【DESCRIPTION】

【Invention Title】

INSULINOTROPIC PEPTIDE DERIVATIVE WHEREIN ITS N-TERMINAL AMINO ACID IS MODIFIED

【Technical Field】

The present invention relates to an insulinotropic peptide derivative having an improved insulinotropic activity. In particular, the present invention relates to an N-terminal amino acid-modified insulinotropic peptide having high stability and insulinotropic activity.

【Background Art】

Since peptides tend to be easily denatured due to their low stability, degraded by in-vivo proteolytic enzymes, thus losing the activity, and have a relatively small size, thereby easily passing through the kidney. Accordingly, in order to maintain the blood level and the titer of a medicament comprising a peptide as a pharmaceutically effective component, it is necessary to administer the peptide drug frequently to a patient to maintain desired blood level and titer. However, the peptide

drugs are usually administered in the form of injectable preparations, and such frequent administration for maintaining the blood levels of the physiologically active peptides cause severe pain for the patients. To solve these problems, many efforts have been made. As one of such efforts, there has been suggested an approach that transmission through the biological membrane of the peptide drug is increased, and then the peptide drug is transferred into the body by oropharyngeal or nasopharyngeal inhalation. However, this approach is still difficult in maintaining the in-vivo activity of the peptide drug due to the remarkably lower in-vivo transfer efficiency, as compared with injectable preparations.

On the other hand, many efforts have been made to improve the blood stability of the peptide drug, and to maintain the drug in blood at a high level for a prolonged period of time, thereby maximizing the pharmaceutical efficacy of the drug. The long acting preparation of such peptide drug therefore is required to increase the stability of the peptide drug, and to maintain the titers at sufficiently high levels without causing immune

responses in patients.

As a method for stabilizing the peptide, and inhibiting the degradation by a proteolytic enzyme, some trials have been performed to modify a specific amino acid sequence which is sensitive to the proteolytic enzyme. For example, GLP-1 (7-37 or 7-36 amide), which functions to reduce the glucose concentration in blood for the treatment of Type 2 diabetes, has a short half-life of the physiological activity of about 4 minutes or less (Kreymann et al., 1987), due to loss of the titers of GLP-1 through the cleavage between the 8^{th} amino acid (Ala) and the 9^{th} amino acid (Asp) by a dipeptidyl peptidase IV (DPP IV). As a result, various investigations have been made on a GLP-1 analog having resistance to DPP IV, and trials have been made for substitution of Ala^8 with Gly (Deacon et al., 1998; Burcelin et al., 1999), or with Leu or D-Ala (Xiao et al., 2001), thereby increasing the resistance to DPP IV, while maintaining its activity. The N-terminal amino acid His^7 of GLP-1 is critical for the GLP-1 activity, and serves as a target of DPP IV. Accordingly, US Patent No. 5,545,618 describes that the

N-terminus is modified with an alkyl or acyl group, and Gallwitz, et al. describes that His^7 was subject to N-methylation, or alpha-methylation, or the entire His is substituted with imidazole to increase the resistance to DPP IV, and to maintain physiological activity. Whereas the resistance to dipeptidyl peptidase is increased to improve its stability, the His^7-modified derivatives are found to have markedly reduced receptor affinity with lower cAMP stimulation at the same concentration (Gallwitz. et al., Regulatory Peptide 79:93-102(1999), Regulatory Peptide 86:103-111(2000)).

In addition to GLP-1, exendins are peptides that are found in the venom of glia monster, a lizard common in Arizona and Northern Mexico. Exendin-3 is present in the venom of Heloderma horridum, and exendin-4 is present in the venom of Heloderma suspectum. The exendins have a high homology of 53% with GLP-1 (Goke, et al., J. Bio. Chem., 268:19650-55(1993)). Exendin-4 reportedly acts at GLP-1 receptors on specific insulin-secreting cells, at dispersed acinar cells from guinea pig pancreas, and at parietal cells from stomach, and the peptide is also said

