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Role of glucagon-like peptide-1 and its agonists on early prevention of cardiac remodeling in type 1 diabetic rat hearts

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Abstract. Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from intestinal L cells upon nutrients ingestion, and is currently used for treating diabetes mellitus. It plays an important role in receptor modulation and cross talk with insulin at the coronary endothelium (CE) and cardiomyocytes (CM) in diabetic type 1 rat heart model. We studied the effects of insulin, GLP-1 analogues (exendin-4), and dipeptidyl peptidase-IV (DPP-IV) inhibitor on GLP-1 cardiac receptor modulation. The binding affinity of GLP-1 to its receptor on CE and CM was calculated using a rat heart perfusion model with [^{125}I]-GLP-1(7-36). Tissue samples from the heart were used for immunostaining and Western blot analyses. GLP-1 systemic blood levels were measured using ELISA. GLP-1 binding affinity (τ) increased on the CE (0.33 ± 0.01 *vs.* 0.25 ± 0.01 min; $p < 0.001$) and decreased on the CM (0.29 ± 0.02 *vs.* 0.43 ± 0.02 min; $p < 0.001$) in the diabetic non-treated rats when compared to normal. There was normalization of τ back to baseline on the CE and CM levels with insulin and DPP-IV inhibitor treatment, respectively. Histological sections and immunofluorescence showed receptor up-regulation in diabetic rats with significant decrease and even normalization with the different treatment strategies. Systemic GLP-1 levels increased after 14 days of diabetes induction (10 ± 3.7 *vs.* 103 ± 58 pM; $p = 0.0005$). In conclusion, there is a significant GLP-1 receptor affinity modulation on the CE and CM levels in rats with diabetes type 1, and a cross talk with GLP-1 analogues in early prevention of cardiac remodeling.

Key words: Binding affinity — Dipeptidyl peptidase-IV inhibitor — Dissociation constant — Exendin-4 — Glucagon-like peptide-1

Abbreviation: CE, coronary endothelium; CM, cardiomyocytes; DPP-IV, dipeptidyl peptidase-IV; GLP-1, glucagon-like peptide-1; STZ, streptozotocin; τ, affinity (residency-time) constant.

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by impaired response to insulin i.e. insulin resistance, followed later by drop in insulin secretion (Poretsky 2002). It affects approximately 170 million individuals worldwide and is expected to alter the lives of at least 366 million individuals within a span of 25 years to come (Maiese et al. 2007). Diabetes mellitus is associated with increased cardiovascular remodeling which warrants an aggressive regimen of treatment leading to reduction in complications (Port et al. 2003). There has been many options for medications that target different physiologic pathways involved in glycemic control including: 1) oral hypoglycemics such as sulfonylureas (insulin-stimulant), glitazones and metformin (insulin sensitizers), and 2) insulin analogues and animal insulin. More recently, glucagon-like peptide-1 (GLP-1), an incretin hormone released from the gut cells, was found to inhibit gluca-

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gon release, stimulate insulin secretion, and promote pancreatic β cells regeneration (Asmar and Holst 2010). Gastric emptying is slowed by GLP-1 administration, thus slowing digestion and absorption moderating blood glucose excursions (Murphy and Bloom 2007). GLP-1 acts by binding to its receptor on β-cells mediating insulin secretion through activation of adenyl cyclase, formation of intracellular cyclic AMP (During et al. 2003), and activation of protein kinase A (PKA) (D'Alessio and Vahl 2004); this chain reaction results in closure of voltage-gated potassium channels (that are activated in case of hyperglycemia), increase in intracellular calcium, and exocytosis at site distal to elevation of intracellular calcium in the same way that insulin does its action. Also a number of kinases are activated upon the binding of GLP-1 to its receptor including phosphatidylinositol 3-kinase (PI3-kinase) and extracellular regulated kinase (Erk) (Gomez et al. 2002). Activation of GLP-1 receptor improves β-cell function and survival following induction of endoplasmic reticulum stress in diabetes (Yusta et al. 2006). In this study, we investigated the effects of a GLP-1 analogue (exendin-4) and a DPP-IV inhibitor (KR-62436), which were approved for clinical use by the FDA in 2005 and 2007, respectively, on GLP-1 receptor modulation and binding kinetics on the coronary endothelium and cardiomyocytes in a diabetic rat model and to assess any possible cross talk with insulin admisinistration and cardiac remodeling. Exendin-4 exhibits glucoregulatory effects as mammalian GLP-1; however, unlike GLP-1, exendin-4 is resistant to DPP-IV degradation (Scott and Moran 2007). Administration of such incretin exerts an antihypertensive effect on the heart thus inducing vasorelaxation, and improving left ventricular contractile action of patients with cardiomyopathy (Combettes 2006).Various studies reported the multiple metabolic effects of DPP-IV inhibitors including enhancement of glucose-dependent stimulation of pancreatic insulin release as well as attenuation of glucagon secretion (Tahara et al. 2009). Moreover, loss of DPP-IV activity is associated with improved glucose tolerance and reduced glycemic excursion following oral glucose challenge (Drucker 2007). a cardinal role of DPP-IV inhibitors is their potential to significantly augment β-cell mass in streptozotocin (STZ)-injected diabetic rats (Moritoh et al. 2008). In contrast to GLP-1 mimetics, there are no data indicating inhibition of gastric emptying, appetite or weight reduction due to a treatment with DPP-IV inhibitors (Inzucchi and McGuire 2008). Inhibition of DPP-IV results in more availability of GLP-1 leading to the relaxation of rat conduit arteries (Yamamoto et al. 2002).

