DESCRIPTION

METHOD FOR DETERMINING GLUCAGON-LIKE PEPTIDE-1, AND KIT FOR USE IN SAID METHOD

TECHNICAL FIELD

[0001]

The present invention relates to a method of readily and accurately measuring glucagon-like peptide-1 (GLP-1) and a kit used therein.

BACKGROUND ART

[0002]

In development of pharmacological agents aiming at inhibiting the activity of dipeptidyl peptidase IV (hereinafter, may be referred to as "DPP-IV") or pharmaceutical products (mainly drugs for diabetes) with glucagon-like peptide-1 (GLP-1) as a pharmacological agent, the measurement of the concentration of GLP-1 that is active in the blood (GLP-1 (active)) or the concentration of total GLP-1 (GLP-1 (total)) is often carried out for the purpose of measuring parameters for drug efficacy evaluation and the concentration of drugs.

Mechanisms of action of DPP-IV inhibitors involve increasing the concentration active form GLP-1, which has a property of being decomposed by DPP-IV, by inhibiting the activity of DPP-IV and promoting insulin secretion by GLP-1 to promote utilization of sugars. Thus, by measuring the concentration of GLP-1, effects of the action of the DPP-IV inhibitor can be evaluated.

[0003]

As the active form GLP-1, there are known to be GLP-1 (7-36 Amide) and GLP-1 (7-37). Measurement of these is widely carried out by an immunoassay method using a specific antibody (ELISA method: a kit manufactured by Merck Millipore ~~Corporation~~ or the like). However, non-specific reactions often take place and it

1-1-3: Acid treatment

To a tube made of polypropylene, 150 μL of 0.2 mol/L glycine-hydrochloric acid buffer solution (pH 1.3) and a standard sample solution for a standard curve or 150 μL of individual blood plasma were added and mixed. The resulting mixture was incubated at 37°C for 10 minutes. Thereafter, 30 μL of 2 mol/L tris hydrochloric acid buffer solution (pH 8.5) was added thereto for neutralization and used as a sample subjected the acid treatment in the following measurement of the amount of active form GLP-1.

[0026]

1-1-4: Measurement of concentration of active form GLP-1

For the measurement of the concentration of active form GLP-1, GLUCAGON-LIKE PEPTIDE-1 (ACTIVE) ELISA KIT 96 Well Plate (manufactured by Merck Millipore ~~Corporation~~) by an anti-GLP-1 monoclonal antibody was used. The measurement method was carried out according to an accompanying explanatory leaflet. As for a measurement apparatus, Veritas microtiter plate luminometer (manufactured by Promega) was used; and the analysis was carried out by SoftMax Pro (manufactured by Molecular Devices Japan).

[0027]

1-2: Procedures of a conventional measurement method by a solid phase column

The procedure was carried out in the same manner as described in 1-1 except that the acid treatment of 1-1-3 was altered to the following solid phase column.

As the solid phase column, Oasis HLB Extraction Plate (manufactured by Waters Corporation) was used. It was used according to the accompanying instruction manual. After the conditioning of column was carried out, 300 μL of the blood plasma prepared in 1-1-1 was diluted 4-fold with PBS to 1200 μL, which was added to a well and then subjected to centrifugation (10 × g, for 3 minutes, room temperature). Thereafter, 10% methanol solution 1 mL was added and then

subjected to centrifugation (10 × g, for 3 minutes, room temperature) to wash the well. This operation was repeated twice. Subsequently, a deep well plate was set up and 0.5 mL of 75% methanol solution containing 0.5% ammonia was added and then subjected to centrifugation (10 × g, 3 minutes, room temperature) for elution. After repeating this operation twice, the eluting solution was completely collected from the well by centrifugation (100 × g, for 1 minute, room temperature). Subsequently, this eluting solution was dried to solidify under a nitrogen stream. To this, 250 μL of Assay Buffer contained in GLUCAGON-LIKE PEPTIDE-1 (ACTIVE) ELISA KIT 96 Well Plate kit (manufactured by Merck Millipore ~~Corporation~~) was added, put through a plate shaker for about 5 minutes for re-dissolution, and used in the measurement of the concentration of active form GLP-1 as a pretreated sample. Because a re-dissolved amount of 250 μL was less than the amount of the sample, which was 300 μL, a measurement value was multiplied by a volume correction coefficient (0.83: a value obtained by dividing the re-dissolved amount by the amount of the sample).

Further, in cases where the pretreatment was not carried out by way of comparison, the blood plasma prepared in 1-1-1 was used as was in the measurement of the concentration of active form GLP-1 (the volume correction was not required either).

[0028]

<<Example 2: Comparison of effects of pretreatment methods in the measurement of the concentration of active form GLP-1>>

When the concentration of active form GLP-1 and the concentration of total GLP-1 are compared in the same individual, individuals whose concentration of active form GLP-1 is higher than their concentration of total GLP-1 were noted in a method without pretreatment. Thus, it was obvious that active form GLP-1 is not able to be accurately measured without the pretreatment and it has been confirmed