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(54) SOMATOSTATIN ANALOGUES

(71) We, the SALK INSTITUTE FOR BIOLOGICAL STUDIES, a Corporation organised and existing under the laws of the State of California, United States of America, of 10010 North Torrey Pines Road, La Jolla, State of California, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

The present invention relates generally to peptides having biological activity in respect to the inhibition of growth hormone, insulin and glucagon secretion. More particularly, the present invention is directed to peptides having fewer amino acid moieties than somatostatin which are effective to inhibit the release of growth hormone by the pituitary gland or the release of glucagon or insulin by the pancreas. Various peptides of the invention have dissociated biological activity in respect to the inhibition of growth hormone, insulin and glucagon secretion.

A peptide having inhibitory effect on the secretion of growth hormone has been characterized and is described in United States Patent No. 3,904,594 to Guillemin et al. This peptide has been named "somatostatin". Somatostatin (also known as somatotropin release inhibiting factor) is the tetradecapeptide:

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

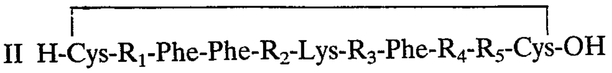
Somatostatin, the linear form of somatostatin (dihydrosomatostatin) and various acylated derivatives of somatostatin and dihydrosomatostatin are described in the aforementioned United States Patent.

Somatostatin and many analogs of somatostatin exhibit activity in respect to the inhibition of growth hormone (GH) secretion from cultured, dispersed, rat anterior pituirary cells *in vitro* and inhibition of insulin and glucagon secretion *in vivo* in the rat. It has been considered highly desirable in the use of somatostatin to selectively inhibit only the secretion of GH, insulin or glucagon. Efforts have been made to develop analogs of somatostatin which possess dissociated biological activity and which inhibit only GH, insulin or glucagon secretion. Although there have been reports citing differences in the amounts of somatostatin required for inhibition of insulin compared to glucagon in the human and the perfused rat pancreas *in vitro*, somatostatin and some somatostatin analogs exhibit similar potencies on the inhibition of these two hormones *in vivo*.

The present invention relates to the discovery that certan amino acids can be removed and/or rearranged in somatostatin and dihydrosomatostatin peptides to provide peptides having fewer amino acid components and which possess biological activity in respect to the inhibition of GH, insulin or glucagon secretion. Some of the peptides of the invention having fewer amino acid components than somatostatin or dihydrosomatostatin are considered to be of great value because of the relative simplicity with which these peptides can be manufactured.

The peptides of the invention are defined by the formulae:

I H-Cys-R_1-Phe-Phe-R_2-Lys-R_3-Phe-R_4-R_5-Cys-OH



II H-Cys-R_1-Phe-Phe-R_2-Lys-R_3-Phe-R_4-R_5-Cys-OH

where R_1 is Asn or a direct bond, R_2 is Trp or D-Trp, R_3 is Phe or Thr, R_4 is Thr or a direct bond, and R_5 is Ser, Phe or a direct bond, provided that at least one of R_1, R_4 and R_5 is a direct bond

The nomenclature used to describe the peptides of the present invention is in accordance with the conventional practice of using the first three letters of the trivial name. Also, in accordance with such practice, it is the L form of the amino acid that is intended, unless otherwise expressly indicated. In this connection, it should be understood that either of the Cys amino acid moieties can be either D-Cys or L-Cys.

Pharmaceutically acceptable or veterinarily acceptable acid addition salts of the peptides are also within the scope of the present invention. Such acid addition salts include but are not limited to hydrochloride, hydrobromide, sulfate, phosphate, maleate, acetate, citrate, benzoate, succinate, malate, ascorbate and tartrate.

The invention also envisages the possbility of pharmaceutically acceptable or veterinarily acceptable bioprecursors of the peptides, i.e. compounds having a structural formula different from the peptides but which nonetheless, upon administration to a human being or other animal, are converted in the body into the peptides.

