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Glucagon-like peptide-1 relaxes rat conduit arteries via an endothelium-independent mechanism

Thomas Nyström[a,*], Adrian T. Gonon[b], Åke Sjöholm[a], John Pernow[b]

[a]*Karolinska Institutet, Department of Internal Medicine, Stockholm South Hospital SE-118 83 Stockholm, Sweden*
[b]*Karolinska Institutet, Department of Cardiology, Karolinska Hospital, SE-171 76 Stockholm Sweden*

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Abstract

A lot of interest has engendered in glucagon-like peptide-1 (GLP-1) as an emerging new drug in the treatment of type 2 diabetes. GLP-1 exerts several effects that reduce glycemia in type 2 diabetes patients. We recently also demonstrated that GLP-1 ameliorates endothelial dysfunction in type 2 diabetes mellitus patients with established coronary heart disease, suggesting a new important cardioprotective role for GLP-1. Because hypertension is overrepresented in diabetes and is adversely influencing survival, we have now investigated direct GLP-1 effects on vascular beds in a rat organ bath model. It was found that GLP-1 relaxed femoral artery rings in a dose-response manner. The relaxant effect from GLP-1 was completely inhibited by the specific GLP-1 receptor antagonist, exendin(9–39). Neither the specific nitric oxide (NO) synthase inhibitor, *N*-nitro-L-arginine, nor removing of endothelium, affected the GLP-1 relaxant effect. In conclusion, we now report a direct vascular action of GLP-1, relaxing conduit vessels independently of NO and the endothelium.

Keywords: GLP-1; Exendin; Endothelial function; Vasorelaxation; Nitric oxide; Cyclic AMP

1. Introduction

Glucagon-like peptide-1 (GLP-1) has attained much interest as an emerging new drug in the treatment of type 2 diabetes mellitus [1,2]. GLP-1 is secreted by enteroendocrine L-cells in the small intestine in response to ingestion of food and augments the insulinotropic effect of nutrients through an incretin effect [3–6]. GLP-1 stimulates insulin secretion and inhibits glucagon secretion which lowers plasma glucose levels in type 2 diabetes patients. Another important effect of GLP-1 on glycemia is through inhibiting small bowel motility, an effect that is nitric oxide (NO)-dependent in the fasted but not fed state [7]. Additional effects of GLP-1, apart from glycemic influence, have been reported and high-affinity receptors for GLP-1 are present in various tissues [8]. In the cardiovascular system, GLP-1 has been shown to increase systolic blood pressure and heart rate in rodents [9–11], to cause vasorelaxation in the pulmonary circulation in rats [12,13] and to improve severe left ventricular heart failure in human suffering from a myocardial infarction [14]. Recently, we demonstrated that GLP-1 ameliorates endothelial dysfunction in type 2 diabetes mellitus patients with established coronary heart disease, suggesting a novel and important cardioprotective effect for GLP-1 (unpublished data). One salient feature in type 2 diabetes mellitus patients is endothelial dysfunction which closely correlates to cardiovascular death [15]. Therefore, any positive influence of GLP-1 on endothelial function may prove useful in preventing premature cardiovascular disease in diabetic patients. However, the involvement of the endothelium for the effect of GLP-1 in systemic arteries remains to be determined.

In the present study, we wanted to characterize the possible direct effects of GLP-1 in the vasculature, thereby gaining mechanistic insights into the salutary actions of

* Corresponding author. Tel.: +46 861 63211; fax: +46 861 63146.
E-mail address: thomas.nystrom@sos.sll.se (T. Nyström).

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GLP-1 on endothelial function. Therefore, the aim of this study was to examine whether GLP-1 has direct vascular effects on conduit vessels and to investigate the mechanism underlying an effect including involvement of endothelium-derived NO.

2. Materials and methods

This study was approved by the regional ethics committee for animal research and conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication No. 85-23, revised 1985).

