancreatic islets have a diminished response to glucose in rats (15). Human neonatal pancreas may develop slower than in rats, and may express enough function during weaning when infants take adult diet containing high contents of carbohydrates.

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