Available online at www.sciencedirect.com
ScienceDirect
Regulatory Peptides 146 (2008) 243–249
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Protective effects of GLP-1 analogues exendin-4 and GLP-1(9–36) amide against ischemia–reperfusion injury in rat heart

David P. Sonne [a], Thomas Engstrøm [b], Marek Treiman [a,*]

[a] Department of Biomedical Sciences and The Danish National Research, Foundation Centre for Heart Arrhythmia, University of Copenhagen, The Panum Institute 12.5, Blegdamsvej 3, DK-2200 Copenhagen, Denmark
[b] Cardiac Catheterization Laboratory, The Heart Centre, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

Received 28 September 2007; accepted 4 October 2007
Available online 13 October 2007

Abstract

Glucagon-Like Peptide-1 (GLP-1) is an incretin peptide secreted from intestinal L-cells, whose potent plasma glucose-lowering action has prompted intense efforts to develop GLP-1 receptor-targeting drugs for treatment of diabetic hyperglycemia. More recently, GLP-1 and its analogues have been shown to exert cardiovascular effects in a number of experimental models. Here we tested exendin-4 (Exe-4), a peptide agonist at GLP-1 receptors, and GLP-1(9–36) amide, the primary endogenous metabolite of GLP-1 (both in the concentration range 0.03–3.0 nM), for their protective effects against ischemia–reperfusion injury (IRI) in an isolated rat heart preparation. When administered, the agents were only present for the first 15 min of a 120 min reperfusion period (postconditioning protocol). Exe-4, but not GLP-1(9–36) amide, showed a strong infarct-limiting action (from 33.2% ±2.7% to 14.5% ±2.2% of the ischemic area, $p<0.05$). This infarct size-limiting effect of Exe-4 was abolished by exendin(9–39) (Exe(9–39)), a GLP-1 receptor antagonist. In contrast, both Exe-4 and GLP-1(9–36) amide were able to augment left ventricular performance (left ventricular developed pressure and rate-pressure product) during the last 60 min of reperfusion. These effects were only partially antagonized by Exe(9–39). We suggest that Exe-4, in addition to being currently exploited in treatment of diabetes, may present a suitable candidate for postconditioning trials in clinical settings of IRI. The divergent agonist effects of Exe-4 and GLP-1(9–36), along with correspondingly divergent antagonistic efficacy of Exe(9–39), seem consistent with the presence of more than one type of GLP-1 receptor in this system.

Keywords: Myocardial; Infarction; Postconditioning; Pharmacological; Cardioprotection; Ischemia

1. Introduction

Glucagon-Like Peptide-1 (GLP-1) is one of several products of posttranslational processing of the proglucagon gene transcript in the intestinal L-cells, the other products comprising glicentin, oxyntomodulin, intervening peptides and Glucagon-Like Peptide-2 [1]. Physiology and pharmacology of GLP-1 have been researched extensively, with a major emphasis on its incretin actions and consequently its application in the treatment of type-2 diabetes [2]. GLP-1 occurs in two biologically active isoforms: GLP-1(7–36) amide and a glycine-extended isoform GLP-1(7–37) [3], with the amidated peptide being the most prevalent form in man. However, both forms are rapidly degraded in circulation at the N-terminus by dipeptidyl peptidase IV (DPPIV), resulting in a short plasma half-life for the intact peptides (approximately 1–2 min) [4]. This cleavage by DPPIV generates metabolites (GLP-1(9-36) amide and GLP-1(9–37), respectively) which are unable to activate the GLP-1 receptor (GLP-1R) [5] and which lack insulinotropic activity [6].

Ischemia–reperfusion injury (IRI) of the heart refers to a range of pathophysiological phenomena spanning from reversible dysfunction to cell death, as induced by reperfusion after a period of major ischemia of an area of myocardium, such as seen when angioplasty or thrombolysis is applied for treatment of acute myocardial infarction (AMI) [7,8]. In the last two decades, a host of experimental studies have brought a

* Corresponding author. Tel.: +45 3532 7510 or +45 5120 1601; fax: +45 3532 7418.
E-mail address: M.Treiman@mfi.ku.dk (M. Treiman).

