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(7-36)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), anti-diabetogenic 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 GLP-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 [125I]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 [125I]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 (ID₅₀) 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 x 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 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 (ID₅₀) 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 x 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...
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 $10^{-7}$ mol/L unlabelled tGLP-1 in this diabetic group, being $1.5 \times 10^{-7}$ mol/L, 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 GLP-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-Pefiacarrillo et al., 1994a), and that these glycogenic effects are associated to an increase of the glycogen synthase activity. It has also been found that both liver and muscle tissues contain a GLP-1(7-36)amide binding protein of an approximately $350$ Mr (Valleverde et al., 1995; Delgado et al., 1995) which likely could be somewhat 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-Pefiacarrillo 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-Pefiacarrillo et al., 1994a).

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 (Naukk 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-Pefiacarrillo 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 pg/g body wt) dissolved in 25 mL of a citrate-Na$_2$OH buffer (0.05 M, pH 4.5); at the age of 6–7 weeks, an intravenous glucose tolerance test (2.8 mol/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 pg/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$_{251}$ (580–600 MBq/µg) was from Amersham International (Aylesbury, Buckinghamshire, United Kingdom); Triton X-100, polyethyleneglycol 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$_{251}$, and 4–8 µg chloramine-T, in a total