Vol. 110, No. 3, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 10, 1983 Pages 866-872

THE EFFECT OF SYNTHETIC FRAGMENT 31-44 OF HUMAN GROWTH HORMONE ON GLUCOSE UPTAKE BY ISOLATED ADIPOSE TISSUE

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Received December 22, 1982

Synthetic tetradecapeptide corresponding to amino acid sequence 31-44 of human growth hormone molecule and possessing a lipotropic activity was tested for the ability to stimulate glucose uptake by isolated epididymal fat pads of fed rats. Tetradecapeptide 31-44 (1 μg/ml), growth hormone (1 μg/ml) and insulin (50 μU/ml) stimulated in about equal degree the uptake of [U-^{14}C]glucose by adipose tissue. Tissue samples were preliminary incubated for 3-4 hours in the absence of hormones to eliminate the refractoriness to the insulin-like effects of growth hormone. Without preincubation the tissue was refractory to the action of growth hormone and tetradecapeptide 31-44, but was sensitive to insulin. The data obtained together with the findings of Lewis et al., which showed that 20K structural variant of human growth hormone having the deletion of residues 32-46 cannot stimulate glucose uptake and lipolysis in rats, make it possible to suggest that both activities are associated with fragment 31-44.

In 1973, from pepsin hydrolizate of human GH, which retained full lipotropic activity of the hormone, we isolated peptide corresponding to amino acid sequence 31-44 of GH and responsible for the activity of the hydrolizate (1). Later, this GH fragment was synthesized and studies on the biological properties of the synthetic tetradecapeptide 31-44 showed that it stimulated the lipolysis in intact fasted rats and rabbits in vivo and in isolated adipose tissue of animals and man in vitro (2-6). In 1979, Frigeri et al. (7), Lewis et al. (8) discovered a

Abbreviations: GH - growth hormone, FFA - free fatty acids, BSA - bovine serum albumin.

0006-291X/83/030866-07$01.50/0

hypophyseal structural variant of human GH (20K-GH) with a 32-46-fragment deletion and lacking certain early insulin-like and late lipotropic hormone effects. Lack of lipotropic activity in 20K-GH may be the direct result of the deletion which practically involves tetradecapeptide 31-44. However, previously we failed to demonstrate the influence of tetradecapeptide 31-44 on carbohydrate metabolism in vivo upon a single or chronic administration to rabbits and in a glucose-tolerant test in normal rats (6). The data of Frigeri et al. on the absence of early insulin-like effects in 20K-GH prompted us to test the effect of synthetic tetradecapeptide 31-44 on glucose uptake by isolated adipose tissue of rats.

MATERIALS AND METHODS

In our work we used human GH isolated by the method of Sairam et al. (9) and having a growth-promoting activity 1.0 IU/mg. Tetradecapeptide 31-44 H-Phe-Glu-Glu-Ala-Tyr-Ile-Pro-Lys-Glu-Gln-Lys-Tyr-Ser-Phe-OH was synthesized by the solid-phase method of peptide synthesis (10). The load of COOH-terminal amino acid (Boc-L-Phe) on the chloromethylated copolymer styrene and 1% of divinylbenzene was 0.6 mmol/g.

For synthesis, the following derivatives of L-amino acids were used Boc-Ser(Bzl)-OH, Boc-Tyr(Bzl)-OH, Boc-Lys(Z)-OH, Boc-Gln-ONp, Boc-Glu(OBzl)-OH, Boc-Pro-OH, Boc-Ile-OH, Boc-Ala-OH and Boc-Phe-OH.

All the condensation stages were performed by using 3-fold excesses of dicyclohexyldicarbodiimide and appropriate derivative of amino acid. The only exception was the addition of Gln-40 which was introduced by the method of n-nitrophenyl esters. The peptide chain was separated from the resin by treating with hydrogen bromide in trifluoracetic acid; This together with the indicated selection of protective groups make it possible to combine peptide chain removal from the resin and its complete deblocking into one stage.

