IDENTIFICATION OF SPARGANUM GROWTH FACTOR BY A RADIORECEPTOR ASSAY FOR GROWTH HORMONE

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Summary: The growth hormone-like substance of Spirometra mansonoides in the incubation medium of the worm as well as in serum from infested rats was studied with the radioreceptor assay (RRA) for growth hormone (GH). Sparganum growth factor (SGF) inhibited the binding of hGH tracer to the rabbit liver receptor in a parallel manner to human GH. Serum concentrations of SGF in normal rats increased to a level equivalent to 1 μg/ml hGH eight days after subcutaneous implantation of 50 worms. Upon gel filtration in G-100 Sephadex, SGF eluted in the fractions corresponding to a molecular weight of approximately 70,000. The Rf of SGF upon polyacrylamide disc gel electrophoresis was 0.32 and after gel isoelectrofocusing, the growth factor was detected in gel segment of pH 6.0.

Introduction: The plerocercoid larvae (sparganа) of the tape worm, Spirometra mansonoides, secretes a factor which stimulates growth in hypophysectomized (hypox) rats (1 - 3). Moreover when serum from infested rats is injected into control hypox rats, growth is also stimulated. When the sparganum of the worm is maintained in culture SGF can also detected in the incubation medium (5,6). The nature and mechanism of action of SGF have remained obscure. It has been recognised that the administration of SGF to hypox rats leads to an increase in somatomedin levels in the serum (4) but that SGF itself does not have intrinsic somatomedin activity. These observations favour the concept that SGF may be related to growth hormone, stimulating the production of somatomedin and hence growth in a manner analogous to the effect of pituitary growth hormone. In the present study we report data that supports this view because SGF competes with hGH for binding sites on rabbit liver membrane preparations.

MATERIALS AND METHODS: The cultivation of the sparganа of Spirometra mansonoides was carried out as described previously (6). The sparganа were pipetted into containers containing Eagle's medium with or without 3% of fetal calf serum (FCS). The volume of incubation medium was adjusted to 1 ml. per worm. The cultures were incubated at 37 C and the medium was changed once every seven days. A cumulative sample was made up of aliquots obtained at weekly intervals for a 6 month period. A single injection of 0.3 to 1.0 ml of the media given intraperitoneally caused an increase in body weight in hypox rats

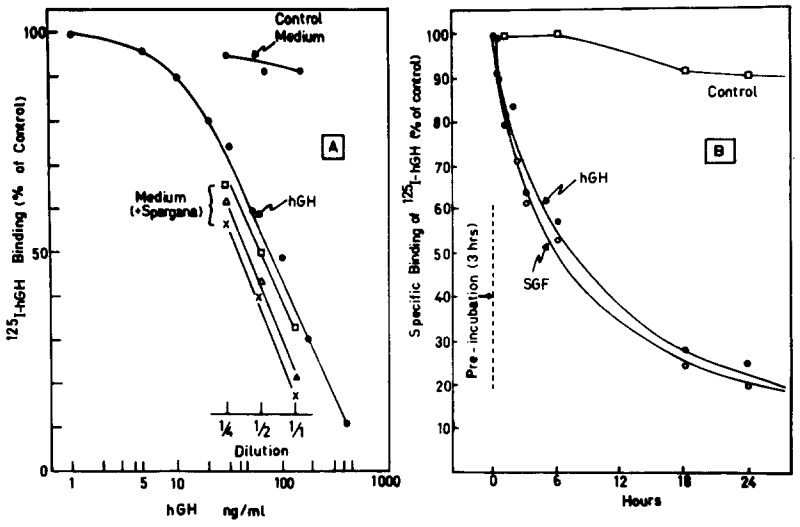

Fig. 1a A standard curve for GH compared with serial dilution response curves of the incubation media of the spargana in the RRA. The displacement by dilutions of 3 different incubation media are shown. Medium alone (open circle) caused only minimal displacement. The ordinate is expressed as per cent of ^{125}I-hGH binding in the absence of cold hGH.

