Partial Characterization of an Insulin-dependent Serum Factor that Regulates Ornithine Decarboxylase in Skeletal Muscle

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SUMMARY

We have previously shown that a factor(s) in rat serum induces ornithine decarboxylase (ODC) in incubated muscle and that its activity is diminished in sera from diabetic rats. To characterize this factor further, we have studied some of its physicochemical and biologic properties. As judged from its ability to induce ODC in the incubated rat soleus muscle, the factor is protease-sensitive and both heat- and acid-stable. In untreated whole serum its activity is associated with a high-molecular-weight fraction whereas after boiling at pH 5.5, activity is principally in a fraction with a molecular weight between 3500 and 12,000 daltons. The activity of the factor is diminished in hypophysectomized, starved, and aged as well as diabetic rats. In diabetic rat serum it is restored to normal by the addition of a purified somatomedin, multiplication-stimulating activity. These findings suggest that the "ODC inducing factor" is a low-molecular-weight peptide and that it has many of the characteristics of a somatomedin. DIABETES *31*:500–505, June 1982.

We have recently reported that the activity of ornithine decarboxylase (ODC), the initial and rate-controlling enzyme in polyamine biosynthesis, is diminished in muscle of diabetic rats[1] and that the activity of a serum factor that can induce ODC activity in skeletal muscle in vitro is also diminished.[2] The finding that pharmacologic, but not physiologic, levels of insulin can substitute for this factor in diabetic serum suggested to us that it may be a somatomedin. The somatomedins are a family of growth hormone-dependent peptides that stimulate growth processes in many tissues and cell culture systems. They have been shown to have a high degree of sequence homology to proinsulin; however, only at supraphysiologic concentrations does insulin interact with the somatomedin receptor and mimic their anabolic effects.[3,4] On the other hand, there is increasing evidence that insulin and nutritional factors may regulate somatomedin production.[5] To explore this issue further, studies were undertaken to characterize physicochemically the "ODC inducing factor" and to compare its properties with those of known somatomedins.

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MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats were used in all studies. Except for the hypophysectomized animals, all rats were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts). They were kept in temperature-controlled animal quarters (23°C) with a 12-h light-dark cycle (8:00 a.m. to 8:00 p.m.) and allowed free access to Purina Rat Chow and water.

MUSCLE INCUBATIONS

Details of this procedure have been described previously.[2,6] Soleus muscles from weanling rats, 40–60 g, were incubated in Krebs Henseleit bicarbonate solution and 10 mM glucose (referred to as KHS) and other additions described in RESULTS. The medium was gassed with 95% oxygen and 5% CO_2 before use. Two solei from an individual rat were placed in the same flask and considered a single determination. The flasks were stoppered and incubated at 37°C for 3 h in a shaking water bath. The muscles were then homogenized and the high speed supernatant was used for determination of ornithine decarboxylase as outlined in earlier reports.[1,2]

SERUM

In some experiments, serum partially replaced the standard incubation medium, i.e., KHS. Rats, fed ad libitum and

weighing 140–180 g, were decapitated between 8:30 and 9:00 a.m. and the blood collected and allowed to clot at room temperature for 1 h. The serum was stored at −70°C. Serum was obtained from diabetic, hypophysectomized, starved and aged rats in an identical manner. Diabetes was produced in 140–160-g rats fasted overnight by an injection of streptozotocin (kindly provided by Dr. William Dulin, Upjohn Company, Kalamazoo, Michigan) 100 mg/kg body wt i.p., 5 days before decapitation. Glucose levels of the pooled serum of diabetic rats ranged between 500 and 700 mg/dl; control values from normal littermates were 130–150 mg/dl. These diabetic rats did not appear severely ketotic, nor were they hyperosmotic as judged by a pooled serum protein concentration of 52 versus 54 mg/ml for normal rat serum. To obtain growth hormone-deficient serum, hypophysectomized male Sprague-Dawley rats weighing 150 g were purchased from Hormone Assay Laboratory (Chicago, Illinois). They were observed for 8 days to document lack of weight gain compared with normal littermates. One group of hypophysectomized rats received 1 mg bovine growth hormone (NIH-GH-B18) dissolved in saline, pH 9, subcutaneously at 5:00 p.m. for 5 days. The average weight gain for the 5-day treatment period for normal and growth hormone-treated hypophysectomized rats was 33 and 22 g, respectively, while hypophysectomized rats lost an average of 3 g during this period. For "starved" serum, rats initially weighing 140–160 g were starved for 24, 48, or 72 h. Weight loss was not recorded. For the aging study, serum was collected from 3-wk-old (45–50 g), 5–6-wk-old (130–140 g), 10–12-wk-old (290–310 g), 6-mo-old (450–540 g), and 2-yr-old (900–1100 g) rats. Except for the weaning rats, these animals arrived at 4 wk old and were housed in our animal facilities.

