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Tissue concentrations of somatomedin C: Further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action

(insulin-like growth factor I/growth factor/growth hormone)

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ABSTRACT **We have validated a method for extracting and measuring the tissue content of somatomedin C (Sm-C)/insulin-like growth factor I (IGF-I), a growth-hormone-dependent, growth-promoting peptide. The Sm-C content of tissue extracts was strongly growth-hormone dependent because most of the tissues studied from hypophysectomized rats contained significantly less Sm-C than normal tissues. The intraperitoneal administration of ovine growth hormone (oGH) to hypophysectomized rats caused tissue extractable Sm-C to increase in kidney, liver, lung, heart, and testes. Tissue Sm-C responses to oGH were maximal after 12 hr, 6 hr before the maximal increment in serum. In liver and lung, the tissue Sm-C response to various doses of oGH fit linear regression models, and the doses of oGH needed to increase the Sm-C are in the range of those required to increase protein synthesis. Although these results do not exclude the possibility that the somatomedins act by hormone-like endocrine mechanisms, they add support to the concept that these peptides act through autocrine or paracrine mechanisms, being produced at multiple sites and acting at or near their sites of production.**

There is now evidence that somatomedins, traditionally considered to originate in the liver, are synthesized at multiple sites (1, 2). Using explant cultures, we have observed that a variety of fetal mouse organs elaborate immunoreactive somatomedin C (Sm-C) into medium (3). Furthermore, it has been shown that Sm-C/insulin-like growth factor I (IGF-I) is synthesized by cultured human and rat fibroblasts (4–6). These findings suggest that the somatomedins might act through paracrine or autocrine mechanisms, having their biologic actions at or near their sites of origin. If such mechanisms are operative, alterations in somatomedin physiology may be better reflected by estimation of tissue concentrations than by measurement of concentrations in blood. Using a newly devised method for extraction of Sm-C, we addressed these possibilities by comparing tissue concentrations of immunoreactive Sm-C in normal rats and their hypophysectomized littermates. In addition, we assessed the tissue Sm-C response to the administration of growth hormone in hypophysectomized rats.

MATERIALS AND METHODS

Animals. Hypophysectomized male Sprague–Dawley rats and their normal littermates were purchased from Zivic–Miller Labs (Allison Park, PA). Hypophysectomy was carried out when the animals weighed ≈100 g. Animals were fed standard laboratory chow ad lib, and provided water, each liter of which contained 2.03 g of NaCl, 83.3 mg of KCl, 2.1 mg of CaCl, 16.7 mg of MgCl, and 50 g of sucrose. Two sets of rats were used for the experiments. At the time of sacrifice, 48–50 days of age and 20 days after operation, the hypophysectomized groups weighed 100.4 ± 9.8 g (mean ± SD; $n = 68$) and 107.2 ± 8.2 g ($n = 25$). The mean weights for the two groups of normal rats were 251.6 ± 17.7 g ($n = 7$) and 263.8 ± 18.6 g ($n = 6$). Completeness of hypophysectomy was verified by direct examination of the sella turcica and by RIA of serum Sm-C. The mean serum Sm-C concentrations in the two groups of hypophysectomized rats were 2.26 ± 0.4% and 2.57 ± 0.44% of the values in the normal rats.

Experimental Design. Groups of six hypophysectomized rats were injected intraperitoneally with ovine growth hormone (oGH; S-11 provided by the Hormone Distribution Program, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases) or vehicle (0.05 M NH_4CO_3, pH 8.2). In the first experiment, 1 mg of oGH in 1 ml of vehicle was administered, and rats were sacrificed by decapitation after 2, 4, 8, 12, 18, and 24 hr. As controls, one to three rats that had been injected with vehicle were sacrificed after each time interval. The injections were timed so that all animals were sacrificed between 0900 and 1200 hr. Six additional hypophysectomized rats that were not injected were sacrificed at the same time of day. In the second experiment, groups of four to six hypophysectomized rats were administered 10, 50, 100, or 250 μg of oGH, or were injected with vehicle, and were sacrificed after 12 hr.