Also considered to be within the scope of the present invention are intermediates of the formula:

III X-Cys(X^1)-R_1-Phe-Phe-R_2-Lys(X^2)-R_3(X^3)-Phe-R_4(X^4)-
R_5(X^5)-Cys(X^6)-R_6.

wherein: X is either hydrogen or an α-amino protecting group. The α-amino protecting groups conteplated by X are those known to be useful in the art in the step-wise synthesis of polypeptides. Among the glasses of α-amino protecting groups covered by X are (1) acyl type protecting groups such as formyl, trifluoroacetyl, phthalyl, p-toluenesulfonyl (tosyl), benzenesulfonyl, p-nitrophenylsulfenyl, tritylsulfenyl, o-nitrophenoxyacetyl, chloroacetyl, acetyl and γ-chlorobutyryl; (2) aromatic urethan type protecting groups such as benzyloxycarbonyl and substituted benzyloxycarbonyl such as p-chlorobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, p-bromobenzyloxycarbonyl and p-methoxybenzyloxycarbonyl; (3) aliphatic urethan protecting groups such as t-butyloxycarbonyl, diisopropylmethoxycarbonyl, isopropyloxycarbonyl, ethoxycarbonyl and allyloxycarbonyl; (4) cycloalkyl urethan type protecting groups such as cyclopentyloxycaronyl, adamantyloxycarbonyl and cyclohexyloxycarbonyl; (5) thiourethan type protecting groups such as phenylthiocarbonyl; (6) alkyl type protecting groups such as triphenylmethyl (trityl) and benzyl; (7) trialkylsilane groups such as trimethylsilane. The preferred α-amino protecting group defined by R is t-butyloxycarbonyl.

X^1 and X^6 are each a protecting group for Cys, e.g. p-methoxybenzyl, p-methylbenzyl, acetamidomethyl, trityl or benzyl. The preferred protecting group is p-methoxybenzyl. X^1 and/or X^5 can be hydrogen which means that there is no protecting group on the sulfur group.

X^2 is a protecting group for the ε-amino substituent of lysine or X^2 is hydrogen which means there is no protecting group on the side chain amino substituent. Illustrative of suitable side chain amino protecting groups are benzyl, 2,6-dichlorobenzyloxycarbonyl, benzyloxycarbonyl, tosyl, t-amyloxycarbonyl and t-butyloxycarbonyl. The selection of such a side chain amino protecting group is not critical except that it must be one which is not removed during deprotection of the α-amino groups during the synthesis. Hence, the α-amino protecting and side chain amino protecting group cannot be the same.

X^3, X^4 and X^5 are protecting groups for the hydroxyl group of Thr and Ser, e.g. acetyl, benzoyl, tert-butyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl or benzyloxycarbonyl. The preferred protecting group is benzyl. X^3 and/or X^4 and/or X^5 may be hydrogen which means there is no protecting group on the hydroxyl group.

R_1, R_2, R_3, R_4 and R_5 are as previously defined, R_6 is an ester group (e.g. methoxy), an amino group, a hydrazide group, a hydroxy group or a group solid resin support represented by the formula:

-O-CH_2-polystyrene resin support

or

-O-CH_2-benzyl-polystyrene resin support

The polymer is preferably a copolymer or styrene with 0.5 to 2.0% by weight divinyl benzene as a cross-linking agent which causes the polystyrene polymer to be completely insoluble in certain organic solvents. In formula III at least one of the X, X^1, X_2, X_3, X_4, X_5 and X_6 is a protecting group.

Included in the present invention is a pharmaceutical or veterinary formulation which comprises a peptide in accordance with the invention or a pharmaceutically acceptable or veterinarily acceptable acid addition salt or bioprecursor thereof formulated for pharmaceutical or veterinary use, respectively.