PHYSICOCHEMICAL CHARACTERIZATION OF THE SERUM FACTOR

The "ODC inducing factor" in serum was characterized with respect to acid and heat stability, protease sensitivity, and molecular weight as follows.

Acid stability. Serum was acidified to pH 5.5 with 5 N and 0.1 N HCl. Prior to incubation, the pH of the medium to which the serum was added was adjusted to neutrality with 5 N and 0.1 NaOH. A comparable amount of 5 M NaCl was added to the control medium to control for ionic change.

Heat stability. Untreated or acidified serum was heated in a boiling water bath for 10 min, then centrifuged at 40,000 *g* for 30 min. The supernatant, neutralized as described above if necessary, was added to the incubation medium.

Protease sensitivity. Serum was incubated ±4 mg/ml pronase (bacterial protease Type VI from *Streptomyces griseus*, Sigma Chemical Corp., St. Louis, Missouri) at 37°C for 60 min. The samples were then heated in a boiling water bath for 10 min to inactivate the pronase, and centrifuged at 40,000 *g* for 30 min. The supernantant was used for incubation. To determine if addition of the pronase or some other aspects of this procedure influenced ODC activity, the effects of 6% bovine serum albumin in KHS ±4 mg/ml pronase on ODC activity was also assessed.

Molecular weight determination. To estimate the molecular weight of the serum factor, untreated or acid-boiled sera were dialyzed using dialysis tubing with molecular weight retentions of 3500, 6000–8000, and greater than 12,000 daltons (Fisher Scientific Company, Medford, Massachusetts). The sera were dialyzed against 100 vol of KHS containing physiologic levels of amino acids.[7]

Other assays. Protein was determined according to the method of Lowry,[8] serum glucose with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, Ohio), and immunoreactive insulin with a Phadebas insulin radioimmunoassay kit (Pharmacia Diagnostics, Piscataway, New Jersey).

Statistics. Analysis of variance (ANOV) and the Dunnett test[9] were employed when comparing multiple groups with a single control. Differences between two groups were analyzed using Student's *t* test for two independent samples. Results were considered statistically significant when $P < 0.05$.

RESULTS

PHYSICOCHEMICAL PROPERTIES

Heat and acid stability. When serum from normal rats was boiled for 10 min, it did not lose its ability to induce ODC activity, despite a 90–95% loss in its protein content (Table 1). Also, its ability to induce ODC was not lost when the serum was acidified to pH 5.5 either before or after boiling (Table 1, experiment I). When serum from diabetic rats was boiled, qualitatively similar changes were observed (Table 1, experiment II). Although the data in Table 1 indicate that

TABLE 1
Physicochemical characteristics of the ODC inducing factor in serum: heat and acid stability

	ODC activity: pmol CO_2 released (mg protein/ml)	Protein concentration in incubation medium (mg/ml)	ODC inducing factor specific activity
Experiment I			
No additions	91 ± 10 (4)		
Normal serum			
Untreated	255 ± 24 (5)	30	8
Boiled	202 ± 9 (9)	2.5	81
Boiled/pH 5.5	228 ± 36 (4)	2.6	88
pH 5.5/boiled	283 ± 18 (6)	1.8	157
Experiment II			
No additions	131 ± 18 (6)		
Normal serum			
Untreated	365 ± 19 (5)	27	14
Boiled	305 ± 20 (4)	1.4	218
Diabetic serum			
Untreated	256 ± 28* (5)	26	10
Boiled	230 ± 23* (5)	1.7	135

Soleus muscles from rats fed ad libitum were incubated in KHS and the indicated sera or sera-derived additions at a 50% concentration. ODC activity was measured after 3 h of incubation. Results are means ± SEM with the numbers of observations in parentheses.
Experiment I: Serum from normal rats was either untreated, boiled, boiled and then acidified to pH 5.5, or acidified to pH 5.5 and then boiled as described in METHODS. No treated serum value was significantly different from that of untreated serum (based on ANOV using the Dunnett procedure).
Experiment II: Untreated or boiled serum from normal or diabetic rats was used. Diabetes was induced by streptozotocin as described in METHODS.
* Indicates a significant difference between normal and diabetic serum (based on Student's *t* test), $P < 0.05$.
ODC inducing factor specific activity = ODC activity/(mg protein/ml incubation medium).