Blood was collected from all rats at the time of decapitation, and immediately chilled in ice; sera were obtained by centrifugation and stored at −20°C until time of assay (within 1 wk). In the first experiment, brains, hearts, lungs, livers, and kidneys were dissected and immediately frozen in liquid nitrogen. The time from sacrifice to completion of dissection was ≈90 sec. In the second experiment, lung, liver, and testes were removed from the hypophysectomized oGH-treated rats. In the normal rats and hypophysectomized rats injected with vehicle, perirenal fat pad, cervical lymph node, iliopsoas muscle, prostate, sternum, submaxillary gland, and thymus were also removed. From sacrifice until collection of all tissues, no more than 4 min elapsed. All tissues were frozen, weighed, and stored at −20°C until extraction (within 4 wk).

Method of Tissue Extraction. Under liquid nitrogen, each tissue was pulverized to a powder with a mortar and pestle, and two aliquots of each tissue were weighed in tared polystyrene conical centrifuge tubes. One aliquot was saved for determination of total protein by the method of Lowry (7) and the other was extracted with acetic acid. Acetic acid was used to extract Sm-C from tissue because plasma somatome-

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Abbreviations: Sm-C, somatomedin C; oGH, ovine growth hormone; IGF-I, insulin-like growth factor I.

dins are liberated from their binding proteins by exposure to acid (8–10) and bioassayable somatomedin has been extracted from rat liver with acetic acid (11). Five milliliters of chilled 1 M acetic acid was added for each gram of tissue, yielding a final pH of 3.6–4.2. The mixture was shaken vigorously, allowed to stand on ice for 2 hr, and, after centrifugation at 600 × *g* for 10 min, the supernate was removed by pipette and saved. The precipitate was then subjected again to extraction with fresh 1 M acetic acid. The two supernates were combined, frozen at −70°C, lyophilized to dryness, and reconstituted with 0.05 M Tris·HCl buffer (pH 7.8) in a ratio of 2 ml of buffer to 1 g of original tissue. Because variable amounts of insoluble material (depending on the tissue) remain after reconstitution of the lyophilized material, the extract was clarified by centrifugation at 600 × *g* for 10 min. Extracts were then frozen at −20°C and stored until RIA for Sm-C (usually within 2 wk).

To determine whether additional Sm-C could be extracted using a different procedure, the residues of liver, kidney, and lung that had been subjected to extraction were sonicated (Branson 185 Cell Disrupter for 45 sec at a setting of 3) in assay buffer, and the supernatant was assayed. In these three organs, the Sm-C measured in the original extract was 80.4–92.2% of that measured from the original extract plus the reextracted precipitate. In an additional experiment, partially purified Sm-C was added to aliquots of the liquid nitrogen frozen powder from liver and heart, and after extraction >80% of the added Sm-C was quantified in the assay. When Sm-C in the form of rat plasma was added to powdered heart, liver, lung, and brain frozen in liquid nitrogen, recoveries of 91–128% were observed.

RIA for Sm-C. The RIA for Sm-C was carried out using a nonequilibrium technique (12, 13). Sm-C used for iodination and other studies had been purified to a specific activity of 27,000 units/mg and was judged by multiple criteria to be no less than 90% pure (14). The standard is a lyophilized pool of plasma from normal adult human blood donors (lot 1778-5; Ortho Diagnostics). The activity in tissue extracts is expressed as units of immunoreactive Sm-C per g of tissue, in which a unit is designated as the activity in 1 ml of the standard. Because normal adult rat serum has more Sm-C activity than human serum, the values reported for normal rat serum are relatively high.

In rat serum our RIA reflects the growth-hormone dependency of Sm-C (15). In the two groups of normal control rats, Sm-C concentrations were 28.5 ± 5.4 units/ml (mean ± SD; $n = 7$) and 28.7 ± 10 units/ml ($n = 6$); in hypophysectomized littermates, they were 0.67 ± 0.12 units/ml ($n = 16$) and 0.74 ± 0.12 units/ml ($n = 6$) ($P < 0.0001$). Binding proteins do not appear to interfere with measurements in unextracted rat serum because 97.5% of added Sm-C is measured when the purified Sm-C is assayed in the presence of rat serum. In addition, when subjected to acid chromatography (see below), the immunoreactivity migrating in the region of free Sm-C represented 99% and 106% of that measured in unextracted serum from normal and hypophysectomized rats, respectively. When rat serum was incubated with acetic acid in a fashion identical to that used to extract tissues (4 hr at 4°C), there was no change in the Sm-C concentration. On the basis of these observations, we routinely assayed fresh unextracted serum.