The invention further provides a pharmaceutical or veterinary composition which comprises a peptide in accordance with the invention or a pharmaceutically acceptable or veterinarily acceptable acid addition salt or bioprecursor thereof together with a pharmaceutically acceptable or veterinarily acceptable, respectively, diluent, carrier or excipient.

The compositions and formulations of the invention may be in unit disage form.

The invention also provides a method of affecting the release of growth hormone, insulin, and/or glucagon in an animal, which method comprises administering to the animal an effective amount of a peptide in accordance with the invention, a pharmaceutically acceptable or veterinarily acceptable acid addition salt or bioprecursor thereof, a formulation of the invention, or a composition of the invention.

The invention further provides a solid phase synthetic method for making the above peptides, which method comprises one or more synthetic steps substantially as described below.

In selecting a particular side chain protecting group to be used in the synthesis of the peptides of formula I or formula II, the following rules should be followed:
(a) the protecting group must be stable to the reagent and under the reaction conditions selected for removing the αamino protecting group at each step of the synthesis, (b) the protecting group must retain its protecting properties and not be split off under coupling conditions, and (c) the side chain protecting group must be removable upon the completion of the synthesis containing the desired amino acid sequence under reaction conditions that will not alter the peptide chain.

The peptides of formula I and formula II can be prepared using solid phase synthesis. The synthesis is commenced from the C-terminal end of the peptide using an α- amino protected resin. Such a starting material can be prepared by attaching an α-amino and S-protected Cys to a chloromethylated resin or a hydroxymethyl resin. The preparation of the hydroxymethyl resin is described by Bodanszky et al., Chem. Ind. (London) 38, 1597-98 (1966). A chloromethylated resin is commercially available from Bio Rad Laboratories, Richmond, California and the preparation of such a resin isdescribed by Stewart et al., "Solid Phase Peptide Synthesis" (Freeman & Co., San Francisco 1969), Chapter 1, pp 1-6. The α-amino and S-protected Cys is coupled to the chloromethylated resin according to the procedure of Monohan and Gilon, Biopolymer 12, pp 2513-19, 1973. Following the coupling of the α-amino and S-protected Cys to the resin support, the α-amino protecting group is removed such as by using trifluoroacetic acid in methylene chloride, trifluoroacetic acid alone or HC1 in dioxane. The deprotection is carried out at a temperature between 0°C. and room temperature.

Other standard cleaving reagents and conditions for removal of specific α-amino protecting groups may be used as described in Schroder & Lubke, "The Peptides", 1 pp 72-75 (Academic Press 1965).

After removal of the α-amino protecting group of Cys the remaining α-amino and side chain protected amino acids are coupled step-wise in the desired order to obtain a compound of formula III or as an alternate to adding each amino acid separately to the synthesis, some of them may be coupled prior to addition to the solid phase reactor. The selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as a coupling reagent is N,N^1-dicyclohexylcarbodiimide.

The activating reagents used in the solid phase synthesis of the peptides are those well known in the peptide art. Examples of suitable activating reagents are: (1) carbodiimides such as N,N-diisopropylcarbodiimide, N-ethyl N^1-(γ-dimethylaminopropylcarbodiimide); (2) cyanamides such as N,N-dibenzylicyanamide; (3) keteimines; (4) isoxazolium salts such as N-ethyl-5-phenylisoxazolium-3^1-sulfonate; (5) monocyclic nitrogen containing heterocyclic amides of aromatic character containing one through 4 nitrogens in the ring such as imidazolides, pyrazolides, 1,2,4-triazolides. Specific heterocyclic amides that are useful include N,N^1-carbonyldiimidazole, N,N^1-carbonyl-di-1,2,4-triazole; (6) alkoxylated acetylene such as ethoxyacetylene; (7) reagents which form a mixed anhydride with the carboxyl moiety of the amino acid such as ethylchloroformate and isobutylchloroformate and (8) nitrogen-containing heterocyclic compounds having a hydroxy group on one ring nitrogen such as N-hydroxyphthalimide, N-hydroxysuccinimide and 1-hydroxybenzotriazole. Other activating reagents and their use in peptide coupling are described by Schroder & Lubke *supra*, in Chapter III and by Kapoor, J. Pharm. Sci., 59, pp 1-27 (1970).