TABLE 2
Physicochemical characteristics of the ODC inducing factor in serum: protease sensitivity

	ODC activity (pmol CO_2 released/mg protein/h)
No additions	90 ± 8* (4)
Albumin	94 ± 11* (3)
Albumin + pronase	40 ± 2* (4)
Serum, untreated	270 ± 30 (5)
Serum ± pronase	48 ± 1* (5)

Soleus muscles from rats fed ad libitum were incubated in KHS with the additions described in METHODS at a 50% concentration. ODC activity was measured after 3 h of incubation. Results are means ± SEM with the numbers of observations in parentheses.
* Indicates a value significantly different from that of untreated serum (based on ANOV using the Dunnett procedure), $P < 0.05$.

absolute values of serum-induced ODC activity can vary from experiment to experiment, the differential sensitivity to normal and diabetic serum represented in experiment II was a consistent observation.

Protease sensitivity. The ability of serum to increase ODC activity was lost when it was incubated with a nonspecific protease (Table 2), suggesting that the "ODC inducing factor"* is either a polypeptide or that it contains an essential protein component. The reason pronase treatment resulted in a lower ODC activity than when muscle was incubated in 6% albumin is unclear but may be related to ionic changes produced by the pronase, which is purified in 30% calcium acetate (see below). Additional experiments with other proteases will be necessary to confirm the factor's peptide nature.

Molecular weight determination. In preliminary experiments we noted that increasing the ionic strength, and particularly the sodium concentration, of the medium decreased ODC activity and that lowering the sodium concentration had the reverse effect. This phenomenon has also been reported by others.[10,11] Because of this, our initial molecular weight determinations by ultrafiltration were difficult to interpret. Thus the molecular weight of the "ODC inducing factor" was estimated by using dialysis tubings with different molecular weight retention limits (MWRL). With this procedure, the electrolyte composition of the samples, dialyzed versus the same buffer, would be comparable (parenthetically the electrolyte profile of serum of diabetic and hypophysectomized rats was analyzed and found to be normal). Untreated or acid-boiled sera were dialyzed for 3 or 24 h against 100 vol of KHS plus amino acids in physiologic concentrations. The results in Table 3 indicate that whole serum maintains its activity when dialyzed in tubing with MWRL greater than 12,000 daltons. Interestingly, after the serum was acidified and boiled, a substantial portion of the activity was lost when dialyzed in tubing with MWRL greater than 12,000 daltons but not when dialyzed in tubing with MWRL of 3500 daltons. When acid/boiled serum was dialyzed in tubing with MWRL of 6000–8000 daltons, the results were less clear-cut; there appears to be some loss of activity but it is not significant. This may indicate that the molecular weight of the "ODC inducing factor" is very close to the retention limits of the tubing. These results suggest that in untreated serum the "ODC inducing factor" is associated with a fraction of molecular weight greater than 12,000 daltons whereas after acidification and boiling it predominantly appears in a fraction with a molecular weight between 3500 and 12,000 daltons.

* Although it is actually serum "activity" that is being assessed in these studies, it will be referred to as an ODC inducing "factor" for convenience.