Materials and Methods

Animals

All experiments were conducted with the approval of the Institutional Review Board/Animal House Committee of the American University of Beirut, and in accordance with the guidelines of the American Association for Laboratory Animal Sciences (AALAS) on "Humane Care and Use of Laboratory Animals".

Male sprague dawley rats (six-weeks old, 200–250 g body weight) were purchased from Harland Netherland (The Netherlands), and bred at American University of Beirut, Medical Center, Animal Care Facility (Lebanon). The rats were distributed into seven groups matched for age, gender, and weight. Each group was composed of 24 animals divided into 4 animals *per* cage. Animals were fed Purina pellets and tap water *ad libitum*. They were at all times kept at constant temperature and had 12 hours of light *per* day for 14 days, after which they were sacrificed.

Treatment and monitor plan

Rats were distributed into 7 groups:

Group N (n = 24): Normal controls gavaged tap water, 4 ml/kg body weight (b.w.), once daily.

Group D (n = 24): Rats with type 1 diabetes injected *intraperitoneally* (*i.p.*) with 3 ml/kg b.w. normal saline solution (NSS), twice daily, and given placebo (water) by oral gavage (4 ml/kg b.w., once daily).

Group DI (n = 24): Rats with type 1 diabetes injected *i.p.* with bovine insulin (Sigma-Aldrich Co., St. Louis, USA), 0.28 unit/ml, 1 unit/kg b.w., twice daily, and gavaged H_2O 4 ml/kg b.w. once daily (placebo).

Group DE (n = 24): Rats with type 1 diabetes injected *i.p.* with exendin-4 (Sigma Aldrich Co., St. Louis, USA), 0.03 μg/kg b.w., twice daily, and gavaged H_2O 4 ml/kg b.w., once daily (placebo) (Hantouche et al. 2010).

Group DIE (n = 24): Rats with type 1 diabetes injected *i.p.* with bovine insulin (1 unit/kg b.w.), twice daily, and exendin-4 (0.03 μg/kg b.w.), twice daily, and gavaged H_2O 4 ml/kg b.w., once daily (placebo).

Group DD (n = 24): Rats with type 1 diabetes gavaged DPP-IV inhibitor (Sigma Aldrich Co., St. Louis, USA), 0.20 mg/kg b.w., once daily, and injected *i.p.* with NSS (3 ml/kg b.w., twice daily).

Group DDI (n = 24): Rats with type 1 diabetes injected i.*p.* with bovine insulin (1 unit/kg b.w.), twice daily, and gavaged DPP-IV inhibitor 0.20 mg/kg b.w., once daily.

Induction of diabetes

After being anesthetized using isoflurane, diabetes was induced in rats by a single intravenous injection of STZ (Sigma Chemical Company, USA; 85 mg/kg b.w.), acidified to pH 4.5 with 0.1 M citrate buffer. Blood glucose was determined two days after STZ injection by Accu-Chek using a drop of blood from tail end (Accu-Chek instant test, Roche Diagnostics,

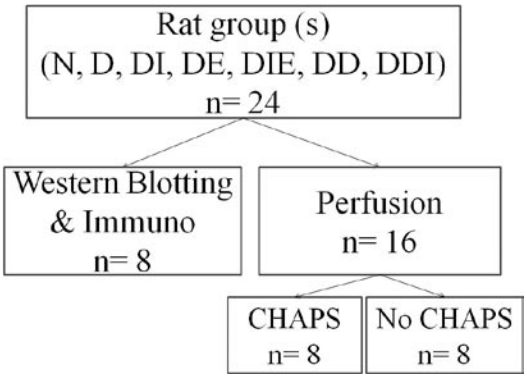


Figure 1. Surgical procedures scheme in an algorethmic way showing the distribution of different rat groups.

Germany), and a non-fasting blood glucose level of above 200 mg/dl indicated diabetes.

Surgical procedures

Sixteen rats out of 24 from each group were sacrificed as subgroup and used to study the binding kinetics. Each subgroup was further divided into two divisions. One division (8 rats) was perfused with buffer, and the other (8 rats) was perfused with 20 mM of 3-[(3-cholamidopropyl) dimethylammonio]-1- propanesulfonate (CHAPS; Sigma Chemical Company, St. Louis, USA) to strip the endothelial lining (Berthiaume et al. 1995). The rats were weighed then anesthetized by a single *i.p.* injection of sodium pentobarbital (50 mg/kg b.w.). Once asleep, each rat was fixed to a heat pad to avoid rapid cooling. The skin was cut in the mid gastric region up to the sternal notch. Then, the abdominal muscle layer was longitudinally opened to the xyphoid. The thoracic cavity was opened by cutting through the mid-clavicular lines of each side of the thorax (Bikhazi et al. 2003). Thus, the whole thoracic cavity was fully uncovered to perform heart perfusion (Fig. 1).

Rat heart perfusion

The right and left superior venae cavae, as well as the inferior vena cava and the pulmonary artery and veins were isolated and tied at their connection with the heart. a catheter was then introduced into the aorta to be fixed near the opening of the coronary artery without altogether reaching the aortic valve. This site was used as the inlet of the perfusate which was collected via another catheter from the right atrium at the opening of the coronary sinus. Both catheters were firmly anchored in place with sutures. The dissected part of the animal and the exposed heart were soaked with saline at 37°C throughout the experiment. All these perfusion experiments were carried out following the procedures reported in previous studies performed in our laboratory (Haddad et al. 1997).

Tissue preparation for histological studies

The cardiac tissues from the different groups were isolated for Western blotting and immunostaining. Each heart was then cut into two halves. One half was used for the Western blot and the other for immunostaining.