Each protected amino acid or amino acid sequence is introduced into the solid phase reactor in about a four-fold excess and the coupling is carried out in a medium of dimethylformamide: methylene chloride (1:1 by volume) or in dimethyl formamide: methylene chloride (1:1 by volume) or in dimethylformamide or methylene chloride along. In cases where incomplete coupling occurred the coupling procedure is repeated before removal of the α-amino protecting group, prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis is monitored by the ninhydrin reaction, as described by E. Kaiser et al., Analyt. Biochem, 34, 595 (1970).

After the desired amino acid sequence of formula III has been synthesized, the peptide is removed from the resin support by treatment with a reagent such as liquid hydrogen fluoride which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups X^1, X^2, X_3, X_4, X_5 and X_6 and the α-amino protecting group X to obtain directly a peptide of formula I. Peptides in accordance with formula II are obtained by oxidizing formula I peptides in accordance with known procedures. As an alternate route, the peptide linked to the resin support may be separated from the resin by alcoholysis after which the recovered C-terminal methyl ester is converted to the acid by hydrolysis. Any side chain protecting group may then be cleaved as previously described or by other procedures such as catalytic reduction (e.g. Pd on $BaSO_4$) using conditions which will keep the Trp moiety intact. When using hydrogen fluoride for cleaving, anisole is included in the reaction vessel as a scavenger.

The solid phase synthesis procedure discussed above is well known in the art and has been essentially described by Merrifield J. Am. Chem. Soc., 85, p 2149 (1964).

The peptides of the present invention having dissociated effects in respect to inhibition of release of growth hormone, insulin and glucagon are considered to be particularly important in connection with the treatment of diabetes. The traditional view of diabetes has been that it is a disease resulting from impaired insulin production alone. As clinical and research experience has become more extensive, it has become apparent that some factor in addition to impairment of insulin secretion is operative in diabetes. It is known that, while insulin is normally deficient in diabetes, glucagon is normally present in excess. it is now believed that the presence of glucagon is at least as important a factor in diabetes as the absence of insulin.

The fact that a deficiency in insulin is normally accompanied by an excess of glucagon has made it difficult to study the role of glucagon in diabetes. While it is easy to add extra quantities of a hormone such as insulin, it has proved very difficult to lower the concentration of glucagon. The discovery of somatostatin has facilitated research in respect to the role of glucagon in diabetes. Somatostatin inhibits the release of both insulin and glucagon. The role of somatostatin in diabetes research is detailed in an article appearing in Science, Vol. 188, pp 920-923, 30 May 1975. However, there are several problems in respect to the use of somatostatin as a treatment in diabetes. Somatostatin inhibits the release of insulin in addition to glucagon. Thus, the need for a peptide having a dissociated effect on the inhibition of release of insulin and glucagon has been recognized in connection with diabetes treatment. The peptides of the present invention provide such dissociative effect. More particularly, certain of the peptides of the present invention are effective to inhibit secretion of glucagon while having less effect on the inhibition of secretion of insulin.

The peptides of the invention provide the benefits of somatostatin and known somatostatin analogs, but have fewer amino acid components, e.g. 8 to 10 amino acid moieties as compared to 12 to 14 amino acid moieties for most known somatostatin analogs. Accordingly, the peptides of the present invention have significant economic advantage due to the relative ease of manufacture of these peptides.

The following Examples illustrates various features of the present invention.