TABLE 3
Physicochemical characteristics of the ODC inducing factor in serum: molecular weight determination

Additions	Dialysis tubing MWRL	ODC activity (pmol CO_2 released/mg protein/h)	Percent of untreated, undialyzed serum
Experiment I			
No additions		82 ± 6*	
Serum, untreated	—	243 ± 14	
Serum, untreated	>12,000 daltons	221 ± 39	91
Serum, acid/boiled	>12,000 daltons	130 ± 20*	53
Serum, acid/boiled	6,000–8,000 daltons	185 ± 45	76
Serum, acid/boiled	3,500 daltons	200 ± 34	91
Experiment II			
No additions		44 ± 11*	
Serum, untreated	—	212 ± 3	
Serum, untreated	>12,000 daltons	168 ± 54	79
Serum, acid/boiled	>12,000 daltons	80 ± 8*	38
Serum, acid/boiled	3,500 daltons	156 ± 33	74

Soleus muscles from rats fed ad libitum were incubated in KHS with the indicated additions at a 50% concentration. Untreated and acid/boiled normal rat sera were dialyzed versus KHS plus amino acids for 3 h (experiment I) or 24 h (experiment II) as described in METHODS. ODC activity was measured after 3 h of incubation. Results are means ± SEM of 4–5 observations.
* Indicates a value significantly different from that of untreated, undialyzed serum (based on ANOV using the Dunnett procedure), $P < 0.05$.
MWRL = molecular weight retention limit.

HYPOPHYSECTOMIZED RATS

Historically a principal criterion for classifying a serum factor as a somatomedin has been its growth hormone dependency.[3] Somatomedin levels are decreased in man and experimental animals after hypophysectomy and can be restored toward normal with growth hormone therapy.[12,13]

As shown in Table 4, serum from hypophysectomized rats was less effective than normal serum in stimulating ODC activity in the soleus, and growth hormone treatment in vivo partially restored ODC inducing activity to normal (Table 4, experiment II). Addition of growth hormone to serum in vitro had no effect.

BIOLOGIC BEHAVIOR

In an earlier study, we found that serum from diabetic rats was only 50% as effective as serum from normal rats in stimulating ODC activity in the incubated soleus.[2] To determine whether ODC inducing activity is diminished in other insulin-deficient states, the soleus was incubated with sera from rats starved from 24, 48, or 72 h. Although circulating insulin

TABLE 4
Effect of serum from hypophysectomized rats on ornithine decarboxylase activity in the incubated rat soleus

	ODC activity (pmol CO_2 released/mg protein/h)
Experiment I	
No additions	92 ± 10* (4)
Normal serum	344 ± 33 (6)
Hypophysectomized serum	222 ± 5* (6)
Experiment II	
No additions	114 ± 4* (3)
Normal serum	378 ± 37 (6)
Hypophysectomized serum	281 ± 18* (6)
Hypophysectomized serum + growth hormone (0.2 mg/ml)	271 ± 36* (5)
Growth hormone-treated hypophysectomized serum	334 ± 34 (6)

Soleus muscles from rats fed ad libitum were incubated in KHS + 50% serum obtained from normal, hypophysectomized, and growth hormone-treated hypophysectomized rats as described in METHODS. In one group, 0.2 mg/ml of the growth hormone was added to 50% hypophysectomized serum. ODC activity was measured after 3 h of incubation. Results are means ± SEM with the numbers of observations in parentheses.
* Indicates a value significantly different from that with normal serum (based on ANOV using the Dunnett procedure), $P < 0.05$.

is lower than in the fed state in all three groups,[14] the ability of serum to induce ODC was reduced significantly only in the 72-h starved rats (Table 5).

Sera from very old rats were also found to be less effective in stimulating ODC activity in the incubated soleus (Table 6). Compared with serum from 5–6-wk-old rats ("normal" serum), ODC induction was diminished by 20% in 6-mo-old animals and by 30% in 2-yr-old rats. The immunoassayable insulin concentrations in the pooled sera from the 6- and 24-mo-old rats were slightly greater than in the younger animals.

EFFECT OF MULTIPLICATION-STIMULATING ACTIVITY

To determine whether a purified somatomedin could restore the ODC inducing activity of diabetic serum, multiplication-stimulating activity (MSA) was added in vitro. MSA (obtained from Collaborative Research, Inc., Waltham, Massachusetts) is purified from the conditioned media of a rat liver cell line. As seen in Table 7, and as reported previously,[2] serum from diabetic rats is only 50–60% as effective as normal serum in stimulating ODC activity. When reported physiologic levels of MSA[15,16] were added, ODC activity was induced to the same extent as with normal serum. Thus, MSA is able to substitute for the "ODC inducing factor" in serum of diabetic rats. Interestingly, when MSA was added to KHS in the absence of serum it had no effect, indicating that another factor(s) in serum is required for MSA stimulation of ODC activity.