Hemoglobin Assay and Determination of Amount of Serum in Each Tissue Extract. Hemoglobin concentrations of individual tissues were determined on samples obtained from 14 rats that were age-matched to the rats used for Sm-C analysis. These determinations were carried out on aliquots of acetic acid extracts that had not been subjected to lyophilization. As with Sm-C extraction, it was necessary to extract hemoglobin on ice to prevent degradation. Hemoglobin concentration was determined using the modified benzidene assay method of Crosby and Furth (16). Rat hemoglobin (Sigma) was used as the standard. The volume (in ml) of serum in each ml of extract was calculated as follows:

$$\frac{[Hg]_{ex}}{0.1495} \times 0.54,$$

where $[Hg]_{ex}$ is g of hemoglobin in 1 ml of extract, 0.1495 is the average hemoglobin concentration (g/ml) of rat blood, and 0.54 is the portion of rat blood that is serum (mean hematocrit = 46%).

To determine the efficiency of extraction of hemoglobin, known amounts of hemoglobin in the form of blood frozen in liquid nitrogen were added to powdered tissue from normal animals before extraction with acetic acid. Recovery of added hemoglobin was 102% for heart and liver, 96% for lung, and 61% for brain.

Column Chromatography. To separate immunoreactive Sm-C from its binding proteins, representative tissue extracts and sera were chromatographed on a 1.6 × 40.5 cm Sephadex G-50 (Pharmacia) column equilibrated in 1 M acetic acid (8, 9). Two fractions were collected, lyophilized, and reconstituted for assay. The first (K_d, 0–0.22) contains the bulk of serum proteins and most of the Sm-C binding proteins, while the second (K_d, 0.22–0.66) contains free Sm-C.

RESULTS

Observations on Extraction Methods. Maintaining pH between 3.6 and 4.2 appeared to be optimal for tissue Sm-C extraction because at pH 2.6, only 65% and 62% as much immunoreactivity was obtained from liver and kidney, respectively, and 53% and 18% was obtained from each at pH 2.2. Pilot experiments also showed that substantially more Sm-C was extracted from tissues frozen and pulverized in liquid nitrogen than from iced and homogenized tissues (about 3-fold for liver, 2-fold for kidney, and 5-fold for lung). Likewise, there was evidence for loss of Sm-C when extraction was not carried out on ice.

To determine whether the immunoreactive Sm-C in tissue extracts was being measured without interference by binding proteins or other substances, experiments were done to show that multiple concentrations of extracts from all tissue studied produce serial dilution curves parallel to the human plasma standard and to purified Sm-C. Also, when fixed volumes of extracts from kidney, liver, heart, lung, and brain were assayed in the presence of each of seven concentrations of purified Sm-C, the mean recovery of the added Sm-C ranged from 95.5% to 100.8%. In addition, the immunoreactive Sm-C recovered from acetic acid G-50 Sephadex columns was equal to that measured in extracts, ranging from 90.5% to 97%.

Finally, to show that proteolytic enzymes in the extracts were not capable of degrading ^{125}I-labeled Sm-C in the RIA, and thereby causing a spurious increase in the apparent Sm-C, ^{125}I-labeled Sm-C was incubated with extracts under conditions identical to the assay (72 hr at 4°C), and then precipitated with an excess of anti-Sm-C antibody. In each case, ^{125}I-labeled Sm-C precipitates incubated with extracts ranged from 96.5% to 100.2% of controls (^{125}I-labeled Sm-C incubated with buffer alone).

