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Tissue and Plasma Somatomedin-C/Insulin-Like Growth Factor I Concentrations in the Human Fetus during the First Half of Gestation

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ABSTRACT. To investigate the possible role of somatomedin-C/insulin-like growth factor I (Sm-C/IGF I) in early human development, we measured this peptide by radioimmunoassay in extracts of multiple tissues and in plasma from fetuses during the first half of gestation (9–19 wk). All tissues contained Sm-C/IGF I far in excess of that which could be accounted for by Sm-C/IGF I derived from blood entrapment. Lung and intestine had the highest concentrations (166 ± 35 mU/g, $n = 25$ and 160 ± 20 mU/g, $n = 19$, respectively; mean ± SEM) and liver the lowest (67 ± 16 mU/g, $n = 26$). Plasma concentrations were 270 ± 20 mU/ml ($n = 20$). Neither fetal weight (6–258 g) nor gestational age correlated with Sm-C/IGF I concentrations in any tissue or in plasma. These findings suggest that Sm-C/IGF I is synthesized in many human fetal tissues from as early as the 1st trimester. They also provide further evidence for an autocrine/paracrine role of this peptide growth factor. (*Pediatr Res* 20: 253–255, 1986)

Abbreviations

Sm-C, somatomedin-C
IGF, insulin-like growth factor
RIA, radioimmunoassay
Hgb, hemoglobin

The somatomedins, SM-C/IGF I and IGF II, are thought to stimulate fetal growth (1–3). Although cell surface receptors for these peptides are present from early in gestation (4–5), it is not known when the somatomedins are first synthesized in human gestation. Because multiple tissues make them, the somatomedins are thought to act in an autocrine or paracrine fashion, on their cells of origin or on nearby cells (6). To determine ontogeny and sites of synthesis, we measured Sm-C/IGF I concentrations in multiple tissues and plasma from human abortuses from 9–19 wk gestation. Our results suggest that Sm-C/IGF I is synthesized in multiple sites from as early as the 1st trimester.

MATERIALS AND METHODS

Tissues. Tissues were obtained from 59 fetuses of prostaglandin-induced elective abortions performed at the Northern General Hospital, Sheffield, England. These fetuses were between 9 and 18 wk gestational age, as calculated from the 1st day of the last menstrual period, and weighed between 6 and 258 g. Although each study fetus was not the source of a complete set of tissues, there were no differences in the mean gestational ages or weights of donors for each tissue (Table 1). In the laboratory, tissues were dissected, rinsed in saline, blotted, and flash frozen in liquid nitrogen within 60 min of delivery. Intracardiac blood was collected (Table 1) into heparinized syringes, iced, and the plasma separated by centrifugation. All samples were stored at −20° C until the time of extraction and assay (within 4 months). Procurement of human fetal tissues for this study was approved by the Ethics Committee of the Northern General Hospital, Sheffield and the Committee for the Protection of the Rights of Human Subjects, the University of North Carolina at Chapel Hill.

Tissue extraction and plasma treatment. Tissues were extracted for Sm-C/IGF I using a modification (6) of a procedure described previously (7). Tissues were pulverized under liquid nitrogen, extracted by incubation at 4° C for 2 h in 1 M acetic acid (final pH = 3.5–4.0), and the supernatant was neutralized with 1 N NaOH. Plasma was also incubated at 4° C for 2 h, with an equal volume of 0.1 M glycine-glycine HCl buffer (final pH = 3.6) before assay. To maximize the amount of immunoreactive Sm-C/IGF I that can be measured in plasma (8), separate aliquots of plasma were exposed to glycine-glycine HCl buffer at 37° C for 24 h.