to stimulate somatostatin release and inhibit gastrin release in isolated stomachs. In addition, exendin-3 and exendin-4 were reportedly found to stimulate cAMP production in pancreatic acinar cells, and to stimulate amylase release from pancreatic acinar cells. Since the exendin-4 (US Patent No. 5,424,686) has a sequence of His-Gly, instead of His-Ala which functions as a substrate of dipeptidyl peptidase in GLP-1, it has resistance to DPP IV, and higher physiological activity than GLP-1. As a result, it had an in-vivo half-life of 2 to 4 hours, which was longer than that of GLP-1. Although the native exendin has an in-vivo increased half-life than GLP-1, its physiological activity is not sufficiently sustained. For example, in the case of a commercially available exendin-4 (exenatide), it needs to be injected to a patient twice a day, which is still difficult for patients.

To improve therapeutic efficacy of the native exendin, trials have been made to prepare its analogs, derivatives and variants. The term "analog or variant" typically refers to a peptide prepared by substitution, deletion or insertion of one or more amino acids into or from the native peptide. The term

"derivative" refers to a chemically modified peptide, prepared by alkylation, acylation, esterification, or amidation of one or more amino acids in the native peptide.

Novel exendin agonist compounds are described in PCT Application No. PCT/US98/16387. Claiming priority thereon, a method for reducing food intake using exendin is disclosed in US Patent No. 6956026. In addition, claiming priority on the PCT application, use of exendins and analogs thereof for the reductions of food intake is disclosed in EP0996459, and exendin agonist compounds are disclosed in US Patent No. 7157555. However, they merely disclose several sequences of exendin analogs. Moreover, there is no mention of activity and property with respect to said analogs, which is also not supported by the detailed description.

[Disclosure]

[Technical Problem]

Accordingly, the present inventors found that His^1-modified exendin derivatives exhibit higher blood stability and insulinotropic activity than a native exendin, thereby completing

the present invention.

[Technical Solution]

It is an object of the present invention to provide insulinotropic peptide derivatives having improved blood stability and insulinotropic activity.

It is another object of the present invention to provide a pharmaceutical composition for the treatment of diabetes, comprising the insulinotropic peptide derivative having improved insulinotropic activity.

[Description of Drawings]

Fig 1 shows stability of exendin-4 derivatives in serum. A : exendin-4, D : DA-exendin-4, H : HY-exendin-4, C : CA-exendin-4.

Fig. 2 shows insulinotropic activities of exendin-4, and exendin-4 derivate, CA-exendin 4.

Fig. 3 shows blood glucose lowering effect of exendin-4, and CA-exendin-4 in diabetic model animals.

[Best Mode]

In accordance with an aspect, the present invention relates to an insulinotropic peptide derivative having improved blood stability and insulinotropic activity.

The derivative according to the present invention is a derivative having a chemically modified N-terminal histidine residue, or a derivative having a chemically modified amino group of N-terminal histidine residue.

Preferably, the insulinotropic peptide according to the present invention is exendin-4, exendin-3 or derivatives thereof. The term "exendin-4 or exendin-3 derivative" as used herein, refers to a peptide prepared by substituting, deleting and/or adding one or more amino acids of exendin-4 or exendin-3, or a peptide having one or more amino acid residues chemically modified, e.g., alkylation, acylation esterification or amidation, whose activity is equivalent to that of native exendin-4.

As examples of exendin-3 or exendin-4 deivatives, an exendin-4 derivative prepared by deleting C-terminus of exendin-4 or substituting an amino acid of exendin-4 with nonnatural amino acid, Norleucine is disclosed in WO97/46584. Also, WO99/07404

discloses exendin derivatives whose amino acids are substituted with nonnatural aminoacids, e.g., pentyl glycine, homoproline or tert-butylglycine, and US2008/0119390 discloses exendin derivatives consisting of shorter amino sequences than the native exendin-4 prepared by deleting some amino acid residues of exendin-4, and prepared by substituting some amino acid residues of exendin-4 with other amino acid residues. These publications are incorporated by references.