Based on the hemoglobin content in the extracts, the mean volume of serum in each gram of tissue from normal animals was 13.3 ± 1.3 μl (mean ± SD; $n = 13$) for liver, 32.9 ± 4.4 μl ($n = 12$) for lung, 6.9 ± 0.6 μl for kidney ($n = 12$), 33.3 ± 4.9 μl ($n = 12$) for heart, and 2.33 ± 0.8 μl for brain ($n = 6$). These are of the same magnitude as that observed using another method for estimating contamination of tissue by blood (17). For the other tissues, the serum concentration was 2.3 μl per g of tissue or less. Using the Sm-C concentration in serum, we calculated the percentage of extract Sm-C that

could have been derived from serum Sm-C. On average, serum Sm-C could have accounted for <0.2–47.8% (17 ± 2.4% for liver, 35.4 ± 11.1% for lung, 7.4 ± 1.7% for kidney, 47.8 ± 3.5% for heart, 24.4 ± 14% for brain, 16.3% for thymus, 12.3% for lymph nodes, 12.3% for muscle, and <3% for other tissue studied) of extractable Sm-C from normal tissue and <0.1–5.7% (3.96 ± 1.3% for liver, 4.4 ± 0.7 for lung, 0.7 ± 0.4% for kidney, 5.7 ± 2.3% for heart, and <2.0 for all other organs) from tissues from hypophysectomized animals. Because the Sm-C in serum from normal rats could have made significant contributions to some tissues, the possible serum contribution was subtracted from the tissue concentration.

Tissue Sm-C Concentrations in Normal and Hypophysectomized Rats. Sm-C concentration in hypophysectomized rats were lower than those in normal rats in all tissues studied except the brain (Table 1). These differences were highly significant ($P < 0.005$) in all nonpooled organs in which statistical tests could be applied. Extracts from livers of hypophysectomized rats had relatively less Sm-C (compared to normal) than any other organ. On the other hand, muscle, thymus, and lymph nodes from hypophysectomized animals exhibited the lowest absolute concentrations. There were no significant differences between normal and hypophysectomized rats in protein content per g of frozen tissue. The results of the study, therefore, are not changed when they are expressed on the basis of tissue protein rather than weight.

Increase in Tissue Sm-C After Administration of oGH. In four of the five solid tissues studied (kidney, liver, lung, and heart), there was a significant increase in Sm-C in response to the intraperitoneal administration of 1 mg of oGH. In each of these tissues the peak response occurred after 12 hr (Fig. 1). In kidney, lung, and heart, a significant increment was observed in animals sacrificed after 2 hr, while a sustained increase began between 4 and 8 hr. The changes in tissue Sm-C after administration of oGH consistently preceded changes in serum Sm-C. In serum, the first significant increase in Sm-C was observed at 4 hr; a sustained increase began between 8 and 12 hr; and the maximal increment was observed at 18 hr. Furthermore, tissue Sm-C concentrations decreased long before serum concentrations.

The greatest Sm-C response to oGH occurred in kidney, followed in descending order by liver, lung, and heart (Fig. 2). In a preliminary experiment directed at defining the dose

Table 1. Extractable tissue Sm-C concentrations in male rats

Tissue	Sm-C, units/g*		
	Normal	Hypophysectomized	
	Mean ± SD	Mean ± SD	% normal
Serum†	28.7 ± 0.98	0.74 ± 0.12‡	2.6
Liver	1.91 ± 0.23	0.23 ± 0.08‡	12.0
Lung	2.04 ± 0.86	0.57 ± 0.13‡	27.9
Kidney	2.59 ± 0.80	0.77 ± 0.29‡	29.7
Heart	0.92 ± 0.33	0.48 ± 0.14	52.2
Muscle (iliopsoas)	0.42 ± 0.05	<0.08	<19.1
Brain	0.26 ± 0.09	0.28 ± 0.04	107.7
Testes	1.88 ± 0.42	0.52 ± 0.32‡	27.7
Prostate§	1.06	0.40	37.7
Thymus§	0.33	0.10	30.3
Lymph nodes§	0.48	0.08	16.7
Cartilage (sternum)§	0.67	0.53	79.1
Fat pad (perirenal)	0.67 ± 0.19	0.25 ± 0.10‡	37.3
Submaxillary gland§	1.73	0.78	45.1

*All values, except for pooled organs, represent six or seven organs.
†Serum values are expressed as units/ml.
‡Differences between Sm-C concentrations of normal and hypophysectomized rats are significant ($P < 0.005$).
§Values are pools of five or six organs.