Western blotting

Protein samples (100 µg) extracts from (n = 8) homogenized cardiac muscle were loaded and separated on 10% polyacrylamide gel electrophoresis for 1 h (Mini-gel electrophoresis unit, Protean III, Bio-Rad Laboratory, USA) (Karam et al. 2005). Protein bands were then transferred for 2 h on nitrocellulose membranes (Transblot unit, PowerPac HC, Bio-Rad Laboratory, USA). The membrane was then blocked with a blocking buffer (0.5% dry milk with TBS/Tween) overnight at 6–8°C. On the second day nitrocellulose membrane was incubated for 1 h with primary polyclonal antibody: Goat Polyclonal Anti-GLP-1 rat receptor (diluted 1/400; Santa Cruz Biotech., USA). Then to remove the unbound antibody it was transferred to three consecutive washes with Tris/Tween 20 solution on the nitrocellulose membrane. This was followed by incubating for 2 h with the secondary antibody, Donkey anti-goat Polyclonal IgG-HRP (diluted 1/2000; Santa Cruz Biotech., USA), then washing the membrane with a Tris/Tween 20 solution. Blotted bands were detected by ECL (Enhanced Chemiluminescence) and developed on an Agfa Medical X-ray film blue (Agfa-Gevaert N.V., Mortsel, Belgium) by autoradiography (Fisher Scientific, Pittsburg, USA). The thickness of each band was determined by a semi-quantitative densitometric analysis (arbitrary unit) using a specific computer software, and expressed as mean of thickness ± SD.

Indirect immunoflourescence

GLP-1 receptors were assessed in the heart using indirect immunoflourescence (n = 8). Snap frozen cardiac tissue was sectioned by cyrostat into 4 µm thick sections and placed on gelatin-precoated glass slides. Non-specific sites on the sections were blocked first with rabit serum for 2 h, then incubated with GLP-1 receptor primary antibody raised in rabbits (Santa Cruz Biotech.) (diluted 1/5000) for 45 min. Then it was washed with flourescein-labeled secondary antibody (Santa Cruz Biotech.) (diluted 1/1000) for 1 h. Between incubations, the slides were washed in phosphate buffered saline (PBS; 0.01 M) for 30 min each time. Finally, the sections were mounted with aqueous glycerin, and the slides viewed using a UV light microscope.

Immunofluorescence was measured using a semiquantitative visual program based on MATLAB software.

Enzyme-linked immune-sorbent assay (ELISA)

Blood samples (100 µl) were obtained from the tongues of the rats from each of the eight animals in the seven different groups. The blood was collected in ice-cooled EDTA-plasma tubes. DPP-IV inhibitor (10 µl) was added immediately (in less than 30 s) to the blood samples to avoid degradation of GLP-1. Blood samples were then centrifuged at 1000 × *g* for 10 min. The supernatant was then collected and stored at ≤ –70°C (ELISA Kit, Linco Research, USA). The ELISA plate microwells were first washed with diluted washing buffer (300 µl). Then assay buffer was added to each of the well. 100 µl of the control serum and an equal volume of the experimental sera were added. The plate was finally sealed and incubated overnight at 4°C. The N-terminal region of active GLP-1 molecule present in the blood samples were bound to the microwells which were coated with the monoclonal antibodies. On the second day, the supernatant was decanted and the wells thouroughly washed to remove them. Then 200 µl of GLP-1-alkaline phosphatase detection conjugate were added. This conjugate binds to GLP-1 fixed at the bottom of the wells. The plates were allowed to incubate for two hours at room temperature. The unbound conjugate was washed off and 200 µl of hydrated dilute substrate (1 : 200) were added to the plates and left to sit for 20 min. Finally, bound detection conjugate was quantified by adding 50 µl of stop solution MUP (methyl umbelliferyl phosphate) which, in the presence of alkaline phosphatase, forms the fluorescent product umbelliferone. Each plate was then read on fluorescence plate reader with an excitation/emission wavelength of 355/460 nm. The amount of fluorescence generated is directly proportional to the concentration (pM) of active GLP-1 in the blood sample, which can be derived by interpolation from a reference standard curve generated in the same assay with reference standards of known concentrations of active GLP-1.

Statistical analysis

To assess the significance of the results obtained on the different experimental groups, and among the seven groups, the Student *t*-test was used for continuous variables, and the analysis of variance for multiple factors (ANOVA) using the Bonferroni penalty method for comparison among all the groups. All results were expressed as mean values ± standard deviation, $p < 0.05$ was considered significant.

Results

Body weight and plasma glucose levels

The mean body weight and mean plasma glucose levels for the seven different rat groups are summarized in Table 1.

Binding kinetics of GLP-1 to its receptor

The binding kinetics of GLP-1 to its receptor are summarized in Table 1. There was a significant decrease in the dissociation constant (K_d) of the group D as compared to the group N at the coronary endothelium (CE) (0.17 ± 0.01 *vs.* $0.19 \pm 0.01 \cdot 10^{-15}$ mol, $p = 0.03$). Treatment with insulin and exendin together (DIE) restored K_d to normal value (0.19 ± 0.01 *vs.* $0.19 \pm 0.01 \cdot 10^{-15}$ mol, $p = 1.0$) (Table 1).

