Vasodilator Effect of Glucagon: Receptorial Crosstalk Among Glucagon, GLP-1, and Receptor for Glucagon and GLP-1

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Abstract

Glucagon is known for its insulin-antagonist effect in the blood glucose homeostasis, while it also reduces vascular resistance. The mechanism of the vasoactive effect of glucagon has not been studied before; thereby we aimed to investigate the mediators involved in the vasodilatation induced by glucagon. The vasoactive effect of glucagon, insulin, and glucagon-like peptide-1 was studied on isolated rat thoracic aortic rings using a wire myograph. To investigate the mechanism of the vasodilatation caused by glucagon, we determined the role of the receptor for glucagon and the receptor for GLP-1, and studied also the effect of various inhibitors of gasotransmitters, inhibitors of reactive oxygen species formation, NADPH oxidase, prostaglandin synthesis, protein kinases, potassium channels, and an inhibitor of the Na^+/Ca^{2+}-exchanger. Glucagon causes dose-dependent relaxation in the rat thoracic aorta, which is as potent as that of insulin but greater than that of GLP-1 (7–36) amide. Vasodilatation by GLP-1 is partially mediated by the glucagon receptor. The vasodilatation due to glucagon evokes via the glucagon-receptor, but also via the receptor for GLP-1, and it is endothelium-independent. Contribution of gasotransmitters, prostaglandins, the NADPH oxidase enzyme, free radicals, potassium channels, and the Na^+/Ca^{2+}-exchanger is also significant. Glucagon causes dose-dependent relaxation of rat thoracic aorta in vitro, via the receptor for glucagon and the receptor for GLP-1, while the vasodilatation evoked by GLP-1 also evolves partially via the receptor for glucagon, thereby, a possible crosstalk between the 2 hormones and receptors could occur.

Introduction

Glucagon, a 29 amino acid peptide hormone, produced by the α-cells of the pancreas, is mainly known for its role in the maintenance of blood glucose level, as it stimulates glycogenolysis and glyconeogenesis from pyruvate, lactate, glycerol, and some amino acids, thereby opposing the effects of insulin [1]. Various extra-hepatic effects of glucagon have been described, such as positive inotropic and chronotropic effects, while in the gastro-intestinal tract it acts as a smooth muscle relaxant, but it also affects the glomerular filtration rate, adipose tissue, thyroid gland, and the central nervous system [1,2].

The effect of glucagon is known to evolve via the G-protein coupled glucagon-receptor, through the activation of adenylyl cyclase, increasing cyclic adenosine monophosphate (cAMP) levels, as well as activating the phospholipase C (PLC)/protein kinase C (PKC) pathway [1,2]. However, besides the activation of the cAMP-dependent protein kinase A (PKA), glucagon has also been shown to activate the extracellular signal-regulated protein kinase 1/2 (ERK1/2) in a clonal cell line of human embryonic kidney cells [3]. The glucagon-induced activation of ERK 1/2 is known to be dependent on PKA activation [3].

It is well known that glucagon decreases vascular resistance in several organs, suggesting its vasodilator effect, while the mechanism of the vasodilator effect of glucagon is still unknown [4]. In strips of rabbit renal artery, the glucagon-induced vasodilatation was dose-dependently inhibited by Ca^{2+}-antagonists, suggesting that its vasodilator effect evolves via the increase of intracellular calcium levels [4]. In rat renal arteries in vivo, the vasodilator response to glucagon was shown to evoke with the contribution of nitric oxide (NO) [5].

Glucagon induces dose-dependent vasodilatation in sympathetically-innervated arterial vas-

cular bed of dog liver in vivo; however, the vasodilator potential of glucagon was shown to be less remarkable, compared to that of other hormones (secretin, prostaglandin E_2), and the onset of action was slow [6]. Glucagon decreases coronary vascular pressure in isolated dog heart [7], while in isolated rat heart it potentiates coronary reperfusion following ischemia, and increases NO production [8].