2.1. Preparation of arterial rings

Male Sprague–Dawley rats (weight 250–350 g) were anesthetized with a mixture of fluanisonum and fentanylum (Hypnorm®, Janssen, Beerse, Belgium) and midazolam (Dormicum®, Hoffman-LaRoche, Basel, Switzerland; 2.5, 0.08 and 1.25 mg/kg, respectively, i.m.). The rats were then killed by excision of the heart. The femoral arteries were carefully dissected free from surrounding tissue, removed and put in Krebs–Henseleit (KH) solution [in mmol/l: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4(H_2O)$ 1.2, $NaHCO_3$ 25.2, $CaCl_2$ 2.5 and glucose 11.1]. Circular segments (1–2 mm in length) of the artery were mounted on two thin metal holders, one of which was connected to a force displacement transducer (model FT03, Grass Instrument, Quincy, MA) and the other to a movable device that allowed the application of a passive tension of 5 mN. The tension was recorded on a polygraph (model 7B, Grass). The mounted vascular segments were kept in 2-ml organ baths containing KH solution at 37 °C and were continuously bubbled with 5% CO_2 in O_2 to maintain a pH of 7.4. After preparation, the vascular segments were allowed to equilibrate for 60 min.

2.2. Functional experiments

The contractile function of the vascular segments was tested by administration of phenylephrine (Sigma, final concentration 10^{-5} mol/l) and with K^+-rich (127 mmol/l) KH solution, prepared by replacing NaCl with equimolar amounts of KCl. Endothelium-dependent and -independent relaxations were determined by administration of acetylcholine (ACh; Sigma) and the NO donor sodium nitroprusside (SNP; Alexis, Läufelfingen, Switzerland), respectively. ACh and SNP were added to the organ baths at cumulatively increasing concentrations (10^{-9}–10^{-5} mol/l and 10^{-9}–10^{-6} mol/l, respectively) during a stable contractile tone induced by phenylephrine (10^{-5} mol/l). The relaxatory response following preincubation with a studied substance was always compared to the preceding control response in the same vascular segment.

GLP-1(7–36)amide (Neosystem, Strasbourg, France) was added to the organ baths at cumulative increasing concentrations (10^{-12}–10^{-7} mol/l) during baseline tension to evaluate contractile effects per se. Furthermore, GLP-1 (10^{-7} mol/l) was in separate experiments added ten minutes before a dose–response curve for phenylephrine to test for potentiations of phenylephrine-induced contractions. The relaxant effects of GLP-1 were evaluated by adding cumulatively increasing concentrations of GLP-1 to artery segments precontracted with phenylephrine (10^{-5} mol/l). The receptor specificity of GLP-1 was investigated by administration of the highly specific GLP-1 receptor antagonist exendin(9–39) (American Peptide, Sunnyvale, CA) 10 min before adding GLP-1.

In separate experiments, the NO synthase inhibitor *N*-nitro-L-arginine (L-NNA) (Sigma) in a final concentration of 10^{-3} mol/l was added 15 min prior to ACh to test for possible NO-dependent actions. Furthermore, in some experiments, the endothelium was removed mechanically by a very gently grip of the artery rings with a stainless forceps and then gently rolling the inner surface of the artery against the organ bath metal holders. This procedure was done under a microscope. Completeness of endothelium removal was checked by the absence of acetylcholine-induced relaxation. All substances were added in 50-μl volumes.

2.3. Statistics

Contractions of the artery segments are expressed as a percentage of K^+-induced contractions. The relaxations induced by ACh were expressed as a percentage of the precontracted tone. The precontracted level was set to 0%, and the baseline level, corresponding to maximal relaxation, to 100 %. All values were expressed as means±S.E.M. Friedman's test (ANOVA) was used to compare related samples between (conditions and concentration). Differences were deemed to be statistically significant at $P<0.05$.

3. Results

All vessels tested responded well to ACh and the NO donor SNP in a dose-dependent manner, demonstrating a valid working model for studies of endothelium-dependent and -independent relaxation effects (Fig. 1a and b). Furthermore, the NO synthase inhibitor L-NNA attenuated the relaxations induced by ACh, further demonstrating the involvement of NO in this action (Fig. 1a).