Table 1. Body weight, blood glucose level, and GLP-1 receptor binding kinetics in normal, diabetic, and diabetic treated rats

		N	D	DI	DE	DIE	DD	DDI
	body weight (g)	301 ± 28^a	235 ± 35^b	243 ± 35^c	257 ± 37^d	247 ± 23^e	240 ± 21^f	256 ± 49^g
	glucose (mg/dl)	106 ± 6^a	539 ± 55^b	425 ± 94^c	423 ± 94^d	413 ± 58^e	417 ± 84^f	428 ± 86^g
coronary endothelium	K_d (10^{-15} mol)	0.19 ± 0.01^a	0.17 ± 0.01^b	0.21 ± 0.01^c	0.18 ± 0.01^d	0.19 ± 0.01^e	0.17 ± 0.01^f	0.18 ± 0.01^g
	τ (min)	0.25 ± 0.01^a	0.33 ± 0.01^b	0.28 ± 0.01^c	0.34 ± 0.02^d	0.30 ± 0.01^e	0.35 ± 0.02^f	0.32 ± 0.01^g
cardiomyocyte	K_d (10^{-15} mol)	0.09 ± 0.01^a	0.22 ± 0.01^b	0.19 ± 0.01^c	0.22 ± 0.02^d	0.19 ± 0.02^e	0.13 ± 0.01^f	0.17 ± 0.01^g
	τ (min)	0.43 ± 0.02^a	0.29 ± 0.02^b	0.34 ± 0.02^c	0.34 ± 0.02^d	0.36 ± 0.02^e	0.42 ± 0.02^f	0.37 ± 0.02^g

All values are expressed as means ± SD. A statistical significance was indicated when p is less than 0.05. N, normal; D, diabetic; DI, diabetic treated with insulin; DE, diabetic treated with exendin-4; DIE, diabetic treated with insulin and exendin-4; DD, diabetic treated with DPP-IV inhibitor; K_d, dissociation constant of GLP-1 to its receptor; τ, binding affinity of GLP-1 to its receptor. For body weight: (a, b), (a, c), (a, d), (a, e), and (a, f) are significant. For blood glucose level: (a, b), (a, c), (a, d), (a, e), (a, f), (a, g), (b, c), (b, d), (b, e), (b, f), and (b, g) are significant. For coronary endothelium: K_d (a, b), (a, c), (a, f), (b, c), (b, e), (c, d), (c, e) (c, f), (c, g), (e, f) are significant and τ (a, b), (a, c), (a, d), (a, e), (a, f), (a, g), (b, c), (b, e), (b, f), (c, d), (c,e) (c, f), (c,g), (d, e), (e, f), (f, g) are significant. For cardiomyocytes: K_d (a, b), (a, c), (a, d). (a, e), (a, f), (a, g) , (b, c), (b, e), (b, f), (b, g), (c, d), (c, f), (c, g), (d, e), (d, f), (d, g), (e, f), (e, g), (f, g) are significant and τ (a, b), (a, c), (a, d), (a, e), (a, g), (b, c), (b, d), (b, e), (b, f), (b, g), (c, e) (c, f), (c, g), (d, e), (d, f), (d, g), (e, f), (f, g) are significant. Others are not significant.

The affinity (residency-time) constant (τ) at the level of CE (Table 1) was significantly increased in all groups as compared to normal ($p < 0.001$). Insulin treatment reduced τ , but was still significantly higher than comparing with the normal group (0.28 ± 0.01 *vs.* 0.25 ± 0.01 min, $p = 0.001$).

At the cardiac myocyte level, there was a significant increase in the K_d in all diabetic groups *vs.* normal (Table 1) ($p < 0.0001$). τ was decreased at the level of cardiomyocytes in all diabetic groups as compared to group N except for the diabetic animals treated with DPP-IV inhibitor (group DD) (0.42 ± 0.02 *vs.* 0.43 ± 0.02 min, $p = 0.99$). Treatment with insulin or exendin was associated with an increase in τ as compared to the non treated diabetic group (0.34 ± 0.02 *vs.* 0.29 ± 0.02 min, for both groups, $p = 0.0002$); similarly, the combined treatment of insulin and exendin together or DPP-IV increased τ when compared to diabetic animals (0.36 ± 0.02 *vs.* 0.29 ± 0.02 min, and 0.37 ± 0.02 *vs.* 0.29 ± 0.0204 min, $p = 0.0001$ for both).

ELISA

There was a significant increase in GLP-1 systemic blood levels in the diabetic non treated group at day 14. In all treated groups, it was significantly reduced and almost normalized ($p < 0.001$) (Fig. 2).

Western blotting analysis

On a polyacrylamide gel after an equal loading of the protein extracts (Fig. 3B), a 56 kD band was detected by a semiquantitative analysis. There was no signifcant increase in the band density of GLP-1 receptor (Fig. 3A).

Immunohistochemistry

There was a significant increase in the value of the GLP-1 receptor binding affinity in the diabetic group compared to its value in the normal animals. Treatment with DPP-IV inhibitor resulted in normalization of the results ($p < 0.001$) (Figs. 4 and 5).