TABLE 5
Effect of serum from starved rats on ornithine decarboxylase activity in the incubated rat soleus

	ODC activity (pmol CO_2 released/mg protein/h)
No additions	103 ± 5(7)
Serum from rats	
Fed ad libitum	283 ± 13 (11)
Starved 24 h	297 ± 28 (6)
48 h	270 ± 26 (6)
72 h	229 ± 8* (11)

Soleus muscles from rats fed ad libitum were incubated in KHS ± 50% serum from ad libitum-fed rats or from rats starved 24, 48, or 72 h. ODC activity was measured after 3 h of incubation. Results are means ± SEM with the numbers of observations in parentheses.
* Indicates a value significantly different from that of the fed ad libitum group (based on ANOV using the Dunnett procedure), $P < 0.05$.

TABLE 6
Effect of serum from rats of different ages on ornithine decarboxylase activity in the incubated rat soleus

	ODC activity (pmol CO_2 released/mg protein/h)	Serum insulin (μU/ml)
No additions	108 ± 22 (3)	0
Serum from rats		
3 wk old	336 ± 22 (3)	ND
5–6 wk old	348 ± 25 (4)	25
10–12 wk old	327 ± 34 (5)	20
6 mo old	274 ± 21 (6)	28
24 mo old	233 ± 29* (6)	30

Soleus muscles from rats fed ad libitum were incubated in KHS ± 50% serum from rats of the indicated ages. ODC activity was measured after 3 h of incubation. Results are means ± SEM with the numbers of observations in parentheses. Insulin was measured in the various pooled sera by radioimmunoassay.
* Indicates a value significantly different from that of the 5–6-wk-old serum group (based on ANOV using the Dunnett procedure) at $P < 0.05$.
ND = not determined.

DISCUSSION

The physicochemical properties of the "ODC inducing factor" are strikingly similar to those of the somatomedins. Like the somatomedins it is both heat- and acid-stable and it appears to have an essential peptide character. Dialysis experiments indicate that in untreated serum the "ODC inducing factor" has a molecular weight greater than 12,000 daltons; however, after the serum has been acidified and

TABLE 7
Effect of multiplication-stimulating activity (MSA) on ornithine decarboxylase activity in the incubated rat soleus

	ODC activity (pmol CO_2 released/mg protein/h)
Normal serum	396 ± 46 (6)
Diabetic serum	246 ± 19* (6)
Diabetic serum + MSA (1 μg/ml)	407 ± 26 (3)
No additions	97 ± 16* (6)
MSA (1 μg/ml)	99 ± 8* (3)

Soleus muscles from rats fed ad libitum were incubated in KHS and the indicated additions. Normal and diabetic sera were present at a concentration of 50%. Diabetes was induced with streptozotocin as described in METHODS. ODC activity was measured after 3 h of incubation. Results are means ± SEM with the numbers of observations in parentheses.
* Indicates a value significantly different from that with normal serum (based on ANOV and the Dunnett procedure), $P < 0.05$.

boiled, its molecular weight is between 12,000 and 3500 daltons. This closely parallels the behavior of somatomedins in serum where they are associated with specific high-molecular-weight binding proteins from which they can be dissociated by acidification. Free somatomedins have a molecular weight between 7000 and 10,000 daltons.[17–19]

The "ODC inducing factor" appears to be at least partially growth hormone-dependent. The changes in its activity following hypophysectomy and growth hormone treatment were small, although the decrease with hypophysectomy was significant. One possible explanation for the relatively small effect of hypophysectomy may be that the serum factor is more dependent on insulin than growth hormone. It has been found that some somatomedins are less growth hormone-dependent than others. For example, there is no increase in circulating IGF-II in acromegalics and the decrease in IGF-II in patients with hypopituitarism is much less than the decrease in IGF I/Somatomedin C.[15,20] Thus a less growth hormone-dependent somatomedin could be responsible for the induction of ODC activity in the soleus muscle. In this regard, it may be relevant that the primary structure of MSA has been recently shown to have 93% homology with human IGF II. Marquardt et al.[21] have proposed the term "rat IGF II" for MSA.

