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GLP-1(7-36)amide binding in skeletal muscle membranes from streptozotocin diabetic rats

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A higher specific binding of GLP-1(7-36)amide is found in skeletal muscle plasma membranes from adult streptozotocin (STZ)-treated rats (insulin-dependent diabetes mellitus model) and from neonatal STZ-treated rats (non insulin-dependent diabetes mellitus model), as compared to that in normal controls; no apparent change in the affinity was observed, that indicating the presence in both diabetic models of an increased number of high affinity binding sites for the peptide. The maximal specific GLP-1(7-16)amide binding in the non insulin-dependent diabetes mellitus model was found to be significantly higher than that in the insulin-dependent diabetes mellitus model. As GLP-1(7-36)amide exerts a glycogenic effect in the rat skeletal muscle, the present data suggest that the action of the peptide in the muscle glucose metabolism may be increased in states of insulin deficiency accompanied or not by insulin resistance.

Keywords: GLP-1(7-36)amide; binding; STZ-diabetic rats; muscle

Introduction

About glucagon-like peptide 1(7-36)amide (tGLP-1) -an insulinotropic intestinal peptide released into the circulation after oral glucose or fat ingestion (Kreymann *et al.*, 1987; Orskov *et al.*, 1991; Takahashi *et al.*, 1991)-, antidiabetogenic effects in normal and diabetic states (Gutniak *et al.*, 1992; Hendrick *et al.*, 1993) and potent glycogenic actions in isolated rat hepatocytes (Valverde *et al.*, 1994) and skeletal muscle (Villanueva-Peñacarrillo *et al.*, 1994a), have been reported. Recently, it has also been documented the presence and characteristics of tGLP-1 receptors in plasma membranes from rat liver (Villanueva-Peñacarrillo *et al.*, 1995) and muscle (Delgado *et al.*, 1995). In view of those findings, we have compared the [^{125}I]tGLP-1 binding to skeletal plasma membranes from non insulin- and insulin-dependent diabetes mellitus rat models to that from normal control rats.

Results

The mean values of the [^{125}I]tGLP-1 specific binding to rat muscle membranes were the same in both normal control groups, all along the displacement curve; in consequence, the data was pooled in a sole group (Figure 1). The maximal specific binding (MB) in the NIDDM model was 5.8 ± 0.6% of total radioactivity (n = 14 from 5 rats assayed in duplicate or triplicate), and this value was significantly higher ($P<0.01$) than that obtained in the control group (3.1 ± 0.4%, n = 22 from 8 rats assayed in duplicate or triplicate); statistical significance of the difference between both groups ($P<0.05$) was also observed along the displacement curve up to 10^{-9} mol/L unlabelled tGLP-1. The 50% inhibition dose (ID_{50}) was close to 10^{-9} mol/L unlabelled peptide in both groups, and the Scatchard plot of the data (Figure 1, inset) revealed the presence of high affinity binding sites, with an estimated Kd of 1.2×10^{-9} M in both groups, and a number of binding sites of 146 and 71 fmol/mg membrane protein for NIDDM model and control rats, respectively.

In the IDDM model, the MB was 4.3 ± 0.3% of total radioactivity (n = 14 from 5 rats assayed in duplicate or