Discussion

Incretin hormone secretion is known to respond to two stimuli: first – food ingestion, and second – more precisely blood levels of glucose reached after a meal are enough to stimulate its secretion (Porte et al. 2003). Two hormones of the incretin hormones are currently under intensive research namely glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. Most studies demonstrated that both hormones play a very important role in glucose metabolism (Berghofer

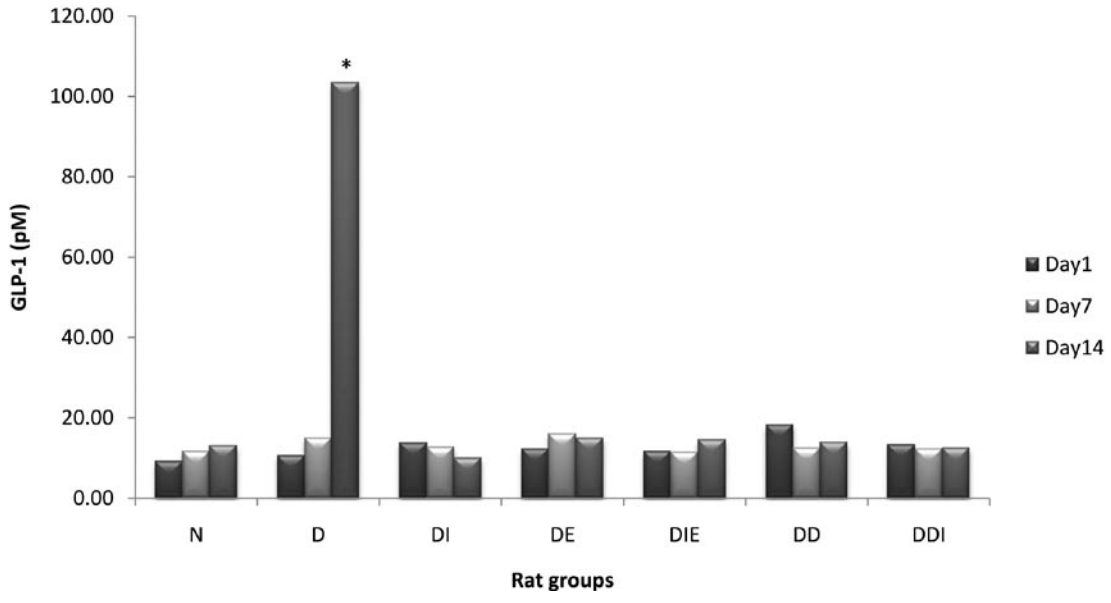


Figure 2. The graph presents the systemic GLP-1 concentration levels determined by ELISA in all rat groups at day 1, 7 and 14. There is significant increase in the [GLP-1] (pM) in the diabetic (D) group at day 14. Treatment with insulin, exendin, or DPP-IV inhibitor has normalized the levels. D, diabetic; DD, diabetic treated with DPP-IV inhibitor; DDI, diabetic treated with DPP-IV inhibitor and insulin; DE, diabetic treated with exendin-4; DI, diabetic treated with insulin; DIE, diabetic treated with insulin and exendin-4; N, normal; GLP-1R, glucagon-like peptide-1 receptor.

et al. 1997). Proglucagon is the precursor of GLP-1 (Drucker 2003). It is secreted in response to the ingestion of fats and carbohydrates (Underwood et al. 2010) by pancreatic α-cells, endocrine L cells located in the intestinal mucosa, in addition to a population of neurons located in the hind brain. Proglucagon is then cleaved, after a meal, in the pancreas by prohormone convertase 1/3 (PC1/3), only found in α-cells, and prohormone convertase 2 (PC2) to yield GLP-1 and GLP-2 (Wei 1995). When it binds to its receptor, it induces pancreatic β-cell action through activation of adenyl cyclase forming intracellular cyclic adenosine monophosphate (cAMP) (Seino et al. 2009). The resulting signaling cascade leads to improvement in glycemic control, which may be cardioprotective (During et al. 2003; Combettes 2006).

Previous studies demonstrated that cardiomyopathic alterations in STZ-induced diabetic rats (Brunton 2009) usually start with apoptosis of cardiomyocytes on day one in the diabetic animals leading to compensatory hypertrophy and a number of cardiomyopathic changes. GLP-1 has been suggested to ameliorate left ventricular function, due to its antiapoptotic and insulin-like properties (Inzucchi and McGuire 2008). In fact, one study confirmed that GLP-1 enhances phosphatydil inositol 3 kinase (pI3K), which plays a key role in activating the antiapoptotic pathway and promotes cardio-protection in the ischemic rat hearts. Therefore, a direct effect of GLP-1 against apoptosis in cardiac cells is possible (Bose et al. 2005). The signaling pathway for GLP-1 receptor is sill poorly defined inspite of the findings that the receptor ligand complex in this instance conveys its action through the activation of G-proteins mimicking the action of ligand receptor complexes such as insulin activity through tyrosine kinase. This similarity results in a sort of cross talk between these complexes. We studied the effects of one of the GLP-1 analogues (exendin-4) and of one of the