In traumatic brain injury, glucagon was shown to be protective against impaired cerebrovasodilation via the activation of the cAMP-PKA pathway [9], while besides the upregulation of cAMP, another study demonstrated that the inhibition of the ERK and mitogen activated protein kinase (MAPK) pathway by glucagon also contributes to its protective effect [10].

Despite the number of studies investigating the mechanism of the glucagon-induced decrease in vascular resistance, the precise mechanism of the vasodilatation regarding the gaseous mediators, protein kinases and ion channels, remains unclear. Here we aimed to demonstrate that glucagon induces dose-dependent vasodilatation of the isolated rat thoracic aorta, and also aimed to determine the gaseous mediators that activate the protein kinases and ion channels involved in the vasodilatation evoked by glucagon using a wire myograph. We aimed to investigate whether GLP-1 and glucagon are able to cross-activate each others' receptors and thereby also lead to vasorelaxation.

Materials and Methods

Animals

Experiments were performed with the permission of the Hungarian Local Animal Experiment Committee in accordance with the 'Principles of laboratory animal care' (NIH publication no. 85–23, revised 1985). Male Sprague-Dawley rats (Charles River Laboratories GmbH, Sulzfeld, Germany), 10–12 week old, weighing 280–340 g, were kept on standard chow ad libitum with continuous water supply. On the day of the experiment rats were killed in diethyl ether narcosis by decapitation.

Vasoreactivity experiments

The rat thoracic aorta was gently excised and placed in oxygenated (95% O_2/5% CO_2), ice-cold Krebs solution (119 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 11.1 mM glucose, 1.6 mM $CaCl_2 \cdot 2H_2O$, pH 7.4). Vessels were carefully cleaned from perivascular fat and connective tissue as described earlier [11, 12], and cut into 2 mm long segments. The vessel rings were mounted on 2 stainless steel wires (40 µm in diameter), and placed in 5 ml organ baths of a wire myograph (Danish Multimyograph Model 610M, DMT-USA Inc., Atlanta, GA, USA). Aortic rings were kept in continuously oxygenated Krebs solution (37 °C, pH 7.4) and placed under a tension of 1 g [13]. Isometric tension was continuously recorded. After 30 min rest, aortic rings were preconstricted with 100 nM epinephrine as described earlier [13, 14], which in our previously performed experiments had shown 60% contraction force of the 60 mM KCl contraction [14]. When all vessel segments had reached a stable contraction plateau, increasing doses (0.1, 1, 2.5, 10, 25 µM) of glucagon (Sigma-Aldrich, St. Louis, MO, USA) were administered to the organ baths, and relaxant responses were assessed (n = 6). The Kd50 value for the activation of the glucagon receptor is in the nanomolar range [15], while the applied dosages of glucagon were adjusted to the dose of epinephrine that we used to preconstrict the vessels. Plasma epinephrine level is approximately 30 pM at rest, while in our experiments we used 100 nM, which is a clear supraphysiological concentration [16]. Since the dose of epinephrine needed to be increased to achieve sufficient preconstriction, also the applied doses of glucagon needed to be increased accordingly.

In one set of experiments, the vasodilator efficacy of glucagon, insulin and glucagon-like peptide-1 amide fragment (7–36) [GLP-1 (7–36)] was compared (n = 4). In another experiment, the endothelium of the vessels was mechanically removed by gently rubbing a hair through it (n = 5). The effect of denudation was verified by the loss of response to 3 µM acetylcholine. A series of experiments were performed in order to identify the extracellular and intracellular mediators of the vasodilator effect of glucagon (n = 4). Prior to contracting the vessels with epinephrine, vessels were preincubated with different materials (n = 4 for each experiment). To determine whether the vasodilatation due to glucagon evoked via the glucagon-receptor, in one set of experiments vessels were preincubated with a specific glucagon receptor antagonist (hGCGR-antagonist; Sigma-Aldrich, St. Louis, MO, USA) (25 µM, 30 min) (n = 4). To investigate whether the glucagon-like peptide-1-receptor (GLP-1R) is involved in the vasodilatation evoked by glucagon, vessels were preincubated with the GLP-1R antagonist exendin (9–39) (25 µM, 30 min, n = 4). To test the hypothesis, that GLP-1 might induce vasodilatation via the glucagon-receptor, some vessels were incubated with hGCGR-antagonist (75 µM, 30 min, n = 4), prior to performing the experiment with GLP-1 (7–36).