Example

The peptides of the present invention were synthesized by solid phase techniques, generally in accordance with the procedure described in United States Patent No. 3,904,595. The synthesis was conducted in a stepwise manner on chloromethylated resin. The resin was composed of fine beads (20 - 70 microns in diameter) of a synthetic resin prepared by copolymerization of styrene with one to two percent (by weight) divinylbenzene. The benzene rings in the resin were chloromethylated in a Friedel-Crafts reaction with chloromethyl methyl ether and stannic chloride. The chlorine thus introduced is a reactive benzyl chloride type of linkage. The Friedel-Crafts reaction is continued until the resin contains 0.5 to 2 millimoles of chlorine per gram of resin. In the further description of the synthesis of the peptides, the reagents used will be first described by their chemical name with their common abbreviation in parenthesis. Thereafter, the reagent will be referred to by the common abbreviation.

A peptide having the structure:

H-Cys-Phe-Phe-D-Trp-Lys-Phe-Phe-Cys-OH

was synthesized by the following solid phase methodology. Other peptides, described hereinafter were synthesized by a similar technique.

The t-butyloxycarbonyl-S-paramethoxybenzyl (Boc-SpOMeBzl) derivative of Cys was linked to the resin by any of three known methods; (1) reflux in ethanol in presence of triethyl amine, (2) Cesium salt of the Boc protected amino acid is kept at 50° C in dimethylformamide (DMF) overnight, (3) the potassium salt of the Boc-protected amino acid is kept at 80° C in dimethyl sulfoxide (DMSO) for 2 hours. Only one milliequivalent of the protected Cys per milliequivalent of C1 on the resin is used.

Method (3) is described hereinbelow in more detal. To a slurry of the resin and the dissolved protected Cys in DMSO is added 0.9 mEq of potassium t-butoxide (KOtBut) per mEq of amino acid. The reaction mixture is exposed to air as little as possible so that no amber coloration is observed. Reaction at 80° C for 2 hours yields a suitable substituted resin for synthesis of the peptides (approx. 0.2 mEq of amino acid derivative per g of resin). After deprotection and neutralization, the peptide chain is built on resin. Deprotection, neutralization and addition of each amino acid is performed in accordance with schedule I. N^{α}-t-butyloxycarbonyl (Boc) derivative of each amino acid is used. After deprotection of the first residue (*i.e.*, SpOMe.Bzl.Cys) according to schedule I (steps 3 to 8 included), the N-Boc derivatives of Thr, when present, is next added along with a coupling agent which is dicyclohexylcarbodiimide (DCC) (step 9 of schedule I). The side chain of Thr is protected with O-benzyl ether (Bzl). Benzyloxycarbonyl (Z) or 2,6-dichlorobenzyloxycarbonyl [Z(2-CL)] was used as the protecting group for the Lys side chain.

I. Schedule for coupling of amino acids in solid phase synthesis (5 - 10 g resin)

Step	Reagents and operations	Mix Times Min.
1	CH_2Cl_2 wash 80 ml (2 times)	3
2	Methanol (MeOH) wash 30 Ml (2 times)	3
3	CH_2Cl_2 wash 80 ml (3 times)	3
4	50 percent trifluoroacetic acid (TFA) containing 5 percent 1,2-ethanedithiol in CH_2Cl_2 70 ml (2 times)	10
5	CH_2Cl_2 wash 80 ml (2 times)	3
6	Triethylamine (Et_3N) 12.5 percent in CH_2Cl_2 70 ml (2 times)	5
7	MeOH wash 40 ml (2 times)	2
8	CH_2Cl_2 wash 80 ml (3 times)	3
9	Boc-amino acid (10 mmoles) in 10 ml DMF (1 times) and 30 ml CH_2Cl_2 plus DCC (10 mmoles) in CH_2Cl_2 (2 M)	30 to 120
10	MeOH wash 40 ml (2 times)	3
11	Et_3N 12.5 percent in CH_2Cl_2 70 ml (2 times)	3
12	MeOH wash 30 ml (2 times)	3
13	CH_2Cl_2 wash 80 ml (2 times)	3

Percentages are by weight.