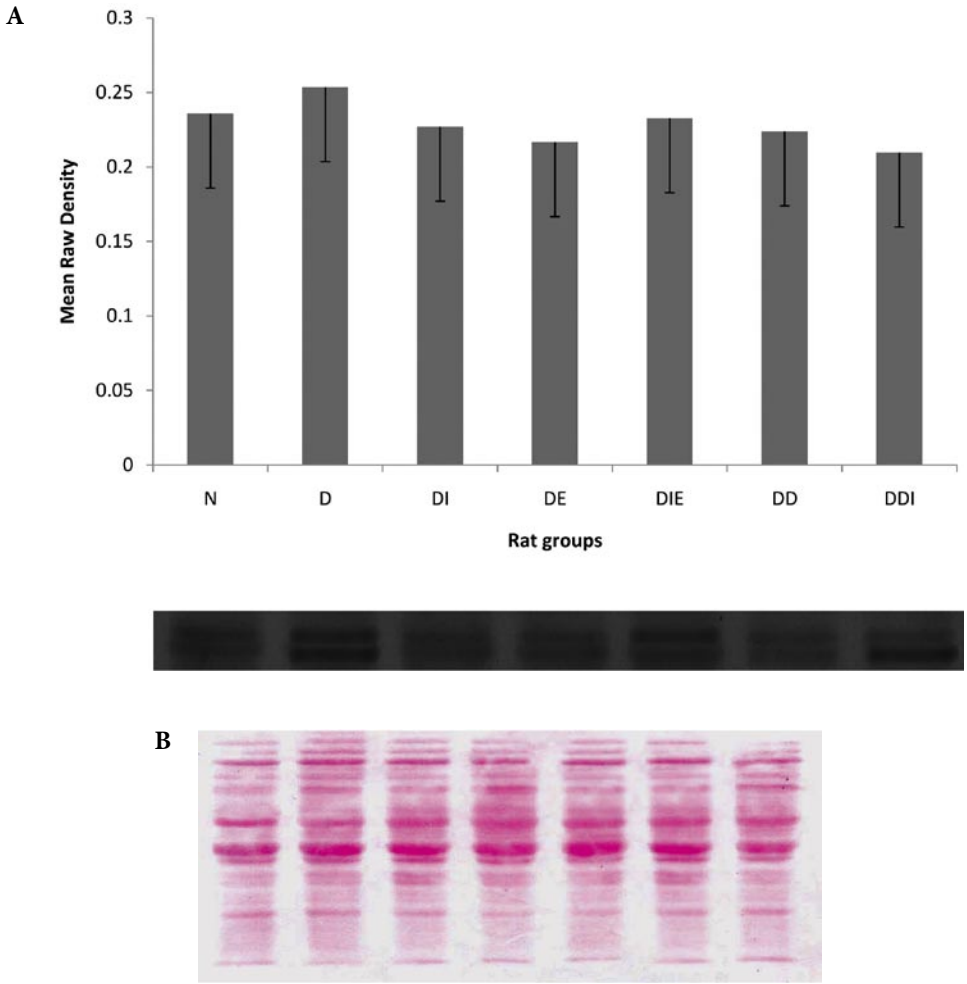
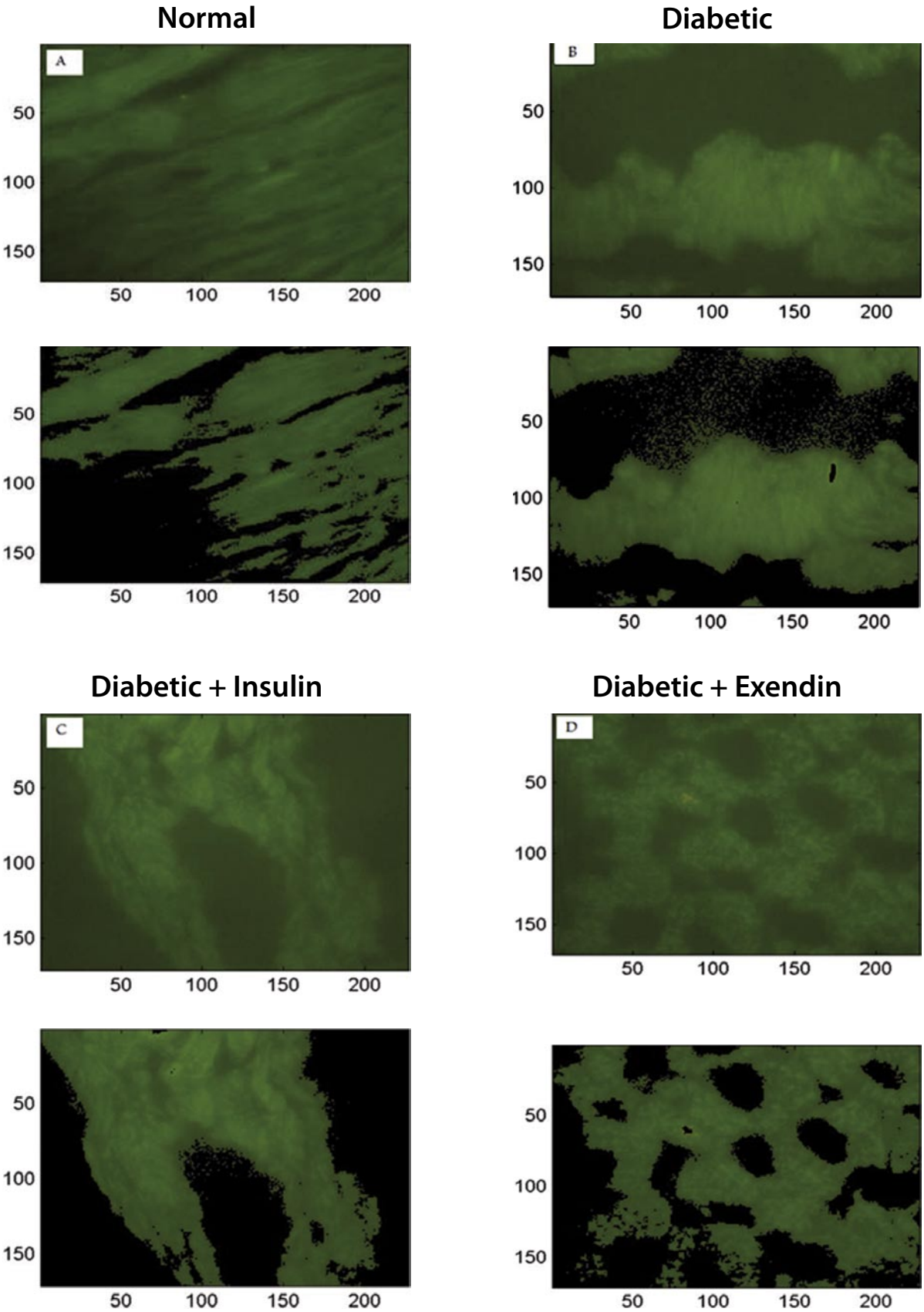
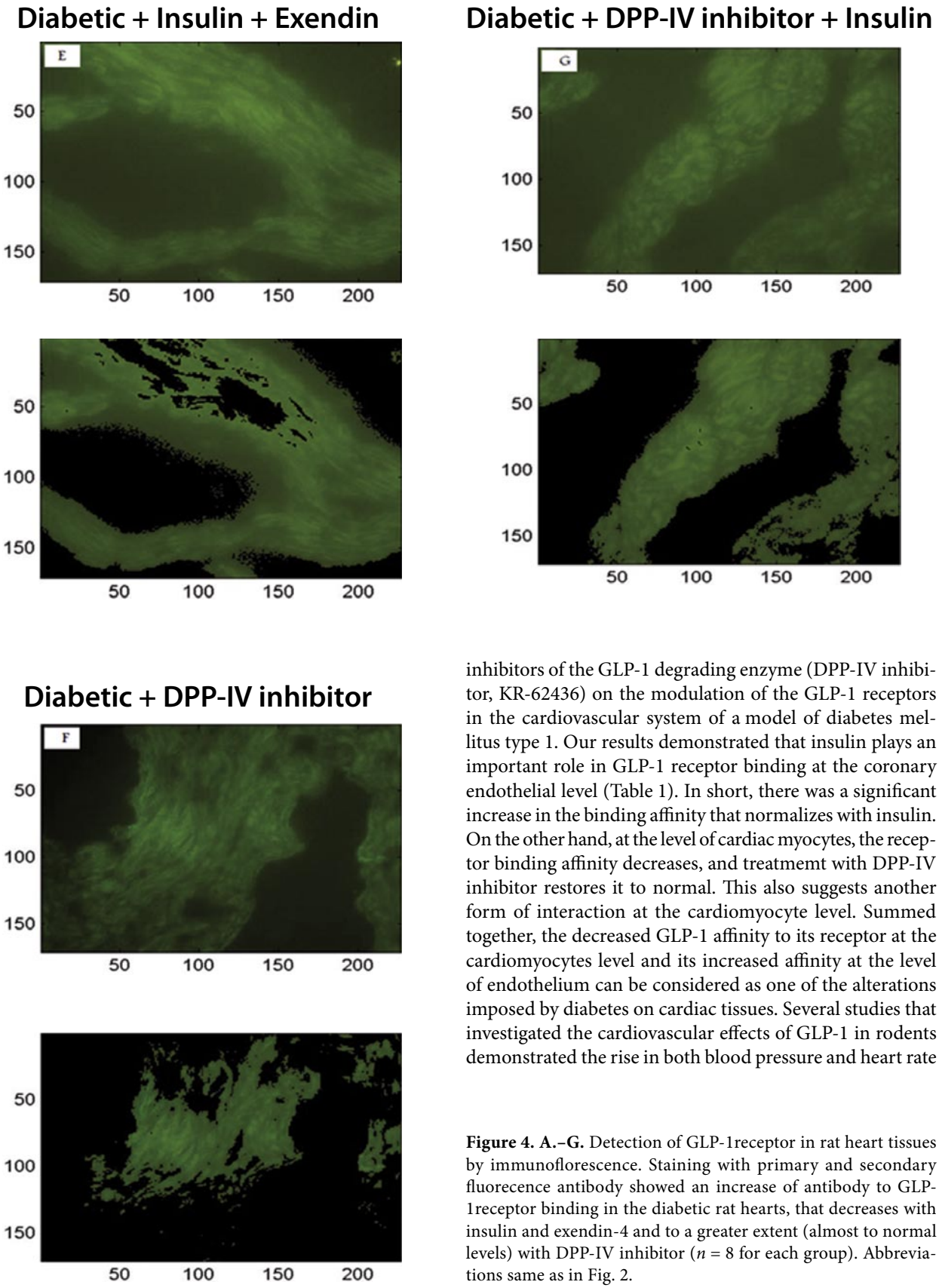