We have previously shown that serum from diabetic rats is less effective than normal serum in inducing ODC activity. This defect can be corrected by injecting the rat with therapeutic amounts of insulin in vivo, but not by adding insulin in physiologic concentrations to serum in vitro.[2] That the "ODC inducing factor" lacking in diabetic serum is a somatomedin is suggested by the finding that the loss of activity can be corrected by adding to the serum, in vitro, either a purified somatomedin such as MSA (this study) or a supraphysiologic concentration of insulin.[2] Somatomedin activity has been shown to be diminished in diabetic man,[22,23] streptozotocin-diabetic rats[24–27] and Macaque monkeys,[25] and pancreatectomized dogs[28] and to be increased by insulin therapy in vivo. In severely ketotic diabetic rats Phillips et al. found that the decrease in somatomedin activity, as measured by cartilage bioassays, was due, in part, to the presence of a heat-labile, nondialysable inhibitor.[29] An inhibitor of this sort was also found in the serum of severely malnourished children and rats.[12,30] However, using sensitive receptor assays for somatomedin activity which are less likely to be influenced by inhibitors, several laboratories[25,27,28] have found an absolute decrease in the levels of somatomedins in serum of streptozotocin-diabetic rats and pancreatectomized dogs. Although in the studies reported here, boiling did not increase the "ODC inducing factor" in diabetic serum, it was not done at reduced pH as in Phillips et al.'s studies[29] and thus does not rule out the possibility of a heat-labile inhibitor.

Serum from rats starved for 72 h was also found to have a diminished ability to stimulate ODC activity. Starvation has been shown to be associated with a decrease in somatomedin activity.[13,31,32] However, the extent of the decrease varies greatly depending on the age of the animal and the assay used to measure somatomedins. Serum somatomedin and muscle ODC activities have been found to be diminished in rats starved for 24 and 48 h.[1,13,31] The finding that ODC inducing activity was depressed only in serum from 72-h starved animals was somewhat surprising if the inducing factor is indeed a somatomedin and if it modulates the decrease in ODC observed in vivo. It must be emphasized, however, that in vivo we are measuring the steady-state activity of muscle ODC after chronic (48 h) exposure to a presumably diminished "ODC inducing factor," whereas in vitro we are monitoring an acute (3 h) effect of serum on ODC induction in muscle from fed rats. It may be that a more drastic reduction in inducing factor such as occurs after 72 h of starvation is necessary to observe the diminished effect of serum in the in vitro system.

The ability of rat serum to induce ODC activity in the incubated soleus diminished with the age of the rat after maturity. Thus, there was a 20% decrease in serum ODC inducing activity at 6 mo and a 30% decrease at 2 yr of age. These decreases were found in spite of higher absolute levels of insulin in the sera of older rats. Florini and Roberts[33] found that somatomedin levels, as measured with a bioassay based on myoblast proliferation in culture, decreased slightly (10%) in 1-yr-old rats and substantially (30–50%) in 2-yr-old rats when compared with rats 2 mo of age. Thus, in older rats and in rats in insulin-deficient states associated with impaired growth and muscle metabolism, the activity of the "ODC inducing factor" is depressed as is that of the somatomedins.

An additional finding of this study was that MSA added to KHS rather than to a medium containing diabetic serum did not induce ODC. This suggests that a second component of serum, which is insulin-insensitive, is necessary for the effect of MSA on ODC induction. In keeping with this notion, whole serum has been found to be much more effective than purified somatomedins in promoting cell growth in many tissue culture systems.[34,35] Cohen and Nissley[36] have reported a heat-stable, low-molecular-weight serum factor that enhances the mitogenic effect of MSA. Additionally, both competence factors present in serum and other unknown components in platelet-poor plasma have been found to be necessary for cells to respond to somatomedins and progress through the cell cycle.[37,38] The relative contribution of other serum factors in the induction of ODC in muscle is unknown. They are clearly necessary to see an effect of MSA but do not seem to account for the physiologic variability between normal and diabetic serum.

In conclusion, the cumulative evidence so far, i.e., physicochemical properties, biologic behavior, and substitution by MSA or supraphysiologic insulin, suggests that a somatomedin-like factor in serum mediates insulin's effect on ODC activity in skeletal muscle. Lack of this factor could account for the impaired polyamine metabolism and growth in insulin-deficient states.