0167-0115/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2007.10.001

substantial degree of understanding of the mechanisms of cardiomyocyte death underlying IRI, and proposed some clinically interesting intervention strategies aiming to limit the extent of IRI [9]. One such strategy, termed pharmacological postconditioning, involves administration of an agent into the coronary circulation at the point of reperfusion for a limited period of time (minutes), to achieve a long-lasting attenuation of IRI [10]. In animal experiments, postconditioning with any of several agents, including insulin, bradykinin, erythropoietin [11] or GLP-1 [12,13], has been found effective. GLP-1R is widely expressed in many organs, including heart and vascular endothelium [14,15]. In isolated cardiomyocytes, GLP-1(7–36) amide has been shown to cause an increase in cAMP levels as well as a chronotropic effect [16]. In rats, heart rate and arterial pressure were augmented by GLP-1(7–36) amide [17], and structural and functional heart abnormalities were demonstrated in mice lacking the GLP-1R [18]. GLP-1(7–36) amide was found to relax femoral artery rings [19]. Infusion of GLP-1(7–36) amide in a dog model of dilated cardiac myopathy resulted in an improvement of myocardial contractility [20]. Following AMI, an increased inotropy effect was noted in patients receiving GLP-1(7–36) amide infusion for up to 3 days after successful reperfusion [21]. An improved left ventricular function was observed in patients with chronic heart failure following a 5 week continuous subcutaneous infusion of GLP-1 (7–36) [22]. Exendin-4 (Exe-4), a peptide constituent of the venom of *H. suspectum* lizard, shows a 53% amino acid homology with GLP-1 and acts as an agonist at GLP-1R [23,24]. Exe-4 is resistant to DPPIV attack, showing plasma half-life of about 26 min [25]. Exenatide, a synthetic version of Exe-4 and the active ingredient of BYETTA®, is presently the only GLP-1R agonist approved for clinical use. In view of the reported efficacy of GLP-1 in limiting infarct size [12,13,26], it seemed worthwhile to evaluate, in a rat heart ischemia–reperfusion model, whether an application of Exe-4 would be beneficial, with a view to a translational potential for treatment of IRI in a clinical setting. In addition, we wished to expand the evaluation to include any postischemic effects of Exe-4 on myocardial performance. In view of somewhat conflicting reports about the activity of the metabolite GLP-1(9–36) amide [5,6,27,28], potential infarct-limiting and myocardial performance effects of this peptide were also tested.

2. Materials and methods

2.1. Pharmacological agents and chemicals

Exendin-4 (Exe-4) and exendin(9–39) (Exe(9–39)) were purchased from Bachem AG (Switzerland). GLP-1(9–36) amide was a generous gift from Dr. Carolyn Deacon, this Institute. All other chemicals were from Sigma-Aldrich (Denmark).

2.2. Animals

Male Sprague-Dawley rats (M&B Taconic, Denmark) weighing 300–400 g were used. The animal studies were conducted in accordance with international guidelines (National Institutes of Health publication no. 85-23, revised 1985 and Danish legislation governing animal experimentation, 1987), and were carried out after permission had been granted by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

2.3. Isolated heart preparation

Rats were anesthetized by a s.c. injection (2 mL/kg body weight) of a mixture of (concentrations in mg/mL) fentanyl citrate (0.315) + fluanisone (10.0), midazolam (5.0) and sterile water at the volume ratio 1:1:2. The animals were injected with heparin (1000 IE/kg) through the femoral vein. Following tracheotomy, rats were connected to a ventilator and the respiratory rate was adjusted to obtain arterial pH 7.35–7.45. Thoracotomy was performed and access to ascending aorta obtained. The aorta was cannulated and secured by means of a suture to a steel cannula connected to a Langendorff perfusion system (ADInstruments, UK), with the flow rate set to 10–12 mL/min. The heart together with the cannulated aorta was excised, attached to the perfusion apparatus, and perfused at a constant pressure of 80 mmHg. The perfusion was with a modified Krebs–Henseleit buffer containing (concentrations in mM): $NaCl_2$ 118.5, KCl 4.7, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, $CaCl_2$ 1.2, KH_2PO_4 1.2, glucose 11.1, equilibrated with 95% O_2–5% CO_2 (pO_2 and pCO_2 550 and 40 mmHg, respectively), pH 7.4. The perfusion solution and the isolated heart were kept at 37 °C. A water/ethanol-filled latex balloon connected to a pressure transducer (ADInstruments, UK) was inserted into the left ventricle via the mitral valve for isovolumetric recordings of left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and heart rate (HR). LVEDP was adjusted to 5–8 mmHg. Myocardial contractility was assessed by the left ventricular developed pressure (LVDP), calculated as LVSP − LVEDP. In addition, the myocardial work capacity was estimated by means of the rate-pressure product (RPP = LVDP · HR). (Since no external work is done by an isovolemic Langendorff heart, RPP is a particularly good measure of the degree of myocardial recovery after ischemia as it correlates well with myocardial oxygen uptake [29]). The data was collected and analyzed by means of an analog-to-digital converter and data acquisition and analysis software (Powerlab 8/30 and ChartPro, ADInstruments, UK).

2.4. Experimental protocols

Hearts were allowed to stabilize for 40 min and subjected to 45 min of global no-flow ischemia, followed by 120 min of reperfusion (Fig. 1A). Exe-4, Exe(9–39) or GLP-1(9–36) amide were diluted to their final concentrations in the perfusion buffer from aqueous stock solutions containing 1% bovine albumin. All agents were administered for 15 min, starting at the point of reperfusion. The hearts were randomly assigned to one of the following 11 experimental groups (drug concentrations in nM): 1) no drug treatment (control); 2)–4) Exe-4 0.03–0.3–3.0, respectively; 5) Exe-4 0.03 + Exe(9–39) 5.0; 6) Exe-4 0.3 + Exe (9–39) 5.0; 7) Exe(9–39) 5.0; 8)–10) GLP-1(9–36) amide 0.03–0.3–3.0, respectively; 11) GLP-1(9–36) amide 0.03 + Exe (9–39) 5.0 (Fig. 1A).