A group of vessels were incubated with the eNOS inhibitor L-NAME (300 µM, 30 min, n = 4). Other vessels were incubated with the potent heme oxygenase inhibitor tin protoporphyrin IX dichloride (10 µM, 30 min, n = 4), others with dl-propargylglycine, inhibitor of cystathionine-γ-lyase (10 mmol/l, 30 min, n = 4), or with the relatively selective cyclooxygenase-1 (COX-1) inhibitor indomethacin (3 µM, 30 min, n = 4).

We tested the effects of superoxide dismutase (SOD; 200 U/ml, 30 min, n = 4) and catalase (1 000 U/ml, 30 min, n = 4). The contribution of the NADPH oxidase enzyme was demonstrated by inhibiting it with diphenyleneiodonium chloride (DPI) (10 µM, 30 min, n = 4). H89 hydrochloride (5 µM, 30 min, n = 4) was used to inhibit PKA and 1*H*-1,2,4-oxadiazolo(4,3-*a*)quinoxalin-1-one (ODQ, 3 µM, 30 min, n = 4) was used to inhibit the effect of soluble guanylyl cyclase (sGC).

To block the large-conductance calcium-activated potassium channels (BK_{Ca} channels) some vessels were incubated with tetraethylammonium bromide (TEA, 2 mM, 30 min, n = 4) for 30 min [15]. To block the ATP-sensitive potassium channels (K_{ATP}), we used glibenclamide (10 µM, 30 min, n = 4) [17]. KCNQ-type voltage-dependent potassium channels were blocked by incubation with XE991 (30 µM, 15 min, n = 4) [12]. The Na^+/Ca^{2+}-exchanger was blocked by incubation with its specific inhibitor SEA0400 (4 µM, 30 min, n = 4) [18].

Untreated time-control experiments were performed to exclude spontaneous vessel relaxation; however, it was not significant. To test the effect of the specific inhibitors on the permanence of the epinephrine-induced plateau, we performed a row of control experiments, and found that most of the chemicals had a slight vasodilator effect, which could not have a significant influence on the results (Effect on the epinephrine-induced plateau: untreated control: 1.02 ± 1.09%; hGCGR-antagonist: 3.56 ± 2.07%); exendin (9–39): 3.35 ± 3.07%; L-NAME: 4.72 ± 2.34%; tin protoporphyrin:

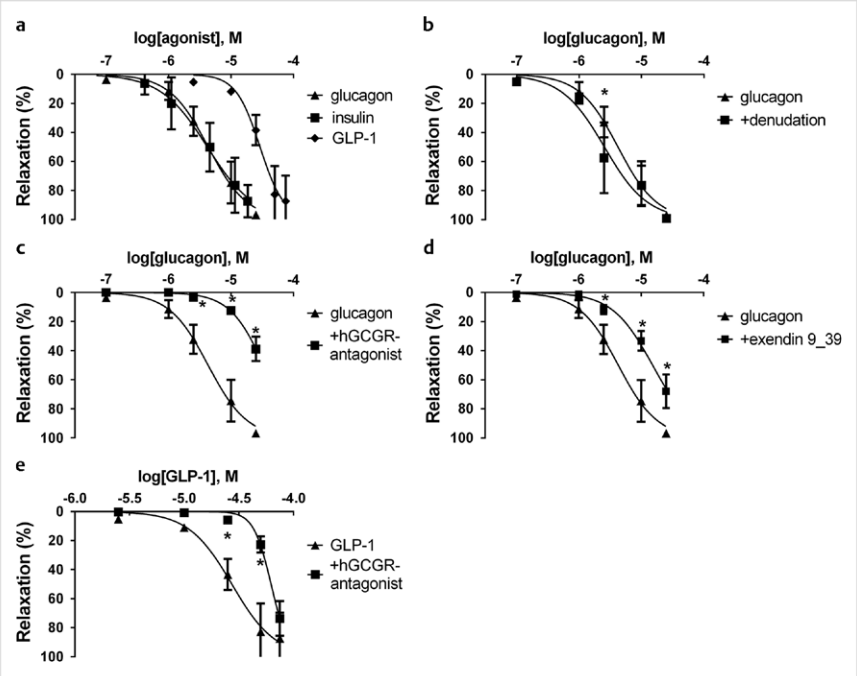

Fig. 1 Concentration-relaxation curves representing a comparison of the vasodilator potential of glucagon, insulin and GLP-1 (7–36) amide **a**. Role of the endothelium in the vasodilatation evoked by glucagon **b**. Receptors in the vasodilator effect of glucagon and GLP-1: effect of a glucagon receptor antagonist (hGCGR-antagonist) **c** and GLP-1R inhibition by the receptor antagonist exendin (9–39) **d**. Inhibition of the GLP-1 induced vasodilatation by glucagon receptor blockade **e**. n = 4, * $p < 0.01$ compared to the relaxation evoked by glucagon only (at respective concentration of glucagon).

10.28 ± 1.99 %; PPG: 5.28 ± 4.00 %; indomethacin: 10.28 ± 2.56 %; SOD: 1.78 ± 0.94 %; catalase: 1.45 ± 0.80 %; DPI: 2.15 ± 0.34 %, H89: 9.05 ± 2.82 %; ODQ: 5.21 ± 4.34 %; TEA: 10.69 ± 4.39 %; glibenclamide 4.34 ± 1.02 %; XE991: 6.62 ± 4.50 %; SEA0400: 2.32 ± 1.64 %, data represent mean ± SD).

The effect of the inhibitors on the epinephrine-induced contraction was also studied; however, no statistical significance could be shown. We compared the effect of inhibitors on the magnitude of the epinephrine-induced contraction with the magnitude of the epinephrine-induced contraction in the control experiments. (The level of constriction in % of maximum constriction of the vessel studied: control: 100 ± 21.32 %, hGCGR-antagonist: 109.67 ± 14.44 %); exendin (9–39): 66.13 ± 4.12 %; L-NAME: 104.03 ± 11.71 %; tin protoporphyrin: 79.16 ± 10.25 %; PPG: 90.53 ± 12.93 %; indomethacin: 97.73 ± 15.72 %; SOD: 74.32 ± 4.00 %; catalase: 61.65 ± 20.02 %; DPI 69.93 ± 6.79 %, H89: 90.2 ± 15.13 %; ODQ: 105.61 ± 12.14 %; TEA: 112.51 ± 25.39 %; glibenclamide 89.01 ± 17.68 %; XE991: 74.33 ± 9.62 %; SEA0400: 97.65 ± 25.75 %, data represent mean ± SD).

Chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA, except for tin protoporphyrin IX dichloride, which was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA); XE991 was purchased from Ascent Scientific Ltd. (Avonmouth, Bristol, UK), while epinephrine was purchased from Richter-Gedeon Hungary (Budapest, Hungary). SEA0400 was synthesized in the Institute of Pharmaceutical Chemistry, University of Szeged, Hungary by Professor Ferenc Fülöp.

Myodaq 2.01 M610 + software was used for data acquisition and display. We expressed the rate of relaxation caused by glucagon, GLP-1, and insulin as the percentage of the contraction evoked by epinephrine.

Statistical analysis

Statistical analysis was performed by using SPSS Version 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was calculated using repeated measures ANOVA with Bonferroni post-hoc test in case of the dose-response curves. Log EC50 values showed non-normal distribution, therefore, nonparametric tests were used (Kruskal-Wallis test followed by Mann-Whitney test). Values are shown as mean ± SD. A value of p less than 0.05 was considered to be significant.