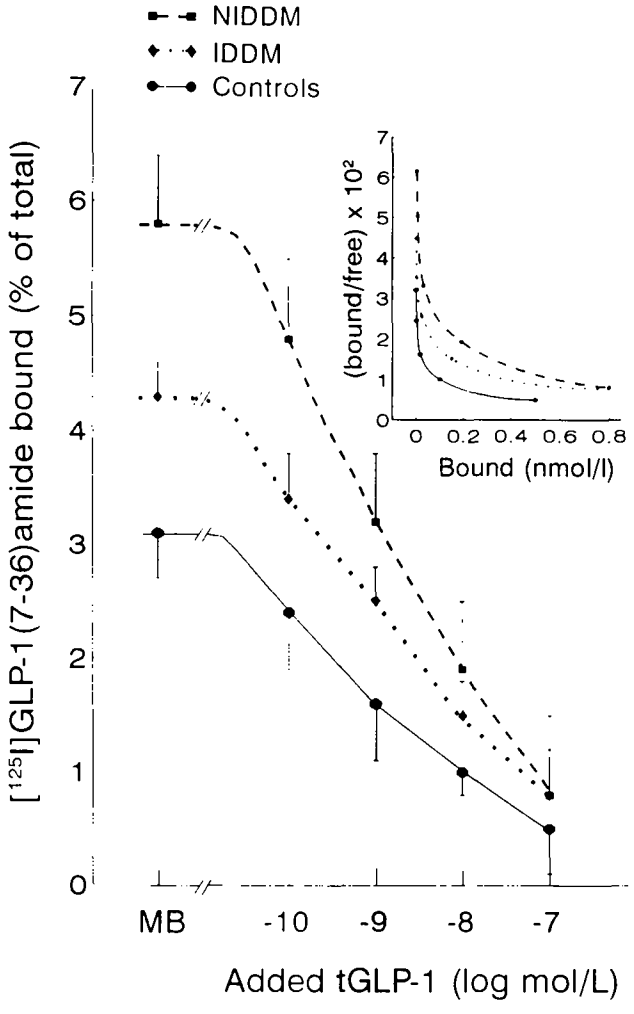

Figure 1 Displacement, by increasing concentrations of tGLP-1, of 3×10^{-11} mol/L [^{125}I]tGLP-1 specific binding to muscle plasma membranes (30 μg/100 μL) during 40 min at 25°C, from five NIDDM model rats (5 individual experiments performed at least in duplicate), five IDDM model rats (5 individual experiments performed at least in duplicate) and eight normal control rats (8 individual experiments performed at least in duplicate); data are shown as mean ± SEM. Inset, Scatchard plot of the data

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triplicate), value which was significantly different ($P<0.05$) from that of the normal rats. Although the mean values along the displacement curve of the diabetic group were higher than those of the normal, the differences did not reach statistical significance. The ID_{50} was also about 10^{-9} mol/L unlabelled tGLP-1 in this diabetic group, being 1.5×10^{-9} M, the high affinity Kd, and 125 fmol/mg membrane protein, the number of high affinity binding sites.

Statistical significant difference ($P<0.05$) was found between the MB of the IDDM and NIDDM models.

Discussion

The present study documents an increased [^{125}I]tGLP-1 specific binding in skeletal muscle membranes from STZ-NIDDM and -IDDM rats, as compared to normal controls.

A plasma glucose-lowering effect of GLP-1(7-36)amide in normal and diabetic state in humans (Gutniak *et al.*, 1992; Nauck *et al.*, 1993a) and rats (Hendrick *et al.*, 1993) has been reported, action which seems to be independent on the circulating insulin levels (D'Alessio *et al.*, 1994). Recent published studies performed in rats, on the effect of tGLP-1 in some extrapancreatic tissues, showed that the peptide potently stimulates the D-glucose incorporation into glycogen in isolated hepatocytes (Valverde *et al.*, 1994) and skeletal muscle (Villanueva-Peñacarrillo *et al.*, 1994a), and that these glycogenic effects are associated to an increase of the glycogen synthase *a* activity. It has also been found that both liver and muscle tissues contain a GLP-1(7-36)amide binding protein of an approximately 63 000 Mr (Villanueva-Peñacarrillo *et al.*, 1995; Delgado *et al.*, 1995) which likely could be somehow different to the pancreatic GLP-1 receptor (Thorens, 1992), as in those two tissues no effect on the adenylate cyclase-cAMP system has been detected (Valverde *et al.*, 1994; Villanueva-Peñacarrillo *et al.*, 1994a). In fat, another extrapancreatic tissue, where tGLP-1 has also a biological effect (Ruiz-Grande *et al.*, 1992; Oben *et al.*, 1991; Miki *et al.*, 1994; Egan *et al.*, 1994), the presence of specific tGLP-1 binding has been detected in rats (Valverde *et al.*, 1993) and humans (Mérida *et al.*, 1993); in addition, it has been reported an increased number of receptors for this peptide in the fat tissue of diabetic patients (Villanueva-Peñacarrillo *et al.*, 1994b).