Fig. 1b The displacement of ^{125}I-hGH from the rabbit liver receptor by hGH standard (solid circle), SGF from culture medium (open circle) and control tubes (open square). 150 µg of rabbit liver membrane fraction was pre-incubated with 100,000 cpm of ^{125}-hGH for 3 hours at 25 C in a final volume of 0.5 ml pH 7.6, 0.025 M Tris-HCl buffer containing 0.1% BSA. Then 0.1 ml of unlabelled hGH (1.5 µg/ml) or 0.1 ml of concentrated incubation medium containing 1.5 µg/ml equivalents of hGH was added, while 0.1 ml of Tris-HCl buffer containing 0.1% BSA was added to the control tubes. At various intervals thereafter, the reaction was terminated by the addition of 3 ml of cold Tris-HCl buffer. The tubes were centrifuged, and specific binding of ^{125}I-hGH was calculated. The ordinate represents specific binding of ^{125}I-hGH expressed as per cent of ^{125}I-hGH binding at the 3 hr preincubation period.

averaging 1 gm/day for period of 7 - 10 days. This growth rate is equivalent to that which is produced by daily injections of 10 µg of purified GH. SGF was measured by RRA for GH, using rabbit liver receptor and hGH tracer (^{125}I-hGH) (7). NIH-GH HS 1652 C (2.0 U/mg) was used for standards and for preparation of the tracer. Lactogenic activity was determined by a RRA using rabbit mammary gland receptor, ovine prolactin tracer (^{125}I-oPRIL) and ovine PRL standard (NIH-PS-10, 25 U/mg) (8). Serum samples from normal or hypox rats were also studied. The polyacrylamide disc electrophoresis was carried out according to the method of Davis using a 7.5% gel and Tris-Glycine buffer pH 8.3 (10). The isoelectrofocusing experiment was carried out as described in the LKB instruction manual (11).

RESULTS: Figure 1a compares the inhibition of binding of ^{125}I-hGH in the RRA with the

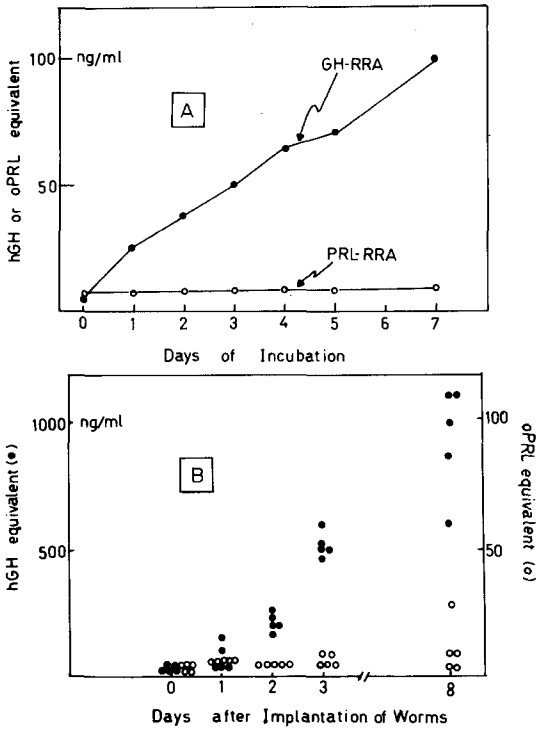

Fig. 2a The concentration of SGF (solid circle) and lactogenic activity (open circle) in the incubation medium containing 3% fetal calf serum. The volume of the medium was adjusted to 1 ml/worm.