GLP-1, applied during basal tension, was without any discernable contractile effect on the femoral artery rings (data not shown). Furthermore, preincubation with GLP-1 did not affect phenylephrine-induced contraction (Fig. 2). In contrast, when GLP-1 was administered during a phenylephrine-induced contractile tone, a dose-dependent vascular relaxation of femoral artery rings was obtained

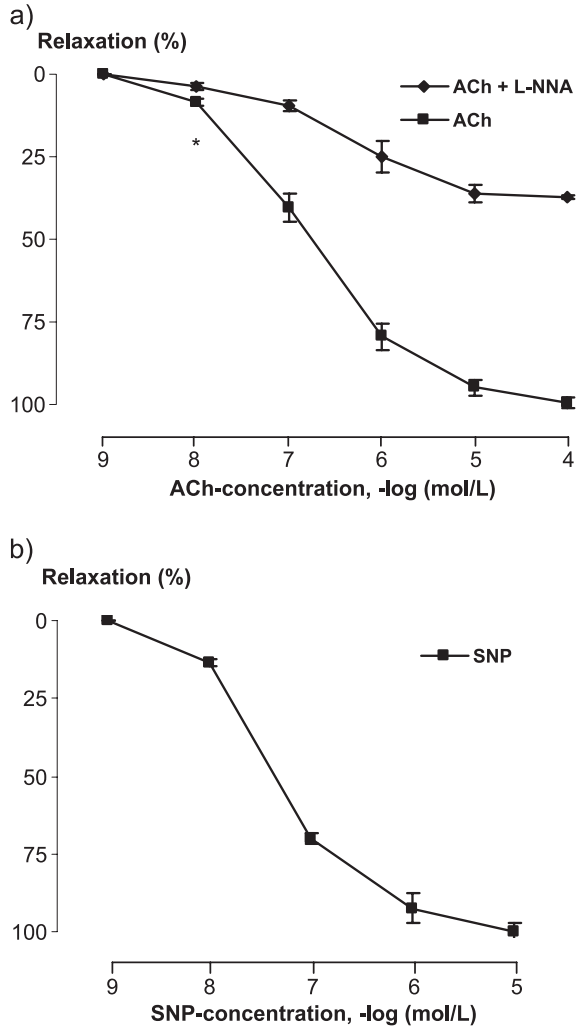

Fig. 1. Nitric oxide mediates the vasorelaxant effect of acetylcholine. (a) Relaxations induced by acetylcholine (ACh) alone (■; n=14) and in the presence of the NO synthase inhibitor N-nitro-L-arginine (L-NNA; ◆; n=8). (b) Relaxations induced by the NO donor sodium nitroprusside (SNP; ■; n=14). *P<0.05 from 10^{-8} mmol/l and onwards by ANOVA. Results are presented as mean±S.E.M.

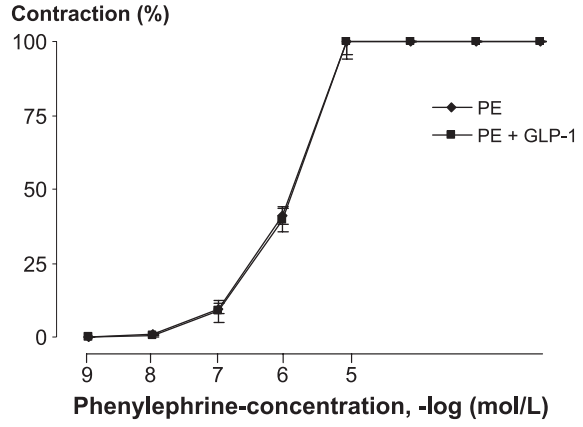

Fig. 2. Glucagon-like peptide-1 does not augment contractility by phenylephrine. Contractions induced by phenylephrine alone and following preincubation with glucagon-like peptide-1 ([GLP-1] 10^{-7} mol/l; n=8). Results are presented as mean±S.E.M.

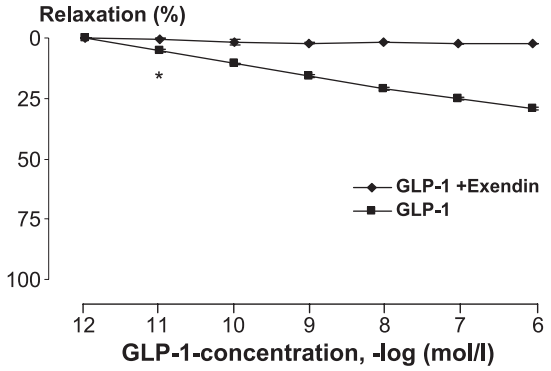

Fig. 3. Exendin(9–39) blocks vasorelaxation by glucagon-like peptide-1. Relaxations induced by glucagon-like peptide-1 (GLP-1) alone (■; n=14) and in the presence of the receptor antagonist exendin(9–39) (10^{-7} mol/l; ◆; n=6). *P<0.05 from 10^{-11} mmol/l and onwards by ANOVA. Results are presented as mean±S.E.M.