Specifically, the present invention may also encompass a derivative thereof with removal of the amino group of N-terminal histidine (desamino-histidyl derivative), a derivative thereof prepared by substitution of the amino group with a hydroxyl group (beta-hydroxyimidazoproionyl derivative), a derivative thereof prepared by modification of the amino group with two methyl residues (dimethyl-histidyl derivative), a derivative thereof prepared by substitution of the amino group with a carboxyl group (beta-carboxyimidazopropionyl derivative), or a derivative thereof with removal of the positive charge of the amino group, in which the alpha carbon of N-terminal histidine residue is removed to remain only the imidazoacetyl group (imidazoacetyl

derivative), and other N-terminal amino group modified-derivatives.

Preferably, the present invention provides exendin-4 derivatives having chemically modified N-terminal amino group or amino acid residue, more preferably exendin-4 derivative in which the alpha amino group or alpha carbon of N-terminal histidine residue (the first amino acid of exendin-4) is substituted or removed, and further more preferably desamino-histidyl-exendin-4 (DA-Exendin-4) with removal of the N-terminal amino group, beta-hydroxy imidazopropionyl-exendin-4 (HY-exendin-4) prepared by substitution of the amino group with a hydroxyl group, beta-carboxy imidazopropionyl-exendin-4 (CX-exendin-4) prepared by substitution of the amino group with a carboxyl group, dimethyl-histidyl-exendin-4 (DM-exendin-4) prepared by modification of the amino group with two methyl residues, and imidazoacetyl-exendin-4 (CA-exendin-4) with removal of alpha carbon of N-terminal histidine residue.

In accordance with a specific aspect, the present invention relates to an insulinotropic peptide derivative comprising an amino acid of the following Formula 1.

R1-X-R2 <Formula 1>

wherein R1 is selected from the group consisting of desamino-histidyl, N-dimethyl-histidyl, beta-hydroxy imidazopropionyl, 4-imidazoacetyl and beta-carboxy imidazopropionyl;

R2 is selected from the group consisting of $-NH_2$, -OH and -Lys,

X is selected from the group consisting of Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser,

Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly, and

Ser-Asp-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser;

Y is selected from the group consisting of Lys, Ser, and Arg, and

Z is selected from the group consisting of Lys, Ser, and Arg.

Preferred insulinotropic peptide derivative has Formula 1, wherein R1 is selected from desamino-histidyl, N-dimethyl-histidyl, beta-hydroxy imidazopropionyl, 4-imidazoacetyl and beta-carboxyimidazopropionyl, Y is Lys or

Ser, Z is Lys, and R2 is $-NH_2$.

In accordance with another specific aspect, the present invention relates to an insulinotropic peptide derivative comprising an amino acid of the following Formula 2.

R3-X'-R4 <Formula 2>

wherein R3 is 4-imidazoacetyl;

X' is

Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser or

Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly;

R4 is $-NH_2$;

Y is selected from the group consisting of Lys, Ser, and Arg; and

Z is selected from the group consisting of Lys, Ser, and Arg.

In terms of its activity, the chemical modification in the N-terminal histidine residue of exendin-4 has a different effect from that in other insulinotropic peptide GLP-1. The chemical modification in the N-terminal histidine residue of GLP-1, for example, α-methyl-GLP-1, n-methyl-GLP-1, or imi-GLP-1, may be expected to inhibit degradation by dipeptidyl peptidase, thereby increasing the stability, and practical reduction in degradation rate was reported. However, it was also reported that they have relatively reduced receptor affinity with lower cAMP stimulation, as compared to the native GLP-1.

In contrast, since exendin-4 is not cleaved by dipeptidyl peptidase, it would be difficult to predict the effect of the chemical modification in the N-terminal histidine residue on its activity, in particular, its effect on receptor affinity and glucose concentration in blood.

Accordingly, the present invention provides an exendin-4 derivative having a chemically modified N-terminal histidine residue or having a chemically modified amino group of N-terminal

histidine residue, which exhibits unexpected excellent insulinotropic activity compared to native exendin-4. These derivatives exhibited excellent blood stability and insulinotropic activity in vitro, compared to exendin-4 (Fig. 2). Practically, it was found in diabetic db/db mouse that they exhibited an excellent effect of reducing the glucose concentration in blood, compared to the native exendin-4 (Fig. 3). It is thought that the change in net charge due to modification in the amino group of N-terminal histidine residue or the change in size of histidine residue causes a difference in sensitivity to proteolytic attack in blood or affects receptor affinity. However, there is still a need for more extensive molecular studies thereon. Such property is expected to maximize the intrinsic insulinotropic activity of exendin-4, that is, a therapeutic effect on type 2 diabetes, and to induce reduction of food intake, delay in gastric emptying or the like.