Fig. 2b The concentration of SGF (solid circle) and lactogenic activity (open circle) in serum from 5 normal rats infested with spargana. Each point represents a sample from a single rat bled on the day indicated.

effect of serial dilutions of an aliquot of the incubation media in which spargana had been cultured. The samples of incubation media inhibited the binding of ^{125}I-hGH to the receptor in a parallel manner to the hGH standard. The apparent GH concentration in the media fortified with 3% FCS was 183 ± 20 ng/ml (mean of 5 samples). In two samples of incubation media containing no FCS the growth hormone-like activity was 24 to 90 ng/ml. Aliquots of media from cultures containing only the tail of the worm or the incubation medium alone did not inhibit the binding of the tracer. When the incubation media containing SGF was assayed for lactogenic activity all the specimens contained less than 20 ng/ml using ovine PRL standard. The displacement of bound ^{125}I-hGH from the rabbit liver receptor by the incubation media and hGH standard was compared. As shown in Figure 1b, the addition of the incubation medium or hGH accerelated the rate of dissociation of ^{125}I-hGH to the same degree.

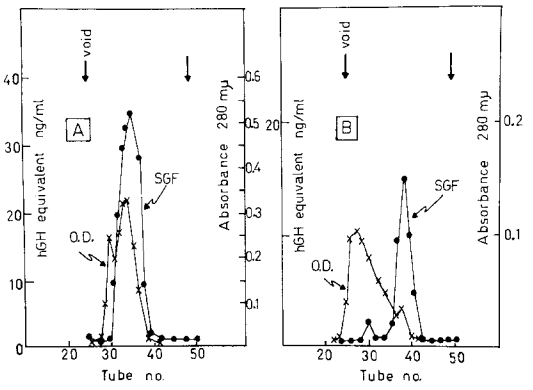

Fig. 3a and 3b Gel filtration of 4 ml of incubation medium with 3% calf serum (Fig. 3a) and the medium without calf serum (Fig. 3b) on Sephadex G-100 column (1.6 X 90 cm). The arrows mark the elution volume of ^{125}I-hGH. Protein was measured by absorbance at 280 mμ. The incubation medium without calf serum (9 ml) was concentrated to 3 ml by Amicon UM-10 before applying the sample to the column. Flow rate: 2 ml/10 min.

The time course of the appearance of GH-like factor in the incubation medium is shown in Figure 2a. The GH-like activity was already detected in the first 24 hours of incubation, and continued to increase over a seven day period. Again lactogenic activity was less than 10 ng/ml in all samples. The concentration of GH-like substance in the serum from 5 normal rats infested with 50 worms was also determined (Figure 2b). Two days after implantation of the worms, serum concentrations of the GH-like substance are definitely higher than in the control period increasing to a level equivalent to 1 μg/ml hGH 8 days after implantation. As in the case of the incubation media, these serum samples displaced ^{125}I-hGH in a parallel manner to the hGH standard. Lactogenic activity in the sera obtained 8 days after implantation was slightly higher than in the control, but the difference was not significant. Samples from hypox rats infested with the spargana showed a similar increase of serum GH-like activity with time (data not shown).

The incubation medium was fractionated on Sephadex G-100 in 0.05 M ammonium bicarbonate pH 8.4 and the eluates were assayed (Fig. 3a and 3b). GH-like activity was eluted in the fractions corresponding to a molecular weight of approximately 70,000. All the fractions collected failed to cross-react in a radioimmunoassay for human GH or PRL.

Figure 4a shows the results of polyacrylamide gel electrophoresis of the incubation medium containing no calf serum. Several components were observed in the stained gel. As shown in the figure, a sharp peak of GH like activity was observed in the segment with an Rf of 0.32. After disc electrophoresis of the incubation medium containing 3% FCS a similar peak of GH-like activity was observed at the same position.