Estimation of tissue extractable Sm-C/IGF I derived from blood. Hgb was determined on extracts prior to neutralization by the method of Cosby and Furth (9), using a human Hgb standard (H 7379, Sigma Chemical Co., St. Louis, MO). The volume of serum that contaminated each extract was calculated as follows:

$$\text{ml serum/ml extract} = \frac{[\text{Hgb}]\text{ex}}{[\text{Hgb}]\text{bl}} \times (1 - \text{hematocrit})$$

where [Hgb]ex is the experimentally determined weight of Hgb (g) in 1 ml of extract, [Hgb]bl is the concentration of Hgb in 1 ml of fetal blood, and hematocrit is expressed as a fraction. Hgb and hematocrit values used for calculations were: fetuses < 14 wk gestation, 0.093 g/ml and 0.73, respectively, 14–17 wk, 0.107 g/ml and 0.67, > 17 wk, 0.115 g/ml and 0.64 (10).

The contamination of tissue Sm-C/IGF I by Sm-C/IGF I in plasma was calculated by subtracting the mean plasma concentrations for the same age (±1 wk) fetuses from the total tissue-extracted Sm-C/IGF I (7). The approximate contribution (mean ± SEM) of plasma to the total Sm-C/IGF I extracted from tissues is as follows: 13 ± 1.3% for liver, 6.9 ± 0.5% for adrenal, 4.2 ± 0.15% for muscle, 4 ± 0.16 kidney, 3.2 ± 0.11% for skin, 2.9 ± 0.14% for heart, and <2% for pancreas, thymus, intestine, lung, and brain. With the exception of two liver extracts, there was no tissue in which plasma contamination accounted for more than 18% of the Sm-C/IGF I extracted.

Sm-C/IGF I RIA. Sm-C/IGF I concentrations were estimated

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Table 1. *Characteristics for each tissue studied*

Tissue	*n*	Gestational age* (wk) Range	Gestational age* (wk) Mean ± SEM	Wt* (g) Range	Wt* (g) Mean ± SEM
Adrenal	19	9–18	14.1 ± 0.52	6–239	91.6 ± 14.6
Brain	6	9–16	13.3 ± 0.97	6–157	12.0 ± 21.0
Heart	20	11–18	14.5 ± 0.42	8–239	103.5 ± 13.3
Intestine	19	9–18	14.2 ± 0.51	6–239	100.6 ± 14.0
Kidney	24	9–18	14.2 ± 0.46	6–239	98.8 ± 12.5
Liver	26	9–18	14.2 ± 0.44	6–239	103.4 ± 12.5
Lung	25	9–18	14.1 ± 0.47	6–239	92.8 ± 12.7
Muscle	22	12–18	14.8 ± 0.38	10–239	114.2 ± 12.1
Pancreas	9	11–17	14.7 ± 0.60	8–188	107.6 ± 17.6
Plasma	20	12–18	15.4 ± 0.43	32–258	111.5 ± 16.2
Skin	17	12–18	14.8 ± 0.45	10–239	113.2 ± 14.6
Thymus	5	11.5–18	15.5 ± 1.05	23–239	134.2 ± 37.0

* There were no significant differences among fetal donors for any tissue.

in tissue extracts and plasma with a homologous RIA (11, 12) which employs a pool of adult human serum as standard. The RIA is highly specific for Sm-C/IGF I and has only 2% cross-reactivity with IGF II (13). Concentrations are expressed as mU/g of wet weight for tissues and mU/ml for plasma.

Validation of technique for measuring Sm-C/IGF I in extracts of human fetal tissues. Several experiments similar to those described previously for adult rat tissues (6, 7) were carried out to validate our methods for measurement Sm-C/IGF I in human fetal tissue extracts. Dose-response curves of extracts from each tissue were found to be parallel to those of the human serum standard. Known amounts of purified Sm-C/IGF I were added to extracts, and the added Sm-C/IGF I was shown to be quantified accurately in the RIA (the range of recovery of all tissues was 86–105% of the added Sm-C/IGF I), indicating that binding proteins were not reducing artificially the measurable peptide. To assure that the extraction procedure was freeing immunoreactive Sm-C/IGF I from binding proteins, and to exclude further any interference by binding proteins, representative extracts of eight tissues were subjected to Sephadex G 75 (Pharmacia) chromatography (bed volume = 65 ml) under acid conditions (0.5 M acetic acid, pH = 3.6). The elution fractions corresponding to the free Sm-C/IGF I (Kav = 0.25–0.65) was collected, lyophilized, reconstituted, and subjected to RIA. These fractions contained between 63 and 95% of the Sm-C/IGF I measured in the acid extract prior to acid chromatography (76, 81, 71, 88, 70, 62, 63, and 95% for adrenal, heart, intestine, kidney, liver, lung, muscle, and skin, respectively), indicating that the extraction procedure was providing a reasonable estimate of the Sm-C/IGF I present.