On the other hand, and while published information in relation to tGLP-1 plasma levels in IDDM patients is lacking, there are controversial results about the circulating levels of the peptide in response to oral glucose in NIDDM patients, as some investigators have found an augmented (Hirota *et al.*, 1990; Orskov *et al.*, 1991; Fukase *et al.*, 1993) and others a decreased (Nauck *et al.*, 1993b) response of GLP-1(7-36)amide as compared to normal controls, discrepancy that can be explained by the problems that in fact exist at measuring it in plasma.

The tGLP-1 binding in skeletal muscle membranes, higher in both IDDM and NIDDM rat models, and apparently not accompanied by changes in the affinity -as estimated from the ID_{50} and from the Scatchard plot, which also indicates an increased number of high-affinity binding sites-, suggests a role of this peptide at the muscle glucose removal, not only in normal state, as we previously reported (Villanueva-Peñacarrillo *et al.*, 1994a), but also in situations of insulin deficiency accompanied or not by insulin resistance (Youn *et al.*, 1994; Blondel *et al.*, 1989), where the action of the peptide in the muscle glucose metabolism could be increased. In fact, we have observed that tGLP-1-induced glucose incorporation into glycogen in the soleus muscle of NIDDM rat model was higher than in normal controls (Alcántara *et al.*, 1995). The knowledge of the intrinsic mechanism of these changes in diabetic states awaits further investigation.

Materials and methods

Biological material

Control rats and those treated with streptozotocin (STZ) were male FJD inbred Wistar, fed with a commercial pelleted chow (UAR, Panlab, Spain) and water *ad libitum*. The non insulin-dependent diabetes mellitus model (NIDDM) was induced in rats as in Portha *et al.* (1979), by intraperitoneal injection, on the day of birth, of STZ (100 μg/g body wt) dissolved in 25 μL of a citrate-Na_2OH buffer (0.05 M, pH 4.5); at the age of 6–7 weeks, an intravenous glucose tolerance test (2.8 μmol/g body wt) was performed in normal and STZ-treated rats. The insulin-dependent diabetes mellitus model was induced in adult rats (172 ± 6 g body wt, $n = 5$) by one dose of STZ (60 μg/g body wt) intraperitoneally administered; the control rats (170 ± 7 g body wt, $n = 4$) were also caged separately, and after 4–7 days, the blood sugar was measured in both groups. All rats were killed by a sharp blow to the head, and the gastrocnemius muscles removed and kept at −70°C until used. The characteristics of the four groups at the time of the study are shown in Table 1.

Chemicals

Synthetic GLP-1(7-36)amide (lot number 010448) was obtained from Peninsula Laboratory Inc. (Belmont, CA, USA); Na[^{125}I] (580-600 MBq/μg) was from Amersham International (Aylesbury, Buckinghamshire, United Kingdom); Triton X-100, polyethylenglycol 6000 (PEG) and dimethylsulfoxide (DMSO) were from Merck (Darmstadt, Germany); bacitracin, phenylmethylsulphonylfluoride (PMSF), leupeptin, pepstatin, Fraction V bovine serum albumin (BSA), and chloramine-T were from Sigma Chemical Co. (St. Louis, MO, USA); Trasylol was from Bayer AG (Leverkusen, Germany), and human gamma-globulin was from Behring, Hoechst Ibérica S.A. (Barcelona, Spain).

Radioactive tGLP-1

[Mono-^{125}I] GLP-1(7-36)amide (70 MBq/nmol) was prepared by the chloramine-T method using 5 μg of the peptide, 29.6 MBq Na[^{125}I], and 4–8 μg chloramine-T, in a total

Table 1 Metabolic and hormonal data in control normal rats and in NIDDM and IDDM rat models (mean ± SEM)

Rats	*Control (n = 4)*	*NIDDM (n = 5)*	*Control (n = 4)*	*IDDM (n = 5)*
Age (days)	47.3 ± 1.8	46.6 ± 1.2	–	–
Weight (g)	179 ± 6	165 ± 5	199 ± 5	182 ± 3
Plasma glucose (mg/dl)	88 ± 12	145 ± 10[b]	107 ± 5	432 ± 46[c]
K (10^{-2}/min)	4.9 ± 1.4	0.9 ± 0.2[a]	–	–
Plasma IRI (ng/mL)	2.7 ± 1.4	3.5 ± 1.2	2.4 ± 0.6	0.8 ± 0.4
Glucose-induced IRI (ng/mL) at 2 min	26.5 ± 5.0	15.7 ± 2.9	–	–