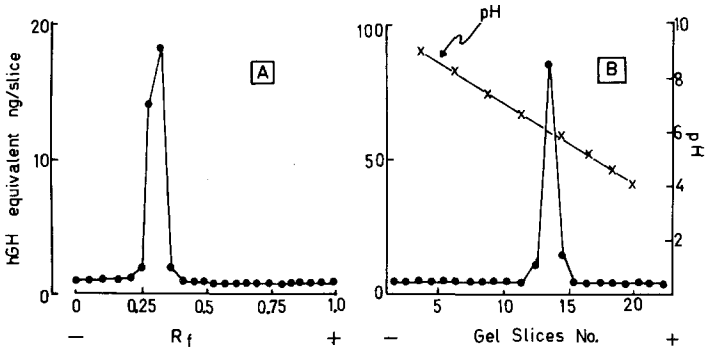

Fig. 4a Polyacrylamide disc gel electrophoresis of the incubation medium of the spargana (no calf serum). The incubation medium was dialyzed against 0.05 M ammonium bicarbonate at 4 C and then lyophilized. 300 µg of the lyophilized material was applied to disc electrophoresis. After the electrophoresis, one gel was stained with Amido black and a gel which was unstained was divided into thin slices (1.5 mm) and each segment was eluted overnight in 0.5 ml Tris-HCl buffer pH 7.6 containing 0.1% BSA and the eluates were assayed.

Fig. 4b Gel isoelectrofocusing of the fraction containing SGF. After gel filtration of the incubation medium, the fractions containing SGF were lyophilized and 500 µg of protein was applied to the gel. After the separation was complete, one channel of the gel was stained with Coomasie blue while the adjacent channel was simply divided into slices, and each of the segments were eluted in 1 ml of Tris-HCl buffer (0.1 M, pH 7.6) containing 0.1% BSA, and the GH-like activity was determined by RRA.

The results of isoelectrofocusing of the fraction containing GH-like activity after gel filtration of the incubation medium on G-100 Sephadex is shown in Figure 4b. A single peak of GH-like activity was detected at a position corresponding to a pI of 6.0.

DISCUSSION: The present study demonstrates that the GH-like substance in the incubation media of the spargana or in the sera of the infested rats can be measured by the RRA for GH. Several factors which might result in a spurious elevation of GH-like activity in the assay were excluded. For example, the tape worm might secrete a proteolytic enzyme into the incubation media which would alter the ^{125}I-hGH to a degree that it would fail to bind to the receptor. However, when ^{125}I-hGH was incubated in this media containing the worm for 3 hours at 37 C, upon subsequent testing no decrease in the binding of the tracer to rabbit liver receptor was observed. Non-specific inhibition of binding of tracer to the receptor by fetal calf serum or medium alone was not observed. Thus, SGF displaces ^{125}I-hGH in the same manner as hGH standard.

On the other hand, the incubation medium of the spargana did not displace ^{125}I-oPRL from the rabbit mammary gland receptor. Recently, Phares and Ruegamer (9) reported that the incubation medium of Spirometra mansonoides has a marked stimulatory effect on the

mucosal epithelium of the pigeon crop sac. The difference in estimates of prolactin like activity of SGF in the two studies may possibly be due to the two different assay procedures employed.

The apparent molecular weight of SGF is greater than that of GH as determined by the gel filtration studies. It remains to be determined whether this greater size is due to aggregation or due to the binding of SGF to a larger protein. The GH-like activity in the incubation media containing serum protein was approximately 200 ng/ml, and seems to be higher than the value in the media containing no protein, which is in accordance with the estimates of growth promoting activity measured by bioassay (5).

Though the data is not shown, the ability of SGF to displace ^{125}I-hGH from the receptor was decreased to 40% upon heating at 56 C for 20 min and disappeared completely when an aliquot of the medium was heated to 100 C for 5 min. The GH-like activity was destroyed by treatment of the incubation media with trypsin (500 μg/ml, 37 C, 3 hours), suggesting that SGF is a protein.

In the RRA for GH, ^{125}I-hGH is displaced only by GH and structurally related substances (7) and we suggest that SGF falls into this category. The growth promoting activity of SGF is striking. A single injection of 1.0 ml of the incubation media containing 200 ng/ml SGF determined by RRA stimulated the growth in hypox rats to the same degree as the daily injection of 10 μg of purified GH. Presumably, the greater growth promoting activity of SGF may be due to the longer half life of the material in vivo as suggested by previous studies (3).

The degree of relatedness of SGF and GH remains unknown but the RRA should prove extremely helpful in the purification and further characterization of SGF.

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