(Fig. 3). A significant relaxation was observed already at 10^{-11} mol/l. However, the maximal relaxation obtained with the highest concentration of GLP-1 was only 29% compared to 97% relaxation induced by Ach. The GLP-1 relaxation effect was completely inhibited by the specific

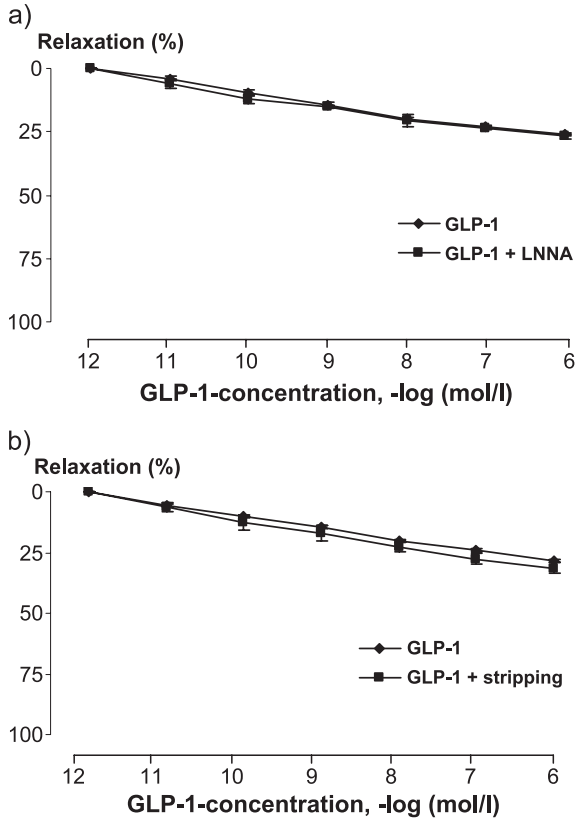

Fig. 4. Vasorelaxation by glucagon-like peptide-1 is independent of nitric oxide and endothelium removal. (a) Relaxations induced by glucagon-like peptide-1 (GLP-1) alone (◆; n=6) and in the presence of the NO synthase inhibitor N-nitro-L-arginine ([L-NNA] 10^{-3} mol/l; ■; n=6). (b) Relaxations induced by glucagon-like peptide-1 (GLP-1; ◆; n=4) and after mechanical removal of the endothelium (stripping; ■; n=8). Results are presented as mean±S.E.M.

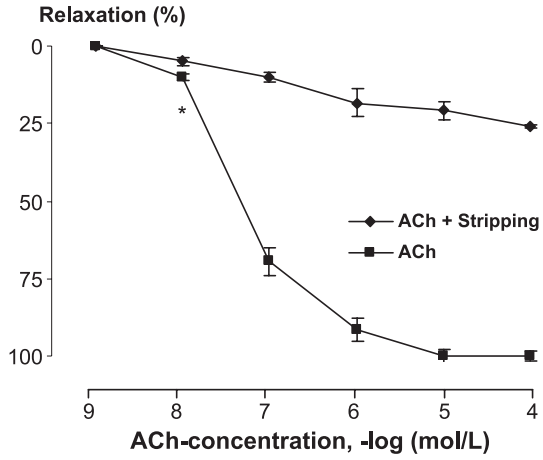

Fig. 5. Vasorelaxation by acetylcholine requires endothelium. Relaxations induced by acetylcholine (ACh) alone (♦; n=6) under control conditions and after mechanical removal of the endothelium (stripping; ■; n=6). *P<0.05 from 10^{-8} mmol/l and onwards by ANOVA. Results are presented as mean±S.E.M.