Figure 3. GLP-1 receptor expression in rat heart tissues. There is no significant difference in the expression of GLP-1receptor (molecular weight 56 kD) among the different groups ($p = 0.88$) (A). Nitrocellulose membranes stained with ponceau red showing equal protein loading (B) ($n = 8$ for each group). Abbreviations same as in Fig. 2.


Normal
Diabetic
A
B
Diabetic + Insulin
Diabetic + Exendin
C
D
50
100
150
200



inhibitors of the GLP-1 degrading enzyme (DPP-IV inhibitor, KR-62436) on the modulation of the GLP-1 receptors in the cardiovascular system of a model of diabetes mellitus type 1. Our results demonstrated that insulin plays an important role in GLP-1 receptor binding at the coronary endothelial level (Table 1). In short, there was a significant increase in the binding affinity that normalizes with insulin. On the other hand, at the level of cardiac myocytes, the receptor binding affinity decreases, and treatmemt with DPP-IV inhibitor restores it to normal. This also suggests another form of interaction at the cardiomyocyte level. Summed together, the decreased GLP-1 affinity to its receptor at the cardiomyocytes level and its increased affinity at the level of endothelium can be considered as one of the alterations imposed by diabetes on cardiac tissues. Several studies that investigated the cardiovascular effects of GLP-1 in rodents demonstrated the rise in both blood pressure and heart rate

Figure 4. A.–G. Detection of GLP-1receptor in rat heart tissues by immunoflorescence. Staining with primary and secondary fluorecence antibody showed an increase of antibody to GLP-1receptor binding in the diabetic rat hearts, that decreases with insulin and exendin-4 and to a greater extent (almost to normal levels) with DPP-IV inhibitor (n = 8 for each group). Abbreviations same as in Fig. 2.