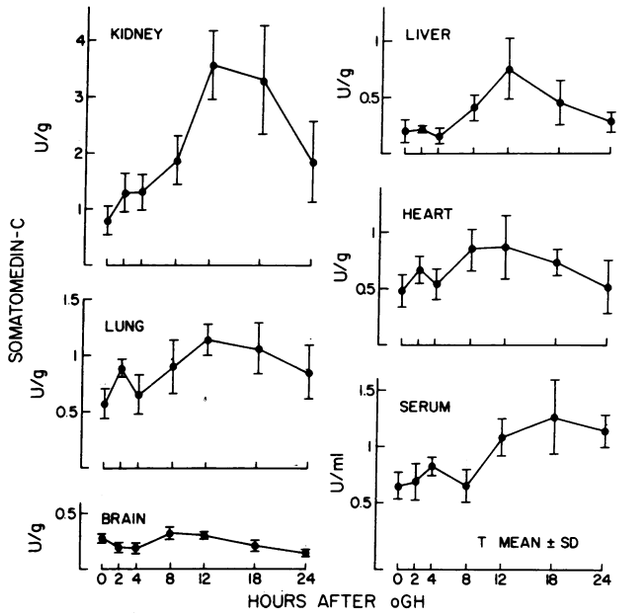

FIG. 1. Tissue and serum Sm-C response to intraperitoneal administration of oGH in hypophysectomized rats. Groups of six male rats were injected intraperitoneally with 1 mg of oGH in 1 ml of vehicle (0.05 M NH_4CO_3, pH 8.2) at time 0 and sacrificed at the times indicated. The time 0 group is composed of rats that were not injected. One to three animals injected with vehicle were sacrificed at each time, and their Sm-C values were not different from the uninjected controls. Tissue Sm-C concentrations are in units/g (frozen weight); serum concentrations are in units/ml. Significant differences between untreated and oGH-injected rats were as follows: kidney, at 2.4, and 24 hr $P < 0.01$ and at 8, 12, and 18 hr $P < 0.0005$; liver, at 8 hr $P < 0.005$, at 12 hr $P < 0.0005$, and at 18 hr $P < 0.0125$; lung, at 2 hr $P < 0.0025$, at 8 hr $P < 0.01$, at 12 and 18 hr $P < 0.0005$, and at 24 hr $P < 0.025$; heart, at 2 hr $P < 0.05$ and at 8, 12, and 18 hr $P < 0.025$; brain, at 2, 4, 18, and 24 hr $P < 0.01$ (lower than control); serum, at 4 hr $P < 0.0125$, at 12 and 24 hr $P < 0.0005$, and at 18 hr $P < 0.0025$. Differences were determined by unpaired Student's t tests.

response of tissue Sm-C, we injected hypophysectomized rats with 0.1, 0.25, 0.5, or 1 mg of oGH. When the rats were sacrificed at 8 hr, it appeared that a maximal or near-maximal response had occurred at all dosages above 0.1 mg (data not shown). In a subsequent experiment (Fig. 3), we injected groups of five rats with 10, 50, 100, or 250 μg of oGH and assessed tissue Sm-C 12 hr later. In liver the mean Sm-C concentrations fit a linear regression model ($r = 0.95$; $P < 0.01$), and in lung the mean values also fit a linear regression model ($r = 0.99$; $P < 0.01$) if the 250-μg oGH dose was excluded. In testes, which were collected as pools of five organs, Sm-C concentrations were 1.13, 1.30, 2.18, and 2.13 units/g for the 10-, 50-, 100-, and 250-μg oGH doses, respectively. In the same group of animals, serum Sm-C concentrations did not change compared to animals injected with vehicle (0.74 ± 0.12 for vehicle controls; 0.74 ± 0.31, 0.84 ± 0.29, 0.82 ± 1.8, and 0.74 ± 0.03 units/ml for oGH doses of 10, 50, 100, and 250 μg, respectively; P value, not significant.