IRI, immunoreactive insulin; K, glucose utilization coefficient. [a]$P<0.02$; [b]$P<0.01$; [c]$P<0.001$ *vs* control

volume of 48 μL 0.3 mol/L phosphate buffer, pH 7.4, for 10–20 sec at 25°C, to yield 45–60% ^{125}I incorporation, and then purified by reversed-phase HPLC on a μBondapack C_{18} column, with a linear gradient of 28–49% acetonitrile in 0.1% trifluoroacetic acid.

Muscle membranes

The protocol followed was based on previously described procedures (Hirshman *et al.*, 1990; Obermaier *et al.*, 1985). The two gastrocnemius muscles from one rat (~2 *g* wet weight) were finely cut with scissors in 7 mL 10 mmol/L sodium phosphate buffer, pH 7.4, containing 5 mmol/L EDTA, 250 mmol/L sucrose, 5 mmol/L PMSF, 500 KIU/mL Trasylol, 2 μmol/L leupeptin, 2 μmol/L pepstatin, 7 500 units/mL bacitracin and 10 mmol/L leucine; after homogenization by Potter Teflon-glass, the homogenate was filtered through a cheesecloth, and the filtrate was centrifuged at 48 000 *g* during 20 min at 4°C. The pelleted plasma membranes were resuspended in 7 mL above media and centrifuged at 200 000 *g* during 50 min at 4°C. Plasma membranes were resuspended in 50 mmol/L HEPES, pH 7.4, containing 150 mmol/L NaCl, 0.01% bacitracin, 0.01 mmol/L PMSF, 2 μmol/L leupeptin, 2 μmol/L pepstatin and 1% Triton X-100, at a final protein concentration of 2–4 mg/mL, and stirred continuously for 1 h at 4°C (Hedo & Simpson, 1984). The undissolved material, pelleted by centrifugation at 200 000 *g* for 30 min at 4°C, was discarded. To remove the presence of the detergent in the solubilized membranes, proteins were precipitated with 12.5% PEG in 50 mmol/L HEPES and 10 mmol/L $MgSO_4$, pH 7.5, at 4°C during 30 min (Marshall *et al.*, 1985). The pellet, representing 20–25% of the total protein content – as determined by the Bradford method (Bradford, 1976) using BSA as standard – in the initial untreated membranes, was reconstituted in 50 mmol/L HEPES, pH 7.4, containing 150 mmol/L NaCl, 0.01% bacitracin, 0.01 mmol/L PMSF, 2 μmol/L leupeptin and 2 μmol/L pepstatin, and stored at −70°C.

Binding studies

The GLP-1(7-36)amide binding assay was performed by incubating aliquot samples of muscle membranes (30 μg protein) with 3×10^{-11} mol/L [^{125}I]GLP-1(7-36)amide, in the absence or in the presence of unlabeled GLP-1(7-36)amide, in a total volume of 100 μL 50 mmol/L HEPES, pH 7.4, containing 10 mmol/L $MgSO_4$, 2% BSA, 0.1% bacitracin and 500 KIU/mL Trasylol, during 40 min at 25°C, as previously reported (Delgado *et al.*, 1995). At the end of the incubation period, the tubes were placed in an iced water bath, and the receptor-bound peptide was separated from the free peptide by precipitation, after the addition of 500 μL iced cold 0.1% human gamma-globulin, with 500 μL PEG (final concentration, 12.5%); after incubating for 10 min at 4°C, the samples were centrifuged at 12 000 *g* for 5 min in a Beckman Microfuge, the supernatants were then removed, and the pellets were counted for gamma-radioactivity. The specific binding was calculated by subtracting the non-specific binding (10^{-6} mol/L unlabeled peptide) from the total binding.

Statistical method

The statistical significance of difference between mean values was assessed by the Student's *t*-test.

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