The exendin-4 derivatives including desamino-histidyl-exendin-4 (DA-exendin-4), beta-hydroxy imidazopropionyl-exendin-4 (HY-exendin-4), beta-carboxyimidazopropionyl-exendin-4 (CX-exendin-4),

dimethyl-histidyl-exendin-4 (DM-exendin-4) and imidazoacetyl-exendin-4 (CA-exendin-4) of the present invention are prepared by removing and substituting the alpha amino group of N-terminal histidine residue or by removing the alpha carbon of N-terminal histidine residue. Therefore, other amino acid sequences are not limited, as long as their activity is maintained. Further, it is obvious to those skilled in the art that the exendin-4 derivatives are modified by a typical method including modification of polymer such as PEG and sugar chain and fusion with albumin or transferrin, so as to enhance their therapeutic effect, being superior to the native exendin-4.

In accordance with another aspect, the present invention provides a pharmaceutical composition for the treatment of diabetes, comprising the insulinotropic peptide derivative.

The term "administration" as used herein means introduction of a predetermined amount of a substance into a patient by a certain suitable method. The conjugate of the present invention may be administered via any of the common routes, as long as it is able to reach a desired tissue. A variety of administration

modes are contemplated, including intraperitoneally, intravenously, intramuscularly, subcutaneously, intradermally, orally, topically, intranasally, intrapulmonarily and intrarectally, but the present invention is not limited to these exemplified administration modes. However, since peptides are digested upon oral administration, active ingredients of a composition for oral administration should be coated or formulated for protection against degradation in the stomach. Preferably, the present composition may be administered in an injectable form. In addition, the pharmaceutical composition of the present invention may be administered using a certain apparatus capable of transporting the active ingredients into a target cell.

The pharmaceutical composition comprising the conjugate of the present invention can further comprise a pharmaceutically acceptable carrier. For oral administration, the pharmaceutically acceptable carrier may include a binder, a lubricant, a disintegrator, an excipient, a solubilizer, a dispersing agent, a stabilizer, a suspending agent, a coloring

agent, and a perfume. For injectable preparations, the pharmaceutically acceptable carrier may include a buffering agent, a preserving agent, an analgesic, a solubilizer, an isotonic agent, and a stabilizer. For preparations for topical administration, the pharmaceutically acceptable carrier may include a base, an excipient, a lubricant, and a preserving agent. The pharmaceutical composition of the present invention may be formulated into a variety of dosage forms in combination with the aforementioned pharmaceutically acceptable carriers. For example, for oral administration, the pharmaceutical composition may be formulated into tablets, troches, capsules, elixirs, suspensions, syrups or wafers. For injectable preparations, the pharmaceutical composition may be formulated into a unit dosage form, such as a multidose container or an ampule as a single-dose dosage form. The pharmaceutical composition may be also formulated into solutions, suspensions, tablets, pills, capsules and long-acting preparations.

On the other hand, examples of the carrier, the excipient, and the diluent suitable for the pharmaceutical formulations

include lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate and mineral oils. In addition, the pharmaceutical formulations may further include fillers, anti-coagulating agents, lubricants, humectants, perfumes, and antiseptics.

The administration frequency and dose of the pharmaceutical composition of the present invention can be determined by several related factors including the types of diseases to be treated, administration routes, the patient's age, gender, weight and severity of the illness, as well as by the types of the drug as an active component. Since the pharmaceutical composition of the present invention has excellent duration of in-vivo efficacy and titer, it can remarkably reduce the administration frequency and dose of pharmaceutical drugs of the present invention.