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REFERENCES

[1] Conover, C. A., Rozovski, S. J., and Ruderman, N. B.: Ornithine decarboxylase in insulin-deficient states. Biochem. J. *192*:725–32, 1980.

[2] Conover, C. A., Rozovski, S. J., and Ruderman, N. B.: Regulation of ornithine decarboxylase in skeletal muscle: evidence for the involvement of an insulin-dependent serum factor. Diabetes *30*:678–84, 1981.

[3] Van Wyk, J. J., and Underwood, L. E.: The somatomedins and their actions. *In* Biochemical Actions of Hormones. Vol. 5. Litwach, G., Ed. New York, Academic Press, 1978, pp. 101–48.

[4] King, G. L., Kahn, R. C., Rechler, M. M., and Nissley, P. S.: Direct demonstration of separate receptors for growth and metabolic activities of insulin and multiplication-stimulating activity using antibodies to the insulin receptor. J. Clin. Invest. *66*:130–40, 1980.

[5] Vassilopoulou-Selin, R., Phillips, L. S., and Reichard, L. A.: Nutrition and somatomedin. VII. Regulation of somatomedin activity by the perfused rat liver. Endocrinology *106*:260–67, 1980.

[6] Maizels, E. Z., Ruderman, N. B., Goodman, M. N., and Lau, D.: Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat. Biochem. J. *162*:557–68, 1977.

[7] Mallette, L. E., and Exton, J. H.: Stimulation by insulin and glucagon of ornithine decarboxylase activity in perfused rat livers. Endocrinology *93*:640–44, 1973.

[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randal, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. *193*:265–75, 1951.

[9] Dunnett, C. W.: A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. *50*:1096–1121, 1955.

[10] Munro, G. F., Miller, R. A., Bell, C. A., and Verderber, E. L.: Effects of external osmolality on polyamine metabolism in HeLa cells. Biochim. Biophys. Acta *411*:263–81, 1975.

[11] Chen, K., Heller, J. S., and Canellakis, E. S.: The inhibition of the induction of ornithine decarboxylase by cations. Biochim. Biophys. Res. Commun. *70*:212–20, 1976.

[12] Van den Brande, J. L., and Du Caju, M. V. L.: Plasma somatomedin activity in children with growth disturbances. *In* Advances in Human Growth Hormone Research. Raitis, S., Ed. Washington, D.C., D.H.E.W. Publication No. (NIH) 74, 1974, pp. 98–115.

[13] Takano, K., Hizuka, N., Kawai, K., and Shizume, K.: Effect of growth hormone and nutrition on the level of somatomedin A in the rat. Acta Endocrinol. *87*:485–94, 1978.

[14] Goodman, M. N., Larsen, P. R., Kaplan, M. N., Aoki, T. T., Young, V. R., and Ruderman, N. B.: Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. Am. J. Physiol. *239*:E277–85, 1980.

[15] Zapf, J., Morell, B., Walter, H., Laron, Z., and Froesch, R. E.: Serum levels of insulin-like growth factor (IGF) and its carrier protein in various metabolic disorders. Acta Endocrinol. *95*:505–17, 1980.

[16] Moses, A. C., Nissley, S. P., Short, P. A., Rechler, M. M., White, R. M., Knight, A. B., and Higa, O. Z.: Increased levels of multiplication-stimulating activity, an insulin-like growth factor, in fetal rat serum. Proc. Natl. Acad. Sci. USA *77*:3649–53, 1980.

[17] Zapf, J., Waldvogel, M., and Froesch, E. R.: Binding of NSILA to human serum. Arch. Biochem. Biophys. *168*:638–45, 1975.

[18] Cohen, K. L., and Nissley, P. S.: Serum half-life of somatomedin activity: evidence for growth hormone-dependence. Acta Endocrinol. *83*:243–58, 1976.

[19] Horner, J. M., Liu, F., and Hintz, R. L.: Comparison of (^{125}I) somatomedin A and (^{125}I) somatomedin C radioreceptor assays for somatomedin peptide content in whole and acid-chromatographed plasma. J. Clin. Endocrinol. Metab. *47*:1287–95, 1978.

[20] Daughaday, W. H., Trivedi, B., and Kapadia, M.: Measurement of insulin-like growth factor II by a specific radioreceptor assay in serum of normal individuals, patients with abnormal growth hormone secretion, and patients with tumor-associated hypoglycemia. J. Clin. Endocrinol. Metab. *53*:289–94, 1981.