The initial purification of tetradecapeptide hydrobromide was performed by precipitation from methanol by ether. For further purification, the precipitated material was subjected to chromatography on the cellulose column (Filtrak-DCS) in the system n-butanol-water-pyridin-acetic acid (15:12:10:3). The same system was used as an eluent. The yield of pure tetradecapeptide 31-44 was 44% of the theoretical one (on the basis of COOH-terminal amino acid attached to the resin). Rf=0.60 (paper chromatography in the above-mentioned system), 0.38 (paper chromatography in a system of n-butanol-acetic acid-water, 4:1:5, upper phase), 0.40 (TLC on "Silufol UV-254" plates, n-butanol-water-pyridin-acetic acid, 15:12:10:3), 0.83 (TLC, pyridin-acetic acid-water, 10.6.3); $[\alpha]_D^{26}$ -21.5° (c=1.0, CH_3CO_2H). It was found, %: C 53.15; H 6.88; N 12.01;

C_{85}, H_{119}, $N_{17}O_{25}$·8 H_2O. It was calculated, % : C 53.01; H 7.09; N 12.38; Amino acid analysis: Phe 2.05 (2); Glu 4.32 (4); Ala 1.00 (1); Tyr 1.76 (2); Ile 0.80 (1); Pro 1.19 (1); Lys 2.06 (2); Ser 0.82 (1).

Apart from the analytical methods indicated above, the individuality of tetradecapeptide was confirmed by determination of the sequence of NH_2-terminal tripeptide by Dansyl-Edman method.

The lipotropic activity of tetradecapeptide 31-44 was tested on non-inbread rabbits (weighing 2-2.5 kg) fasted for 18 hrs. The preparation was introduced intramascularly in a dose of 50 μg/rabbit in 0.5 ml of saline. Control animals were given injections of 0.5 ml saline. The experimental and control groups consisted of 6 rabbits each. Blood samples were taken before and 30 min after administration of the preparation. The concentration of FFA in the serum was determined by the method of Falholt et al. (12).

The effect of tetradecapeptide 31-44 and GH on the accumulation of [U-^{14}C] glucose in adipose tissue was tested in the system proposed by Goodman and Coiro (13) for inducing the sensitivity of adipose tissue of intact rats to the insulin-like action of GH. Segments of the epididymal fat pads taken from fed intact rats (weighing 220-300 g) were preincubated for 3-4 hours in Krebs-Ringers bicarbonate buffer containing 1% BSA and 1 mg/ml glucose in an atmosphere of 95%O_2-5%CO_2 (after 2 hours the incubation mixture was regassed). The tissue weight in each probe ranged from 32 to 42 mg. After preincubation the tissue samples were transferred to a fresh medium containing 1% BSA, 1 mg/ml glucose, 0.2 or 0.08 μCi/ml [U-^{14}C] glucose and the preparations tested. Incubation lasted 1 hour, then the accumulation of [U-^{14}C] glucose by the tissue was determined by the difference of glucose content in the medium before and after incubation.

Experiments 4 and 5 were performed without preincubation and the tissue samples were directly placed in the medium containing hormones and [U-^{14}C] glucose, incubated for 1 hour followed by determination of glucose uptake by the tissue. In experiment 6, the tissue samples were preincubated for 2 hours in the absence of hormones, then for 1 hour in the presence of 1 μg/ml GH to induce refractoriness to GH and then were transferred to a fresh medium containing [U-^{14}C] glucose and substances tested.

RESULTS

The influence of tetradecapeptide 31-44 on the serum FFA level in rabbits. 30 min after injection of 1 μg tetradecapeptide 31-44 to rabbits serum FFA concentration increased on the average from 0.417±0.037 (prior to injection of the preparation) to 0.610±0.063 μeq/ml ($p < 0.05$; increase by 46.3%). After 60 min the FFA level in most animals returned to normal (0.411±0.084 μeq/ml). Infusion of saline to the control rabbits did not influence the serum FFA cohcentration (0.406±

0.024 and 0.418±0.039 μeq/ml before and 30 min after saline administration, respectively). The short-time action of tetradecapeptide 31-44 is explained apparently by its rapid degradation in the organism since its effect can be prolonged up to 3-6 hours by administration of tetradecapeptide in the form of Zn-suspension (5).

The influence of tetradecapeptide 31-44 on the uptake of [U-^{14}C] glucose by isolated adipose tissue of rats. Table 1 presents the data on determining the effect of human GH (1 μg/ml), synthetic tetradecapeptide 31-44 (1 μg/ml) and insulin (50 μU/ml) on the accumulation of [U-^{14}C] glucose by isolated epididymal fat pads of intact fed rats. Since the adipose tissue of intact rats, unlike the tissue of hypophysectomized rats, is unsensitive to early insulin-like effects of GH, the tissue was preincubated for 4 (experiments 1 and 3) or 3 (experiment 2) hours in the absence of hormones to eliminate the refractoriness. After preincubation, all the three preparations in the indicated doses stimulated the glucose uptake by the tissue approximately to the same degree (by 1.5-2 times in different experiments).