Results

Glucagon induces endothelium-independent vasodilatation of the rat aorta, comparable to the vasodilatation caused by insulin and GLP-1

Glucagon caused dose-dependent vasodilatation of the rat thoracic aorta, which was as effective as the vasodilatation evoked by insulin. Glucagon and insulin proved to be more potent vasodilators in the rat thoracic aorta than native GLP-1 (7–36) (**Fig. 1a**). Log EC50 values for the glucagon-induced vasodilatation were not significantly different from that of insulin, [median (IQR) log EC50 values − 5.336 (0.27); − 5.313 (0.21); respectively, p = 0.958]; while it was significantly lower than that of GLP-1, [− 5.336 (0.27) vs. − 4.385 (0.27), p = 0.003].

Vessels with mechanically damaged endothelium showed no decrease in the vasodilator response to glucagon, moreover, the vasodilatation in endothelium-denuded vessels was more pronounced than that in endothelium-intact vessels [log EC50 values − 5.336 (0.27) vs. − 4.78 (0.21) p = 0.013] (**Fig. 1b**). However, the low number of cases limits the interpretation of these findings.

Glucagon causes vasodilatation via the receptor for glucagon and GLP-1

Inhibition of the glucagon-receptor with its antagonist significantly decreased the vasodilator response to glucagon (**Fig. 1c**). On the other hand, GLP-1R inhibition with its specific antagonist, exendin (9–39) also caused a significant reduction in the vasodilatation caused by glucagon (**Fig. 1d**). However, the effect of the glucagon receptor blocker was more pronounced than that of the GLP-1 receptor blocker.