GLP-1 receptor antagonist exendin(9–39) (Fig. 3), indicating the need for specific GLP-1 receptor occupancy for this action of GLP-1. To further examine if the relaxation induced by GLP-1 was NO-dependent, the artery rings were preincubated with L-NNA at a concentration that markedly inhibited the relaxation induced by ACh. L-NNA did not attenuate the relaxation induced by GLP-1, however (Fig. 4a). Furthermore, the relaxant effect of GLP-1 remained intact also after mechanical removal of the endothelium (Fig. 4b). The successful removal of the endothelium was demonstrated by the significant attenuation of Ach-induced relaxation (Fig. 5).

4. Discussion

We show in the present study that GLP-1 potently and dose-dependently relaxes rat conduit arteries. Even if the magnitude of the is relaxant action was threefold less than that of for ACh, GLP-1 was very potent with a significant relaxation already at 10^{-11} mol/l, although at this low concentration the vasorelaxant action was modest (5 %). The specific GLP-1 receptor antagonist exendin(9–39) completely blocked the relaxant action of GLP-1, indicating the specificity of the GLP-1 relaxant effect. Furthermore, neither the specific NO synthase inhibitor L-NNA or mechanical removal of the endothelium affected the relaxant action of GLP-1, indicating that this effect is independent of NO or any other endothelium-derived substance.

The effects of GLP-1 on regulation of glucose homeostasis and glucose metabolism in diabetic patients are well documented; however, little is known about the vascular effects of the peptide. Nonetheless, any such vascular action may be of significance as both endothelial dysfunction and hypertension are frequently accompanying diabetes and adversely influencing survival of diabetic patients. Various studies have demonstrated that GLP-1 increases arterial blood pressure and heart rate in rodents [9–11]. Therefore, concerns have arisen as to the potential consequences of such effects when using GLP-1 in the treatment of type 2 diabetic patients, in whom hypertension is a salient feature [11]. However, in humans, GLP-1 tends to decrease arterial blood pressure [16]. Furthermore, in a salt-sensitive rodent model, GLP-1 treatment demonstrated antihypertensive, cardiac and renoprotective effects, as well as improvement in endothelial function [17]. Recently, a dose dependent relaxation by GLP-1 was observed in isolated perfused preparations of porcine ileal arteries [18]. The present study lends further support to a highly potent vasorelaxant effect of GLP-1 in rodents.

GLP-1 receptor expression has been shown in several organs; that is, lung tissue is abundantly expressing GLP-1 receptor in mucous glands in the trachea and on smooth muscle [8]. Richter et al. [12] demonstrated that GLP-1 receptors are located in smooth muscle of the rat pulmonary artery. They also showed that GLP-1 relaxes pulmonary artery rings in an endothelium-dependent manner [12]. An additional study demonstrated a NO-dependent relaxing effect of GLP-1 of precontracted rat pulmonary arteries [13]. Obviously, these findings are contradictory to our current results as we were unable to block the GLP-1 relaxant action with L-NNA or mechanical removal of the endothelium. The reasons for this apparent discrepancy remain elusive. One possible explanation is that involvement of NO or other endothelium-derived substances in the relaxant effect of GLP-1 differ between pulmonary and systemic arteries. Nonetheless, the fact that L-NNA or endothelial stripping markedly attenuated the relaxation induced by ACh (but not by GLP-1) in our system makes us confident that NO synthase was adequately blocked by the inhibitor and indicates that the vasorelaxant effect of GLP-1 in the rat femoral artery is NO- and endothelium-independent.

Finally, we demonstrate a complete inhibition of the relaxant effect of GLP-1 by the receptor antagonist exendin(9–39), indicating that the vasorelaxant action of GLP-1 occurs specifically through its receptor binding. To this end, very recently Nikolaidis et al. [14] demonstrated, when added to standard therapy, GLP-1 infusion improved left ventricular function in patients with AMI and severe systolic dysfunction, speculating that GLP-1 may improve endothelial function and microcirculatory integrity. As far as we know, only two other studies have directly examined GLP-1 actions on the endothelium and both of them were undertaken in pulmonary arteries addressing lung physiology issues [12,13]. Therefore, our findings are the first to show that GLP-1 directly relaxes conduit vessels in a dose-dependent manner.

In conclusion, GLP-1 induces potent relaxation of rat conduit arteries via a specific receptor independently of NO and the endothelium. This direct vascular effect of GLP-1 may be of hemodynamic importance in type 2 diabetic

patients in whom endothelial dysfunction and hypertension are both salient features that adversely affect their survival.

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