Competitive Binding of Somatomedin to the Insulin Receptors of Adipocytes, Chondrocytes, and Liver Membranes

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abstracT the action of growth hormone on skeletal tissue is mediated through somatomedin, a low molecular weight peptide found in serum. This peptide, formerly known as “sulfation factor” or “thymidine factor,” produces marked insulin-like effects in various target tissues. Since at least some of the metabolic effects of insulin on target cells are initiated by a highly specific interaction with receptors on cell membranes, this study was undertaken to determine whether somatomedin might interact with the same binding sites. It was found that somatomedin, at physiological concentrations, competes with [125I]insulin for receptor sites on isolated fat cells, liver membranes, and isolated chondrocytes, and that the relative binding affinities of insulin and somatomedin reflect the in vitro biological potencies of the two hormones in these tissues. This is the first demonstration of a peptide other than insulin, proinsulin, or derivatives of insulin competing for insulin-binding sites, and implies a structural, as well as a functional, homology between somatomedin and insulin. Somatomedin may be one of a larger group of homologous pleiotropic peptides with different target organ specificities.

In 1957, Salmon and Daughaday observed that although normal serum or serum from hypophysectomized animals treated with growth hormone stimulated the in vitro uptake of 35S by cartilage, growth hormone itself was completely inactive in this respect (1). They postulated that there was a substance in serum stimulated by growth hormone, but distinct from it, that mediated the skeletal effects of growth hormone. This “sulfation factor” also stimulates the incorporation of thymidine into DNA (2), uridine into RNA (3), and the conversion of proline to hydroxyproline (4) in cartilage. Purified preparations of “sulfation factor” exhibit insulin-like effects in at least four different bioassay systems: incorporation of amino acids into rat diaphragm (5), glucose degradation by epidermal fat pads (6), antipolytic action in epinephrine-stimulated epidermal fat pads (7), and stimulation of protein synthesis and cell replication in HeLa cells (8).

Van Wyk et al. found that a substantial portion of the sulfation factor activity in human plasma could be attributed to a neutral peptide with a molecular weight of about 7000 (9, 10). Sulfation factor and thymidine factor activities could not be dissociated after purification about 25,000-fold. Hall and Uthne were unable to dissociate sulfation factor activity from nonimmunoreactive insulin-like activity during similar purification procedures (6). Because of their chemical and biological similarities, it was proposed that sulfation factor, thymidine factor, and the acid–ethanol soluble component of nonsuppressible insulin-like activity in serum described by Jakob et al. (11) may, in fact, be a single substance with broad physiological significance. The more general term “somatomedin” was proposed to replace the previously used operational terms (12). Since then, Temin et al. (13) have partially purified the insulin-like Multiplication Stimulating Activity from fetal-calf serum, and have demonstrated chemical properties closely related to those described for somatomedin.

The striking insulin-like effects of somatomedin prompted the present studies to determine whether or not this peptide might interact with insulin receptors on plasma membranes. Evidence has been advanced that many, and perhaps all, of the metabolic effects of insulin result from interaction of the hormone with specific receptors on the outer surface of target cells (14, 15). Cuatrecasas has shown that even at concentrations as high as 40 μg/ml, ACTH, growth hormone, prolactin, vasopressin, oxytocin, and glucagon do not compete with insulin for binding sites on fat cells or liver membranes (15).

Materials and Methods

A purified preparation of somatomedin was prepared from an acid–ethanol extract of Cohn Fraction IV of retroplacental blood. This was further processed by chromatography on Sephadex G-75 and Sephadex G-50 in 1% formic acid, and assayed for its ability to stimulate 35S0 and [methyl-3H]-thymidine into cartilage segments of hypox rats (16). This preparation contained 18 μg of protein and 0.25 μunits of IR insulin per unit of somatomedin. Some of the studies were repeated with a more highly purified preparation containing <0.1 μunits insulin per unit of somatomedin. This further purification was achieved by free-flow electrophoresis on a Brinkmann Elphor apparatus, model FF-1.

[125I]Insulin, with a specific activity of 120-225 μCi/μg, was prepared by the method of Cuatrecasas (15). Fat cells were prepared from rat epidymal fat pads by the method of Rodbell (17). Chondrocytes were prepared by the method of Garland et al. (18) from pelvic leaflets of 12-day fetal chickens. Rat liver membranes were prepared by the method of Cuatrecasas (19). Specific [125I]insulin binding was determined in the well of a gamma spectrometer (Packard Instruments). Samples were assayed for insulin by radioimmunoassay.

Results

The displacement of [125I]insulin specifically bound to adipocytes by different amounts of unlabeled insulin was exactly reproduced by comparable amounts of somatomedin (Fig. 1). The apparent ratio of somatomedin to insulin in this

* One unit of somatomedin is defined as the amount of sulfation factor activity in one ml of a standard reference plasma.
Fig. 1. Each tube contained $5 \times 10^6$ adipocytes per ml and 10 μunits/ml of $[1^25I]$insulin (120 μCi/μg), in a final incubation volume of 0.5 ml of Krebs–Ringer bicarbonate buffer, pH 7.4. Unlabeled insulin (● — ●) or somatomedin (○ — ○) were added to displace bound $[1^25I]$insulin. Specific binding was determined by subtraction of the amount of $[1^25I]$insulin remaining bound in the presence of 20 μg of native insulin.

The study was 50 μunits of insulin per unit of somatomedin. Scatchard plots of $[1^25I]$insulin binding in the presence of 0, 0.5, and 4.0 units/ml of somatomedin were compatible with simple competition for the same primary receptor sites (Fig. 2). The presence of lower-affinity, higher-capacity receptors was suggested by the change in slope at the higher dosages. Accurate calculations of $K_{assoc}$ for somatomedin cannot be made, however, until it becomes possible to transpose biological units into molar concentrations.