After step 13, an aliquot is taken for a ninhydrin test: if the test is negative, go back to step 1 for coupling of the next amino acid; if the test is positive or slightly positive, go back to steps 9 through 13. Schedule I was used for coupling of each of the amino acids of the peptide to Cys.

Cleavage of the peptides from the resin (5 grams) and deprotection of the side chain protecting groups of the peptide was performed in hydrofluoric acid (75 ml) in the presence of anisole (8 ml). After elimination of hydrofluoric acid under high vacuum, the resin-peptide was washed with diethyl ether.

The dried resin was immediately extracted with 25% (by weight) acetic acid (150 ml) and diluted to 3000 ml with degassed H_2O (N_2). The pH of the solution was adjusted to 6.6-7.0 with NH_4OH. The solution was titrated dropwise under stirring with potassium ferricyanide solution (1.g/500 ml H_2O) until a permanent yellow color was observed. The solution was left for 10 minutes and pH was adjusted to 5.0 with glacial acetic acid; Bio-Rad (Registered Trade Mark) AG 3-X4A [100-200 mesh, U.S. standard sieve size, chloride form, 10-15 g] was added to the turbid solution and stirred for 15 minutes. The solution was filtered over Celite (Registered Trade Mark) and applied successively onto two columns; a) Bio-Rad AG 3-X4A resin chloride form (10 ml); b) Bio-Rex (Registered Trade Mark) 70 resin (100 ml) cation form. The Celite + resin cake was thoroughly washed with water 500 ml) which was applied onto columns a) and b) as a wash. The peptide material was then eluted from the Bio-Rex- 70 resin column with pyridine; acetic acid: water (30:4:66 by volume) or 50% (by weight) acetic acid. Fractions were collected; only the ones containing peptide (ninhydrin positive) were diluted with water and immediately lyophilized. 950 mg of crude cream colored material was obtained. It was applied onto a Sephadex (registered Trade Mark) G-25 F gel column (3 × 200 cm) equilibrated and eluted with 2 N acetic acid. The elution pattern as observed at 280 nm showed one major symmetrical peak. After lyophylization the center cut yielded 550 mg which were submitted to counter current distribution (solvent system n-butanol: acetic acid: water, 4:1:5 by volume) 10 ml lower phase per tube. 100 transfers were performed and the major peak was found in tubes 57-68. The compound (250 mg) appeared homogeneous on tlc.

The specific optical rotation was $[\alpha]^{23}$ = -67.8° ± 2°: [c=1 in 1% (by weight) acetic acid]. Amono acid analysis of this material showed the expected ratio for the different amino acids.

Active esters can be used in solid phase synthesis and the classifical method of synthesis can also be used to prepare the peptides of the invention.

In vitro Bioassay: The effects of the various peptides of the invention were tested *in vitro* on the secretion of growth hormone by primary cultures of ensymatically dissociated rat anterior pituitary cells by the method of Vale et al., Endocrinology 91: p 562-571 (1972). The assay is made by treating pituitary glands removed from rats to separate cells therefrom. The cells are placed in culture dishes in Dulbecco's Modified Eagle Medium (Dulbecco et al., *Virology*, Vol. 8, p. 396, 1949). Carbon dioxide gas and oxygen are supplied to thecell cultures which are maintained at 37° C for 4-5 days prior to use in the assay. Following media changes, cell cultures are incubated for a period of 4 hours and particular somatostatin peptides are added thereto. Radioimmunoassay analysis is used to determine the rate of growth hormone secretion which is expressed in nanograms per hour.

An investigation of the effect of somatostatin, dihydrosomatostatin, (as controls) and the peptides of the invention to inhibit the release of glucagon and insulin was made as follows:

In vivo Bioassay: Male Sprague-Dawley-CD rats weighing 180-200 g housed in temperature and humidity controlled quarters with 14 h light and 10h darm (light 0700-21100) were used in all experiments. Animals were fed a standard ration and tap water *ad libitum*. Experiments were carried out at least 5 days after arrival of rats from the supplier between the hours 1400 and 1600. After ether anesthesia, peptides or saline were administered in a volume of 0.2 ml. via the external jugular vein. Animals remained anesthetized until the time of blood collection from the portal vein. The blood samples were placed into chilled tubes contaning 10 mg EDTA and 50 μl of M Benzamidine per ml of blood.