DISCUSSION

Virtually all studies on the regulation of the somatomedins have focused on their concentrations in serum. Although no enriched tissue source has been found, these growth factors have been considered to act by hormone-like endocrine mechanisms, exerting their actions at locations distant to their site(s) of origin. The observation that explants of multiple fetal tissues and a variety of cultured cells produce immunoreactive somatomedins (4–6) led to the proposal that

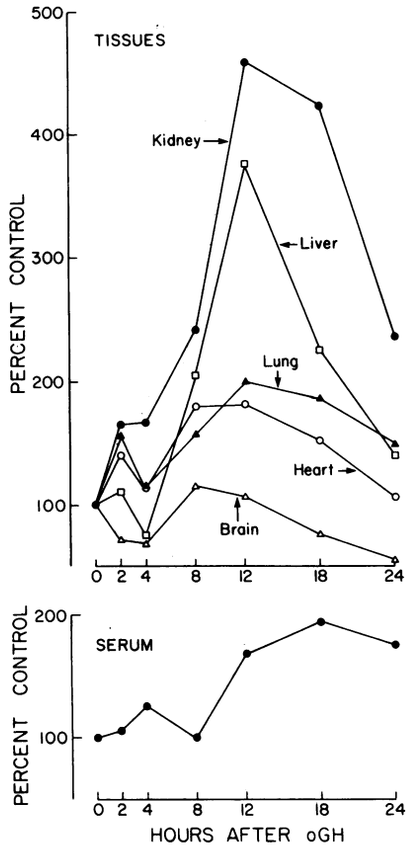

FIG. 2. Magnitude of tissue and serum Sm-C response to intraperitoneal injection of 1 mg of oGH in hypophysectomized rats. Data from the same animals shown in Fig. 1 are displayed as the percent of control (time 0, noninjected animals). Note that the rise and fall in the tissue concentrations of Sm-C precede similar fluctuations in serum concentration.

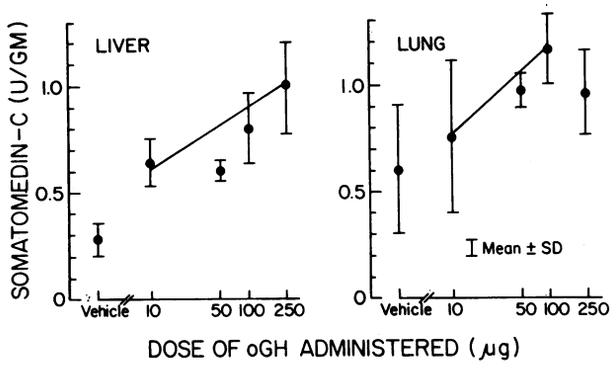

FIG. 3. Tissue concentrations of Sm-C in liver and lung in response to oGH. Groups of hypophysectomized male rats ($n = 5$) were injected intraperitoneally with 1 ml of vehicle or with 10, 50, 100, or 250 μg of oGH (in 1 ml of vehicle) and sacrificed 12 hr later. Results are expressed as units of Sm-C per g of frozen weight. In liver, oGH increased Sm-C concentrations at all doses ($P < 0.0005$), and the mean responses to each dose fit a linear regression model ($r = 0.95$, $P < 0.01$; $y = 0.59 + 0.0016x$). In lung, the 50-, 100-, and 250-μg doses resulted in increased Sm-C concentrations ($P < 0.005$, $P < 0.005$, and $P < 0.05$, respectively). The mean responses to the 10-, 50-, and 100-μg oGH doses also fit a linear regression model ($r = 0.99$, $P < 0.01$; $y = 0.72 + 0.0045x$).

these peptides exert their biological effects at or close to their sites of origin (2), acting by paracrine or autocrine mechanisms (18). The results reported in this study provide further evidence that many tissues are capable of Sm-C synthesis and by inference support the concept of a paracrine or autocrine mechanism of action. The finding that concentrations of Sm-C in tissues are growth-hormone dependent is consistent with our understanding of somatomedin regulation. Furthermore, because the time course of changes in tissue Sm-C concentration in response to oGH administration precede those observed in serum, it appears that the Sm-C derived from multiple tissues determines the serum concentration. Although our data support the paracrine–autocrine concept, they do not exclude an endocrine function because somatomedin in the circulation could act on cells distant from its site of origin.