Displacement of $[1^25I]$insulin specifically bound to liver membranes by somatomedin was also closely similar to that of insulin (Fig. 3). In this instance, the ratio appears to be 200 μunits of insulin per unit of somatomedin.

Isolated chondrocytes differed from adipocytes and liver cell membranes in that a smaller percentage of $[1^25I]$insulin was specifically bound, and the curves were not parallel over the range of dosages studied. Somatomedin was much more effective than even large amounts of unlabeled insulin in displacing $[1^25I]$insulin (Fig. 4). This finding suggests that the specific binding of $[1^25I]$insulin was determined by subtraction of the amount of $[1^25I]$insulin remaining bound in the presence of 3.2 units/ml of somatomedin was subtracted to obtain the specific binding.

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**Fig. 2.** $5 \times 10^6$ adipocytes per ml were incubated in the presence of 10 μunits/ml of $[1^25I]$insulin (225 μCi/μg). Native insulin—10, 40, 160, 640, and 3200 μunits/ml—was assayed in the presence of 0 (● — ●), 0.5 (▲ — ▲), or 4.0 (■ — ■) units/ml of somatomedin. Specific binding was determined by subtraction of the amount of $[1^25I]$insulin remaining bound in the presence of 20 μg of native insulin. $B$, bound; $F$, free.

**Fig. 3.** 100 μg of liver-cell membrane protein was incubated with 10 μunits/ml of $[1^25I]$insulin (225 μCi/μg) in a final volume of 0.2 ml. Various amounts of native insulin (● — ●) or somatomedin (○ — ○) were added to displace bound $[1^25I]$insulin. Specific binding was determined by subtraction of the amount of $[1^25I]$insulin remaining bound in the presence of 20 μg of native insulin.

**Fig. 4.** $3 \times 10^6$ chondrocytes per ml were incubated with 30 μunits/ml of $[1^25I]$insulin (120 μCi/μg) in a final incubation volume of 0.5 ml. Various amounts of native insulin (● — ●) or somatomedin (○ — ○) were added to displace $[1^25I]$insulin. Specific binding was determined by subtraction of the amount of $[1^25I]$insulin remaining bound in the presence of 3.2 units/ml of somatomedin.
chondrocyte receptor is more highly keyed for somatomedin than for insulin. The very shallow curve of \(^{125}\)I insulin displacement by native insulin is compatible with a low-affinity, high-capacity receptor.

The amount of insulin contamination in the somatomedin preparation used in most of these studies was 200-fold less than could account for the displacement of specifically bound \(^{125}\)I insulin seen with fat cells and liver membranes by somatomedin. Furthermore, an identical displacement curve of insulin from liver membrane was obtained with a more purified preparation of somatomedin that contained no detectable insulin. Thus, these studies show that somatomedin competes for the insulin receptor of all three cell types at concentrations within the range found in normal humans.

Since the material used in this study was not absolutely pure, it is possible that the effects seen may be attributable to more than one molecular species that are very similar in size and charge. This question cannot be resolved until a sufficient supply of somatomedin of unquestioned purity becomes available. This uncertainty does not diminish the importance of the present observation, however, since heretofore only insulin, proinsulin, and substituted insulin have been shown to compete with \(^{125}\)I insulin for binding to receptor sites.

**DISCUSSION**

Affinity for insulin-binding sites provides a rational explanation for the marked insulin-like effects of somatomedin. In both isolated fat cells and in isolated chondrocytes the potency ratio between somatomedin and insulin in displacing \(^{125}\)I insulin from membrane-binding sites is in line with the in vitro biologic potency ratios of these two hormones. When glucose degradation in isolated fat cells is measured, 1 unit of somatomedin is in our hands equivalent to about 20 \(\mu\) units of insulin, whereas in a rat rib cartilage assay for sulfation and thymidine activity, 1 unit of somatomedin is equivalent to 30 milliunits of insulin (unpublished results).

Although the exquisite sensitivity of the cartilage receptor to somatomedin is consonant with the original tissue-specific, growth-promoting role originally postulated by Daughaday, both fat and liver receptors are responsive to concentrations of somatomedin that are well below the concentrations normally found in serum. These findings further sharpen the paradox that Froesch (20) has attempted to deal with: the existence of large amounts of insulin-like activity in the serum of animals dying in diabetic coma. It is possible that factors operating in vivo, but not in vitro, limit the interaction of somatomedin with receptors in nonskeletal tissue.

The competitive binding of somatomedin and insulin at low concentrations implies that at least some segments of the somatomedin molecule must be very similar to the receptor recognition sites on the insulin molecule. Impressive structural homologies have recently been demonstrated by Frazier et al. (21) between nerve growth factor and insulin, and it was postulated (21) that nerve growth factor was derived from the same ancestral protein as was insulin.

Nerve growth factor, somatomedin, and insulin all share the capacity to stimulate in their respective target tissues the set of biochemical reactions characteristic of rapid cellular proliferation. These reactions have been collectively described by Herskho et al. (22) as a positive "pleiotropic response." It remains to be shown whether the pleiotropic responses of nerve growth factor and other tissue-specific growth factors such as erythropoietin (23), epidermal growth factor (24), and Multiplication Stimulating Activity (13) can be related to competition with insulin for common membrane binding sites. If so, it can be predicted that their respective binding affinities in various tissues, as in the case of somatomedin, will reflect their tissue specificities. It may indeed be possible that there exists a family of biologically active peptides homologous to insulin that differ in their target organ specificities and hormonal control mechanisms.

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