Plasma was stored at -20°C for insulin and glucagon determinations. Insulin levels were determined by the method of Herbert *et al*, J. Chem. Endocr. Metab. 25:1375, 1965, utilizing porcine insulin antisera and (125I) iodinated insulin tracer. Human insulin standard was obtained from Schwarz-Mann, Orangeburg, New York. Glucagon was determined by the method of Faloona and Unger, in Jaffe et al ed., Methods of Hormone Radioimmunoassay, Academic Press, New York, 1974, p. 317, utilizing glucagon antisera 30K. Cellulose was determined by the glucose oxidase method, utilizing a Beckman (registered Trade Mark) Glucose Analyzer.

GH determinations were performed on tissue culture media utilizing the following reagents: NIAMMD rat GH standard (GH-RP-1), NIAMDD monkey anti-rat GH (GH-Serum-3), and highly purified for iodination.

All experiments were carried on in a randomized block design. Following analysis of variance difference between treatments were determined by the multiple range tests of Dunnett and Duncan. Potency values were calculated from four or six point bioassays.

Various peptides in accordance with the invention were prepared in accordance with the solid phase methodology described above. The composition of the peptides is reported hereinbelow in Table I. Tagle I also sets forth the percent effectiveness of the peptide for inhibiting secretion of growth hormone (GH), insulin and glucagon, with somatostatin taken as the base.

TABLE I

					Growth Hormone	Insulin	Glucagon
Somatostatin (control)					100	100	100
Peptides of invention							
R_1	R_2	R_3	R_4	R_5			
a direct bond	D-Trp	Phe	a direct bond	a direct bond	6	80	100
a direct bond	D-Trp	Phe	a direct bond	a direct bond (D-Cys)^{11}	14	100	100
a direct bond	D-TRP	Phe	Thr	a direct bond	35	10-100	100
Asn	D-Trp	Phe	a direct bond	a direct bond	1	100	1
Asn	Trp	Phe	Thr	a direct bond	3	100	1
Asn	D-Trp	Phe	Thr	a direct bond	10	200	10-100

It should be clearly understood that we make no claim herein to the prevention or treatment of disease in human beings.

Subject to the foregoing disclaimer,

WHAT WE CLAIM IS:-

1. A peptide selected from those of the formulae:

I $\text{H-}\overline{\text{Cys-R}_1\text{-Phe-Phe-R}_2\text{-Lys-R}_3\text{-Phe-R}_4\text{-R}_5\text{-Cys}}\text{-OH}$
II $\text{H-Cys-R}_1\text{-Phe-Phe-R}_2\text{-Lys-R}_3\text{-Phe-R}_4\text{-R}_5\text{-Cys-OH}$
III $\text{X-Cys(X}^1\text{)-R}_1\text{-Phe-Phe-R}_2\text{-Lys-(X}^2\text{)-Phe-R}_4\text{(X}^4\text{)-R}_5\text{(X}^5\text{)-Cys(X}^6\text{)-R}_6$;

wherein R_1 is Asn or a direct bond; R_2 is Trp or D-Trp; R_3 is Phe or Thr; R_4 is Thr or a direct bond. R_5 is Ser, Phe or a direct bond; provided that at least one of R_1, R_4 and R_5 is a direct bond. X is H or an alpha-amino protecting group; X^1 and X^6 are each independently H or a thio protecting group for Cys; X^2 is H or an ε-amino protecting group; X^3, X^4 and X^5 are each independently H or a hydroxyl protecting group for Thr or Ser with the proviso that at least one of X, X^1, X^2, X^3, X^4, X^5 and X^6 is other than hydrogen; R_6 is hydroxy, an ester group, an amine group, a hydrazide group or a solid resin support of the formula -O-CH_2-polystyrene resin support or -O-CH_2-benzyl-polystyrene resin support; and wherein Cys either represents L-Cys or D-Cys.