Our ability to detect differences between the Sm-C content of tissue from normal and hypophysectomized rats, as well as increments in Sm-C after administration of oGH, may be due to the techniques used to preserve and extract this peptide. Flash freezing in liquid nitrogen is likely to minimize *in vivo* degradation, and it is possible that the Sm-C in pulverized tissue is more accessible to acid extraction than it would be in homogenates. We observed previously that liberation of Sm-C from serum-binding proteins is optimal when the pH is between 3.6 and 4.2 (10). The pH studies reported here suggest that the same pH range is optimal for extraction of somatomedin from tissue. Other evidence that these procedures extract Sm-C from tissue and that the activity measured in the RIA is not artifactual includes the observations that (*i*) extract serial dilution curves parallel those of pure Sm-C, (*ii*) pure Sm-C and Sm-C from rat plasma are accurately measured in the presence of extracts, and (*iii*) the amount of extracted Sm-C measured in the RIA is equal to that measured after extracted Sm-C has been separated from binding proteins by gel chromatography in acid.

Perhaps the best evidence that the Sm-C measured in a tissue extract actually originates in that tissue is the response to oGH in hypophysectomized rats. Basal Sm-C concentrations in kidney, lung, and heart of hypophysectomized rats exceed those of whole blood, assuming that a gram of tissue has a volume of 1 ml of blood and that serum occupies 54% of the whole blood volume. At the time of the maximal response to oGH (12 hr), the mean tissue levels in kidney, lung, heart, and liver are, respectively, 6.1, 1.4, 1.5, and 1.3 times higher than whole blood.

In hypophysectomized rat tissue, maximal concentrations of Sm-C were reached 12 hr after the administration of oGH. This presumably is the result of local synthesis occurring 4–12 hr after oGH administration. In heart, lung, and kidney, significant increases in Sm-C also occurred after 2 hr and conceivably caused the increase in serum concentrations observed 2 hr later (at 4 hr). In light of the relatively long interval between oGH injection and maximal increment in Sm-C, we believe that the early increase of Sm-C does not represent *de novo* synthesis, especially since this response is not sustained. It might be that the increment in Sm-C measured after 2 hr represents peptide that has been released from a precursor. Only in kidney was the early increase in Sm-C sustained. Because kidney is probably the major site of Sm-C degradation (19), this sustained increase as well as the striking increment observed after 12 hr might represent in part Sm-C filtered from serum for the purpose of degradation. In brain, the maximal Sm-C was not significantly different from the basal concentration (at time 0). However, significant fluctuations occurred because brain Sm-C concentrations fell below basal concentrations at 2 and 4 hr after oGH administration. It is not clear how growth hormone could directly effect these changes. In addition, they appear not to result from serum-derived Sm-C because the two do not coincide temporally.

To estimate the relative contribution of individual organs to the serum Sm-C, we assumed that the plasma volume of a 100-g hypophysectomized rat was 3 ml. We calculated that between 8 and 18 hr after the administration of oGH, the

total Sm-C content of hyophysectomized rat serum increased by 2 units. During this same interval, tissue concentrations peaked and declined. If one assumes that the decrement in tissue Sm-C occurring between 12 and 18 hr results from movement of Sm-C from tissue to blood, and calculates the decrement in Sm-C per organ, the liver could then account for 55% of the peptide in the circulation, kidney for 6%, lung for 4%, and heart for 1%. Other tissues would have to contribute the remaining 34%.

The observation that tissue Sm-C concentrations increase in response to the administration of oGH in dosages between 10 and 250 μg is consistent with reports of the dose needed to stimulate *de novo* protein synthesis (20). Specifically, rats injected intraperitoneally with 200 μg of bovine growth hormone had an increase in protein synthesis in muscle and liver beginning 30 min after hormone administration (21). Furthermore, bovine growth hormone in concentrations as low as 0.1 μg/ml stimulates the *in vitro* uptake of amino acids in rat diaphragm (22).

The techniques we used to measure tissue concentrations of Sm-C may be better for studying the regulation of this peptide than measurement of levels in serum. Tissue Sm-C concentrations appear to be quite dependent on growth hormone status and, when growth hormone is administered, exhibit changes greater than those that occur in serum. Studies of changes in tissue concentration after alterations in nutrient intake, administration of hormones or drugs, and other manipulations of experimental animals may provide new insights into the regulation of this growth factor and the control of growth.

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