RESULTS

Each of the eleven tissues studied contained Sm-C/IGF I (Fig. 1), and the mean tissue concentrations (67–166 mU/g) were at least seven times more than could be accounted by blood contamination. Concentrations were greatest in lung and intestine, and least in liver. When Sm-C/IGF I concentrations in tissues were correlated by linear regression analysis with body weight or gestational age of donors, no statistically significant associations were found.

The mean concentration (±SEM) of Sm-C/IGF I in fetal plasma that had been incubated for 2 h in acid was 200 ± 20 mU/ml (n = 20). Exposure to acid for 24 h resulted in a mean plasma concentration of 270 ± 20 mU/ml (p = NS by the Student's t test). There was also no correlation between plasma Sm-C/IGF I concentration and fetal body weight.

DISCUSSION

This study shows that human fetal tissues from the first half of pregnancy contain immunoassayable Sm-C/IGF I far in excess of that which can be accounted for by contamination from fetal blood. Previous studies have reported that somatomedin peptides are released during *in vitro* culture of tissue explants from fetal mice (14), fibroblasts (15), myoblasts (16) and liver explants from fetal rats (17), and human fetal fibroblasts (18). We have found that human fetal connective tissues also release immunoassayable Sm-C/IGF I *in vitro* (19). These observations suggest that the Sm-C/IGF I detected in human tissues might be derived from *in situ* synthesis. If this is the case, Sm-C/IGF I may stimulate early human growth by autocrine or paracrine mechanisms.

In human fetal fluids (20, 21) and culture medium conditioned by human fetal tissues (19), the somatomedins are associated with binding proteins of approximately 40,000 molecular weight. Because these binding proteins may interfere with the measurement of somatomedin peptides (22), we extracted all tissues in acetic acid under conditions shown previously to separate Sm-C/IGF I from the binding proteins (6, 7). Our studies demonstrating complete recovery of purified Sm-C/IGF I added to the extracts, together with acid gel filtration studies show that there is no interference of binding proteins in the RIA, and that the differences among fetal tissue Sm-C/IGF I concentrations are not due to binding proteins. It also is unlikely that exposure to prostaglandins has altered the fetal serum Sm-C/IGF I concentrations, because results on samples collected *in utero* prior to