2. A peptide as claimed in claim 1, wherein X^1 and/or X^6 are each independently p-methoxybenzyl, p-methyl-benzyl, acetamidomethyl, trityl or benzyl.

3. A peptide as claimed in claim 1 or claim 2, wherein X^3, X^4 and/or X^5 are each independently acetyl, benzoyl, tert-butyl, trityl, benzyl, tetrahydropyronyl, 2,6-dichlorobenzyl or benzyloxycarbonyl.

4. A peptide as claimed in any one of claims 1 to 3, wherein R_6 is methoxy.

5. A peptide as claimed in claim 1, wherein R_1 is a direct bond, R_2 is D-Trp, R_3 is Phe, R_4 is a direct bond and R_5 is a direct bond.

6. A peptide as claimed in claim 1, wherein R_1 is a direct bond, R_2 is D-Trp, R_3 is Phe, R_4 is a direct bond, R_5 is a direct bond and C-terminal Cys is D-Cys.

7. A peptide as claimed in claim 1, wherein R_1 is Asn, R_2 is D-Trp, R_3 is Phe, R_4 is a direct bond and R_5 is a direct bond.

8. A peptide as claimed in claim 1, wheren R_1 is Asn, R_2 is Trp, R_3 is Phe, R_4 is Thr and R_5 is a direct bond.

9. A peptide as claimed in claim 1, wherein R_1 is Asn, R_2 is D-Trp, R_3 is Phe, R_4 is Thr and R_5 is a direct bond.

10. A peptide as claimed in claim 1, wherein X, X^1, X^2, X^3, X^4, X^5, X^6, and/or R_6 is/are (a) group(s) referred to hereinbefore in general or specific exemplification of said group(s).

11. A peptide as claimed in claim 1 and referred to hereinbefore.

12. A pharmaceutically acceptable or veterinarily acceptable acid addition salt or bioprecursor of a peptide of formula I or formula II and as claimed in any one of claims 1, 5 to 9 or 11.

13. A solid phase synthesis for a peptide as claimed in claim 1, which synthesis comprises one or more synthesis steps substantially as hereinbefore described.

14. A synthesis as claimed in claim 13 and substantially as hereinbefore described in any one of the individual runs of the specific Example.

15. A peptide as claimed in claim 1 which has been synthesized by a synthesis as claimed in claim 13 or claim 14.

16. A pharmaceutically acceptable or veterinarily acceptable acid addition salt or bioprecursor of a peptide of formula I or formula II and as claimed in claim 15.

17. A pharmaceutical or veterinary formulation which comprises a peptide of formula I or formula II as claimed in any one of claims 1, 5 to 9, 11 or 15 or a salt or bio-precursor as claimed in claim 12 or claim 16 formulated for pharmaceutical or veterinary use, respectively.

18. A pharmaceutical or veterinary composition which comprises a peptide of formula I or formula II as claimed in any one of claims 1, 5 to 9, 11 or 15 or a salt or bioprecursor as claimed in claim 12 or claim 16 together with a pharmaceutically acceptable or veterinarily acceptable, respectively, diluent, carrier or excipient.

19. A formulation as claimed in claim 17 or a composition as claimed in claim 18 and in unit dosage form.

20. A method of affecting the release of growth hormone, insulin, and/or glycagon in an animal, which method comprises administering to the animal an effective amount of a peptide of formula I or formula II as claimed in any one of claims 1, 5 to 9, 11 or 15 or a salt or bioprecursor as claimed in claim 12 or claim 16, a formulation as claimed in claim 17 or claim 19, or a composition as claimed in claim 18 or claim 19.

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