In order to ascertain the presence of refractoriness in intact rats to the insulin-like effect of the tetradecapeptide in experiments 4 and 5 the hormones and [U-^{14}C] glucose were added to a freshly isolated tissue without preincubation. Under these conditions, as would by expected, the tissue reacted to insulin and was insensitive to GH. The adipose tissue was found to be also refractory to the stimulating effect of tetradecapeptide 31-44 on glucose uptake.

According to the data of Goodman and Coiro (13), the addition of GH to the medium even for a short period at any moment of preincubation is sufficient to reverse refractoriness

Table 1. The effect of GH and tetradecapeptide 31-44 on the uptake of [U-^{14}C] glucose by adipose tissue of fed intact rats

Experiment No	Uptake of glucose, Cpm/min/g of tissue for 1 hr						
	Control	GH (1 μg/ml).	Increase %	Tetradecapeptide 31-44 (1 μg/ml)	Increase %	Insulin (50 μU/ml)	Increase %
1	189100±16030	382300±28700[a]	102	294500±18200[a]	55	398000±37100[a]	110
2	28320±2130	45050±4540[b]	52	64410±6320[b]	116		
3	18270±3360	30540±3910[b]	67	30290±3180[c]	65		
4	31080±5050	30920±4380	-	37630±9240	-	45870±3870[c]	47
5	26260±2200	25590±2430	-	28390±3040	-	33700±2790[c]	29
6	27750±4010	10470±2320	-	24670±8620	-		

Number of animals in experiments 1, 3 and 4 = 10, in experiment 5 = 8, in experiments 2 and 6 = 6. In experiments 1-3 the adipose tissue was preincubated for 3-4 hours in the absence of GH, in experiments 4 and 5 the hormones were added to the tissue without preincubation; in experiment 6, during the 3rd-hours of preincubation, GH (1 μg/ml) was added in the medium. Incubation time in all the cases was 1 hour; incubation volume - 1 ml, dose of [U-^{14}C] glucose - 0.2 μg/ml (exper. 1) and 0.08 μg/ml (exper. 2-6). Statistical processing was performed by the Student method. All values are the mean ± SEM.

a - $p<0.001$, b - $p<0.01$, c - $p<0.05$; in all the other cases the difference was insignificant.

to the subsequent action of hormone. It was interesting to find out whether the responsiveness of the tissue to the insulin-like effect of tetradecapeptide 31-44 vanishes or not after tissue pretreatment with GH during the last stage of preincubation. In experiment 6, the tissue samples were preincubated for 2 hours in the absence of hormones (time sufficient for elimination of refractoriness to GH), then GH (1 μg/ml) was added to the incubation medium followed by incubation for 1 hour. After this the tissue samples were transferred to a fresh medium containing GH or tetradecapeptide 31-44. Following a preliminary treatment with hormone, the tissue was found to be insensitive both to the stimulatory action of GH and tetradecapeptide 31-44 on glucose uptake.

DISCUSSION

Stimulation of glucose uptake by adipose tissue relates to early insulin-like effects of GH. The presence of this

activity in tetradecapeptide 31-44 and its lack in 20K-GH, in the structure of which this part of sequence is missing, suggests a possible role of this fragment in the manifestation of the insulin-like effects of usual 22K-GH. These GH effects found in the absence of endogenic GH in hypophysectomized rats in vivo and in vitro are not revealed in intact rats. It is believed that the pulsating secretion of GH maintains the tissues of intact animals in the state of constant refractoriness to the insulin-like action of hormone, which, however, can be eliminated by a long incubation of the tissue in the absence of hormone (13). A similar picture we observed for tetradecapeptide 31-44. Freshly isolated tissue is refractory to the stimulatory effect of tetradecapeptide 31-44 on glucose uptake, but after preincubation the tissue becomes sensitive to the action of the peptide. Revesal of refractoriness to the insulin-like action of GH by the addition of hormone to the medium during the last stage of preincubation makes the tissue insensitive to the subsequent action of tetradecapeptide 31-44 either. Appearance and disappearance of the adipose tissue refractoriness simultaneously to the stimulating action of GH and tetradecapeptide on glucose uptake suggests that the action mechanisms of both preparations are similar. Refractoriness is apparently also responsible for the absence of the hypoglycemic effect of tetradecapeptide 31-44 in intact rats or rabbits in vivo (6).

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