[21] Marquardt, H., Todaro, G. J., Henderson, L. E., and Oroszlan, S.: Purification and primary structure of a polypeptide with multiplication-stimulating activity from rat liver cell cultures. J. Biol. Chem. *256*:6859–65, 1981.

[22] Yde, H.: Growth hormone-dependent sulfation factor in serum from patients with various types of diabetes. Acta Med. Scand. *186*:293–97, 1969.

[23] Winter, R. J., Phillips, L. S., Klein, M. N., Trausman, H. S., and Green, O. C.: Somatomedin activity and diabetic control in children with insulin-dependent diabetes. Diabetes *28*:952–54, 1969.

[24] Phillips, L. S., and Orawski, A. T.: Nutrition and somatomedins. III. Diabetic control, somatomedins and growth in rats. Diabetes *26*:864–69, 1977.

[25] Baxter, R. C., Brown, A. S., and Turtle, J. R.: Decrease in serum receptor-reactive somatomedins in diabetes. Horm. Metab. Res. *11*:216–20, 1979.

[26] Franklin, R. C., Rennie, G. C., and Cameron, D. P.: Serum levels of acid-ethanol soluble component of non-suppressible insulin-like activity in untreated and treated streptozotocin diabetic rats. J. Endocrinol. *81*:331–37, 1979.

[27] Takano, K., Hizuka, N., Shizume, K., Hasumi, Y., Megumi, K., and Tusushima, T.: Effect of insulin and nutrition on serum levels of somatomedin A in the rat. Endocrinology *107*:1614–19, 1980.

[28] Eigenmann, J. E., Becker, M., Kammermann, B., Leemann, W., Heimann, R., Zapf, J., and Froesch, E. R.: Decrease of NSILA after pancreatectomy and normalization by insulin therapy. Acta Endocrinol. *85*:818–22, 1977.

[29] Phillips, L. S., Belosky, D. C., and Reichard, L. A.: Nutrition and somatomedin. V. Action and measurement of somatomedin inhibitors in serum from diabetic rats. Endocrinology *104*:1513–18, 1976.

[30] Salmon, W. D., Jr.: Interaction of somatomedin and a peptide inhibitor in serum of hypophysectomized and starved, pituitary-intact rats. Adv. Metab. Disorders *8*:183–99, 1975.

[31] Phillips, L. S., and Young, H. S.: Nutrition and somatomedin. I. Effect of fasting and refeeding on serum somatomedin activity and cartilage growth activity in rats. Endocrinology *99*:304–14, 1976.

[32] Yeh, J. F., and Aloia, J. F.: Dietary constituents and somatomedin activity. Metabolism *27*:507–509, 1978.

[33] Florini, J. R., and Roberts, S. B.: Effect of rat age on blood levels of somatomedin-like growth factors. J. Gerontol. *35*:23–30, 1980.

[34] Morell, B., and Froesch, E. R.: Fibroblasts as an experimental tool in metabolic and hormone studies. II. Effects of insulin and NSILA-s on fibroblasts in culture. Eur. J. Clin. Invest. *3*:119–23, 1973.

[35] Smith, G. L., and Temin, H. M.: Purified multiplication-stimulating activity from rat liver cell conditioned medium: comparison of biological activities with calf serum, insulin and somatomedin. J. Cell Physiol. *84*:181–92, 1975.

[36] Cohen, K. L., and Nissley, S. P.: Cohn Fraction VI enhances the growth stimulating effect of multiplication stimulating activity (MSA) on chick embryo fibroblasts in culture. Horm. Metab. Res. *12*:164–68, 1980.

[37] Stiles, C. D., Capone, G. T., Scher, C. D., Antoniades, H. N., Van Wyk, J. J., and Pledger, W. J.: Dual control of cell growth by somatomedins and platelet-derived growth factor. Proc. Natl. Acad. Sci. USA *76*:1279–83, 1979.

[38] Clemmons, D. R., Van Wyk, J. J., and Pledger, W. J.: Sequential addition of platelet factor and plasma to BALB/c 3T3 fibroblast cultures stimulates somatomedin-C binding early in cell cycle. Proc. Natl. Acad. Sci. USA *79*:6644–48, 1980.