The insulinotropic derivatives according to the present invention are not disclosed by former inventors, or are broadly disclosed without any specific amino acid sequences and their activities are never compared to those of the native exendon-4, other derivatives and variants. Therefore, it never be expected that exendin-4 derivatives whose alpha amino group or alpha carbon of N-terminus is substituted or deleted exert remarkably excellent activities. Accordingly, the excellent stability in serum and insulinotropic activity of the insulinotropic peptide derivatives according to the present invention maximize a therapeutic effect on type 2 diabetes.

[Mode for Invention]

Hereinafter, a better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

Example 1. Plasma stability of exendin-4 derivative

To measure plasma stability of exendin-4 derivatives, each

of a native Exendin-4 and Exendin-4 derivatives was exposed to plasma, and the amounts of remaining proteins not denatured were measured by reversed phase HPLC to perform a test for denaturation depending on exposure time.

In the present experiment, to analyze the samples being exposed to plasma, the plasma mixed samples were deproteinised, and then analyzed.

The native exendin-4, desamino-histidyl-exendin-4 (DA-Exendin-4), beta-hydroxy imidazopropionyl-exendin-4 (HY-exendin-4), beta-carboxy imidazopropionyl-exendin-4 (CA-exendin-4), dimethyl-histidyl-exendin-4 (DM-exendin-4), and imidazoacetyl-exendin-4 (CA-exendin-4) were prepared at a concentration of 1 mg/ml, respectively. 200 ㎕ of each exendin-4 derivative sample was mixed with 200 ㎕ of rat serum, and the reaction was performed at 37℃and at each sampling time. 100 ㎕ of each sample was taken at each time point of 0 hr, 1 hr, 2 hr, 4 hr, 6 hr, 8 hr, 18 hr, and 24 hr. 400 ㎕ of ice-cold methanol was added to 100 ㎕ of the sample to terminate the reaction, followed by vortexing for 20 sec. Each mixture was centrifuged at 15,000 rpm for 30 min, and the supernatant was taken for

analysis.

A reversed phase HPLC was performed using a gradient of TFA in ACN as a mobile phase and using a C18 column.

The results were calculated from a ratio (%) of a major peak area of exendin-4 to total peak area, and the result of each derivative at a time point of 0 hr was taken as 100%, resulting in a graph plotting a pattern that the ratio of a major peak area decreases, as exposure time increases.

Up to the time point of 24 hr, whereas the purity of native exendin-4 decreased by about 70%, the purity of three derivatives (D, H, C form) decreased by about 77%, 78%, 77%, respectively (FIG. 1).

Example 2. Measurement of in vitro activity of exendin-4 derivative

To measure the efficacy of exendin-4 derivatives including desamino-histidyl-exendin-4, their in-vitro cell activity was examined. The native exendin-4 and exendin-4 derivatives were synthesized by American Peptide Corporation. Insulinoma cells or islets of Langerhans, which are generally used for measurement

of in-vitro activity of GLP-1, were isolated, and changes in the cAMP production were analyzed upon GLP-1 treatment.

In the present experiment, the in-vitro activity was measured using RIN-m5F (ATCC CRL-11605), which is known as a rat insulinoma cell and has a GLP-1 receptor, thereby being generally used for measurement of in-vitro activity of GLP-1. RIN-m5F cells were treated with GLP-1, native exendin-4, and exendin-4 derivatives including N-terminal-α-desamino-histidyl-Exendin-4 at varying concentrations, and then cAMP production due to the test materials was examined to determine EC_{50} values.

【Table 1】

test materials	EC_{50} (nM)	ratio vs Exendin4
Exendin-4	1.21	100
Desamino-histidyl(DA)-Exendin-4	0.95	127.4
Dimethyl-histidyl(DM)-Exendin-4	1.31	92.4
imidazoacetyl(CA)-exendin-4	1.2	100
beta-hydroxypropionyl(HY)-exendin-4	1.3	92.4