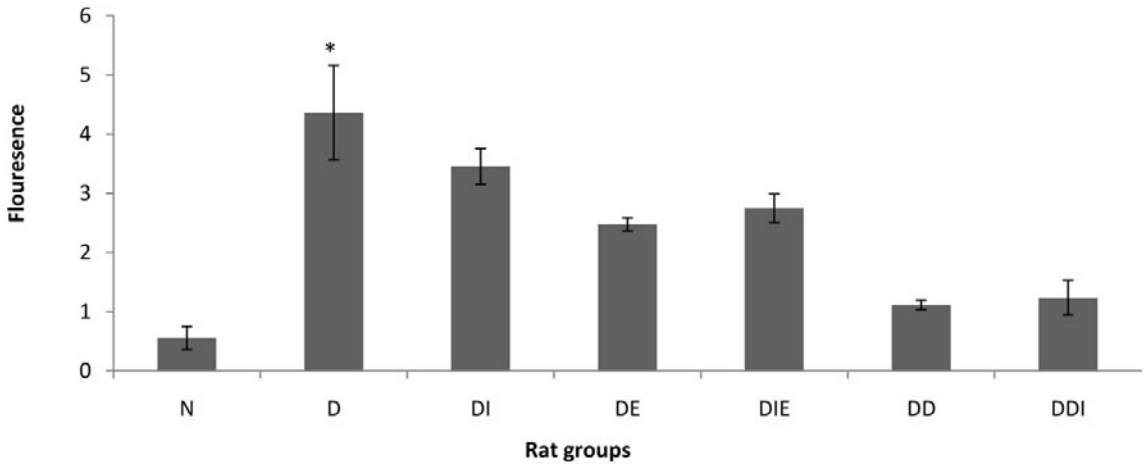


Figure 5. Quantified immunofluorescence of GLP-1 receptor binding expressed in different groups of rats ($n = 8$). There was a significance increase in the fluorescence in diabetic animals when compared to the normal. DPP-IV inhbitor was able to reduce the effect of diabetes and bring down to near normal the expression of GLP-1 receptor ($p < 0.001$). Abbreviations same as in Fig. 2.

(Vila et al. 2001) through sympatho-stimulatory effects. Furthermore, GLP-1 augments basal myocardial glucose uptake and improves left ventricular and systemic hemodynamics in conscious dogs with dilated cardiomyopathy, in the absence of significant increases in plasma insulin levels, indicating an insulinomimetic *versu*s an insulinotropic effect of GLP-1. Therefore, the enhancement in myocardial glucose uptake is independent of the increases in plasma insulin rather due to the important insulinomimetic effect of GLP-1 to stimulate glucose uptake as well as its potential to reduce plasma glucagon (Nikolaidis et al. 2005).

Thus, a possible explanation for the changes in the binding affinity is that there is an alteration in the structure of GLP-1 receptor or its shuttled-incorporation has been altered. Western blotting did not show significant change in GLP-1receptor density. There may be two possible explanations: 1) the protein extract was from both the endothelium and the cardiomyocytes, rather than separate sites, and 2) although the density may not have significantly changed, the binding affinity did, and the two terms are obviously quite different. In fact, the immunoflorescence staining did show increase in GLP-1receptor florescence in the diabetic group, probably secondary to increased binding affinty of GLP-1 to its receptor. a change in the receptor affinity without change in receptor density on the cardiomyocaytes and coronary endothelium has been described in diabetic rat models. The increase in GLP-1 systemic levels in diabetic rats is expected, and is probably due to compensatory oversecretion of this incretin hormone. It is quite interesting that in all groups, GLP-1 concentration is normalized, although one might expect an increase especially with DPP-IV inhibitor. Further investigation on the pharmacokinetics of endogenously produced GLP-1 in the various treatment modalities is needed. There is significant interaction among the GLP-1 receptor on the CE and the CM with insulin, GLP-1 analogues and DPP-IV inhibitors. GLP-1 induces myocardial glucose uptake by increasing myocardial nitric oxide (NO) generation, p38 MAP kinase activity, and GLUT-1 translocation rather than through the typical insulin-signaling cascade consisting of Akt-1 phosphorylation and GLUT-4 translocation. Previous studies have also shown that GLP-1 is associated with augmented NO production in vascular smooth muscle. It is likely that an improved glycemic control in GLP-1 treated patients may have been critical in the enhancement of function. Nevertheless, the enhancement was similar in non-diabetic subjects, indicating effects beyond the glycemic control (Sokos et al. 2006). Thus, GLP-1 and insulin have similar effects on myocardial glucose uptake although the underlying cellular mechanisms are unlike. GLP-1 proved to be crucial in the case of acute myocardial infarction. Moreover, GLP-1 is an insulinotropic and insulinomimetic peptide that ameliorates left ventricular ejection fraction (LVEF) and functional conditions in patients with systolic heart failure. The cross talk between the GLP-1 receptor and the different hormones is likely mediated through various signaling pathways. Further studies are warranted to elucidate this complex interaction. Also, while GLP-1 agonists and DPP-IV inhibitors are mainly activated in type 2 diabetic subjects in the presence of endogenous insulin, it may be interesting to see if they play the same role in animal models of type 1 diabetes with exogenous insulin as happenned in our experimental model.

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