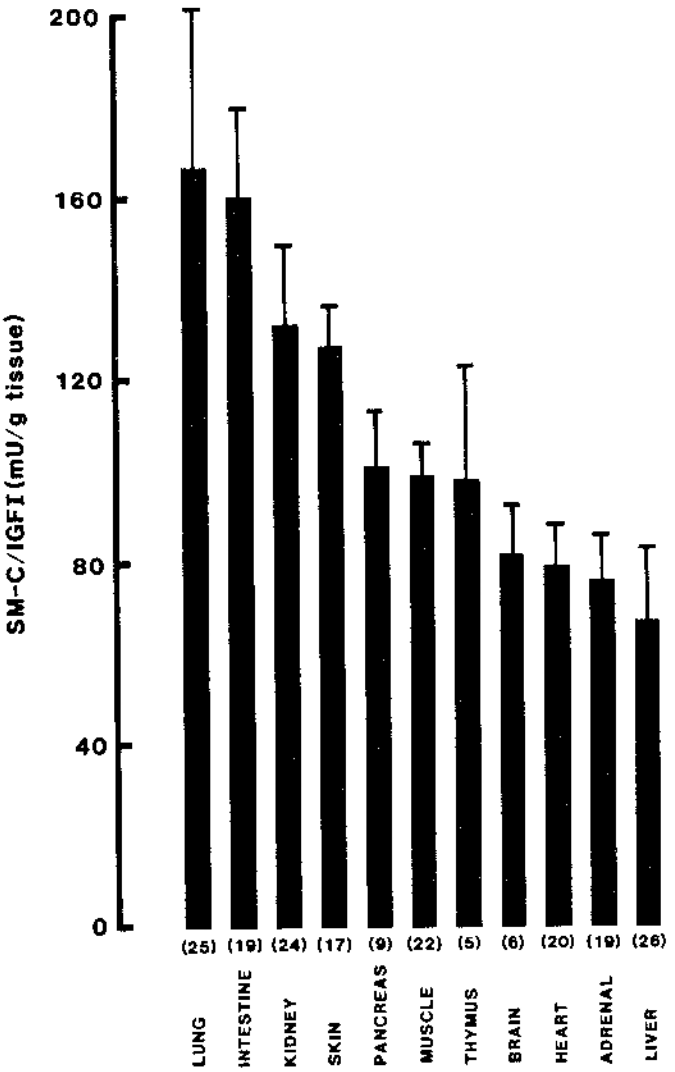

Fig. 1. Sm-C/IGF I concentrations (mean ± SEM) in 11 human fetal tissues from 9–19 wk gestation. The number of samples, each from a separate fetus, are shown in parentheses. Brain tissue is predominantly cerebrum; intestine represents duodenum and jejunum; muscle (skeletal) is from the hamstring group, and skin is from the abdomen.

prostaglandin-induced abortion (23) are similar to those reported herein.

The results in the present study, suggesting that many human fetal tissues have the capacity to produce Sm-C/IGF I, are similar to those reported previously for conditioned media of fetal mouse tissue explants (14). While the liver seems to be the major source of Sm-C/IGF I postnatally (7, 24), we believe that in the fetus, other tissues may be important for the production of this peptide. While the liver contributes approximately 5% to the weight of the mid-trimester fetus (25), another 3% can be accounted for by the lungs and 4–6% by skeletal muscle. Because both of the latter have two to three times more extractable Sm-C/IGF I/g tissue, their contribution to the Sm-C/IGF I in blood may be significant. The validity of this assumption depends on whether the peptide that can be extracted from a tissue reflects that which might be secreted. The lung appears to be a secretor of Sm-C/IGF I, since this peptide has been identified in tracheal fluid of human newborns (26).

For a variety of reasons, including the observation that IGF II is higher in fetal than in adult rat serum (27), IGF II has been postulated to be the somatomedin most important for fetal growth (15). We know of no evidence that this is the case in man. Specifically, the IGF II content of mid- (23) and late (28) gestation human fetal plasma is low relative to adult values (29). Because Sm-C/IGF I is present in cord blood of human newborns and correlates positively with birth size and gestational age (1, 28, 30), it might have a role in growth late in fetal life. However, little is known about the possible role of Sm-C/IGF I in early fetal growth. Human fetal brain and liver possess specific cell membrane receptors to Sm-C/IGF I from as early as 6 wk gestation (5), and Sm-C/IGF I increases the uptake of [^{3}H] thymidine by fetal cartilage explants *in vitro* (31), suggesting that somatomedins may be anabolic early in fetal life.

This study does not provide direct evidence that Sm-C/IGF I stimulates early fetal growth. The presence of this peptide in tissues and blood, however, makes this a possibility. We found no evidence for change in Sm-C/IGF I content of any tissue or in plasma even though the fetuses studied varied in age, had a 40-fold range of weights (6–258 g) and were experiencing brisk increases in the velocity of weight gain and linear growth (32). This suggests that Sm-C/IGF I is not the only factor that stimulates growth early in fetal life. Growth factors such as somatomedins, epidermal growth factor, and platelet-derived growth factor act synergistically to produce optimal rates of cell replication *in vitro* (33). Therefore, fetal growth in the presence of a constant stimulus of Sm-C/IGF I may be influenced by fluctuations in the concentrations of other peptide growth factors, changes in growth receptors, or changes in cellular responsivity.

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