Example 3. Measurement of insulinotropic activity of exendin-4 derivative

The insulinotropic activities of exendin-4 derivatives were compared in RINm5F cells. RINm5F cells were thawed, and subcultured at least once, followed by inoculation into 96-well plates at a density of 1 x 10^5 cells/well with culture media containing FBS (Gibco, #11082). Then, the cells were cultured in a 5% CO_2 incubator at 37℃ for 48 hrs. The culture media were replaced with fresh media containing 0.5% FBS, and then incubated for 1 hr. Each of CA-exendin-4 and exendin-4 (byetta, Amylin) was diluted with culture media containing 0.5% FBS and glucose to yield concentrations from 10 nM to 0.001 nM. Except for the exendin samples, diluted solutions were prepared, and used as a control group. The culture media of RINm5F cells were removed, and the prepared samples were added thereto, followed by culturing in a 5% CO_2 incubator at 37℃ for 1 hr. Then, the media were recovered from each well. A rat insulin ELISA kit (Mercodia) was used to determine the insulin concentrations of the recovered media, and the results are shown in FIG. 2 and Table 2.

【Table 2】

Sample	Ratio of max insulin secretion to control group
CA Exendin-4	83.6%
Exendin-4	43.3%

As shown in FIG. 2 and Table 2, it was found that one of exendin-4 derivatives, CA exendin-4 exhibited about 2-fold higher insulinotropic activity than native exendin-4 at the same concentration.

Example 3. Comparison of in vivo efficacy of exendin-4 derivative

To measure in vivo efficacy of exendin-4 derivatives, their blood glucose lowering effect was measured in diabetic animal model, as compared with native exendin-4. The db/db mice (Jackson Lab, 10-12 week-old) were fasted for 2 hrs, and then administered with exendin-4 and CA exedin-4 at an amount of 0.01 - 1000 mcg.kg, respectively. After 1 hr, blood samples were collected from tail blood vessel to measure blood glucose levels using a glucometer.

Exendin-4, CA exendin-4, and vehicle were administered via subcutaneous route, and %change of blood glucose vs the vehicle was calculated at each concentration. At each concentration, the ED_{50} for the blood glucose lowering effect was calculated using Prism program (FIG. 3, Table 3).

【Table 3】

Sample	ED_{50} (mcg/kg)	R^2
CA exendin-4	2.30	0.99
Exendin-4	9.92	0.98

As shown in FIG. 3 and Table 3, it was found that CA exendin-4 exhibited about 5-fold higher blood glucose lowering effect than native exendin-4 in the diabetic animal model.

【Industrial Applicability】

The insulinotropic peptide derivatives according to the present invention maximize the intrinsic insulinotropic activity of exendin, that is, a therapeutic effect on type 2 diabetes, and induce reduction of food intake, delay in gastric emptying

or the like, being superior to native and other insulinotropic peptide analogs. Therefore, the insulinotropic peptide derivatives and the pharmaceutical composition comprising the same according to the present invention can be effectively provided for the treatment of the diseases.

CLAIMS

1. An insulinotropic peptide derivative, wherein N-terminal histidine residue of a native insulinotropic peptide is substituted with N-dimethyl-histidyl, wherein the insulinotropic peptide is exendin-4 or exendin-3.

2. The insulinotropic peptide derivative as set forth in claim 1, wherein the insulinotropic peptide is exendin-4.

3. An insulinotropic peptide derivative consisting of an amino acid of the following Formula 1:

R1-X-R2 <Formula 1>

wherein R1 is N-dimethyl-histidyl;

R2 is selected from the group consisting of $-NH_2$ or -OH;

X is selected from the group consisting of Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser, Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly and Ser-Asp-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Y-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Z-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser;

Y is selected from the group consisting of Lys, Ser, and Arg; and

Z is selected from the group consisting of Lys, Ser, and Arg.

4. The insulinotropic peptide derivative as set forth in claim 3, wherein R1 is N-dimethyl-histidyl, Y is Lys or Ser, Z is Lys, and R2 is $-NH_2$.

5. A pharmaceutical composition comprising the insulinotropic peptide derivative of any one of claims 1 to 4.

6. The insulinotropic peptide derivative of any one of claims 1 to 4 or the pharmaceutical composition of claim 5 for use in the treatment of diabetes.

7. Use of the insulinotropic peptide derivative of any one of claims 1 to 4 in the preparation of a pharmaceutical composition for treating diabetes.