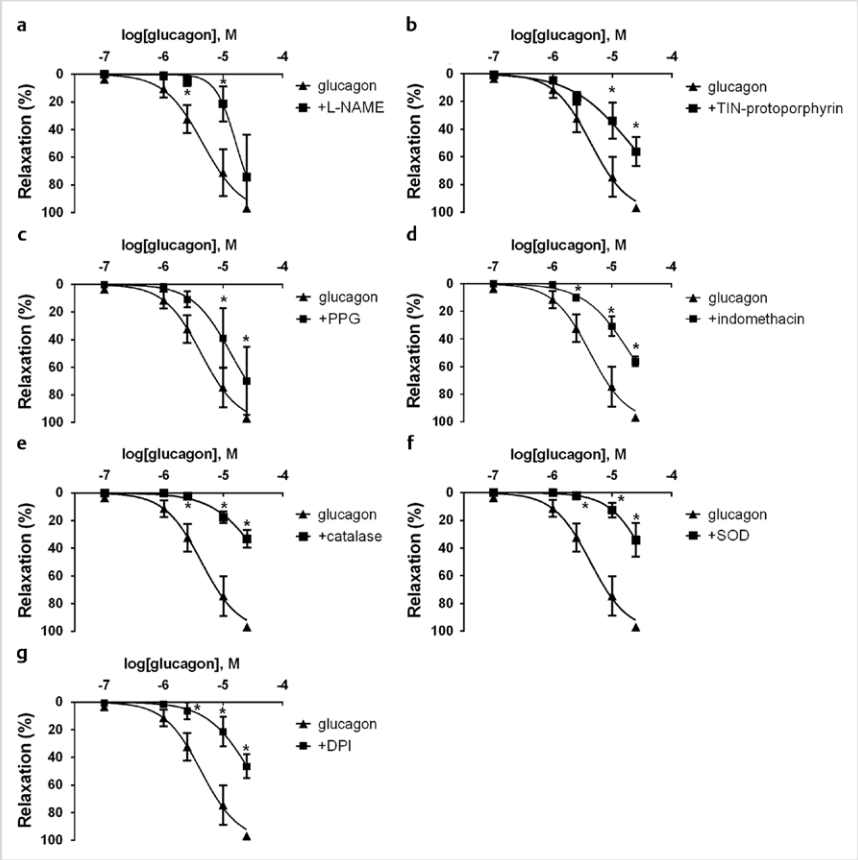

Fig. 2 Concentration-relaxation curves showing the possible role of gasotransmitters and prostaglandins in the vasodilator effect of glucagon: eNOS inhibition with N^{ω}-nitro-l-arginine methyl ester hydrochloride (L-NAME) **a**. Blocking CO production via the inhibition of the enzyme heme oxygenase with tin protoporphyrin IX dichloride **b**. Inhibition of H_2S production by inhibiting cystathionine-γ-lyase with dl-propargylglycine (PPG) **c**. Inhibition of prostaglandin synthesis with indomethacin **d**. Concentration-relaxation curves of glucagon alone and with the addition of catalase **e**, and superoxide dismutase (SOD) **f** or NADPH oxidase inhibition with diphenyleneiodonium chloride (DPI) **g**. n = 4, $p < 0.01$ compared to the relaxation evoked by glucagon only (at respective concentration of glucagon).

Glucagon-like peptide-1 causes vasodilatation via the glucagon-receptor

The concentration-dependent vasorelaxation caused by GLP-1 (7–36) amide was significantly reduced in vessels preincubated with a glucagon-receptor antagonist (hGCGR-antagonist) (**Fig. 1e**), although the glucagon receptor blocker inhibited the GLP-1-induced vasodilatation only at smaller concentrations, but it did not inhibit the vasodilatation when the highest dosage of GLP-1 was applied.

Contribution of gasotransmitters and the effect of COX-1 inhibition in the vasodilatation evoked by glucagon

Inhibition of NO production with the eNOS inhibitor L-NAME significantly inhibited vasodilatation when lower dosages of glucagon were applied, however, it had no effect when higher concentrations of glucagon were used (**Fig. 2a**). The blockade of CO formation with the heme oxygenase inhibitor tin protoporphyrin (**Fig. 2b**) and the inhibition of the H_2S generating cystathionine-γ-lyase with dl-propargylglycine (**Fig. 2c**) both significantly inhibited the vasodilator effect of glucagon. Prostaglandin synthesis inhibition with indomethacin resulted in a significantly reduced vasodilatation to glucagon (**Fig. 2d**).

Involvement of NADPH oxidase enzyme in the vasodilatation induced by glucagon

Glucagon-induced vasodilatation was significantly decreased when vessels were preincubated with superoxide dismutase (**Fig. 2e**), or catalase (**Fig. 2f**), or the NADPH oxidase inhibitor DPI (**Fig. 2g**).

Role of protein kinase G and protein kinase A in the vasodilation caused by glucagon

Soluble guanylyl cyclase inhibitor ODQ almost completely abolished the vasodilator effect of glucagon (**Fig. 3a**). Using H89, an inhibitor of PKA, the vasodilator response to glucagon significantly decreased (**Fig. 3b**).

Role of ion channels and transporters in the vasodilator effect of glucagon

Blockade of the large-conductance calcium-activated potassium channels by TEA significantly reduced the vasodilatation induced by glucagon (**Fig. 3c**). ATP-sensitive potassium channels were blocked with glibenclamide, which almost entirely abolished the vasodilatation in response to glucagon (**Fig. 3d**). KCNQ-type K_v channel inhibition by XE991 (**Fig. 3e**) also significantly reduced the vasodilator effect of glucagon.
Inhibition of the NCX with SEA0400 significantly decreased the vasodilatation evoked by glucagon (**Fig. 3f**).

Discussion

The major novel findings of this study are as follows: glucagon dose-dependently relaxes the rat thoracic aorta in vitro. The vasodilator potential of glucagon is the same as that of insulin and it is greater than that of GLP-1 (7–36) amide. The vasodilatation in response to glucagon evokes mostly via the glucagon-receptor, but it is also mediated by the GLP-1R. GLP-1 (7–36) amide also dilates the rat thoracic aorta, which is partially mediated by the glucagon receptor. According to our findings the further mediators of the vasodilatation evoked by glucagon are

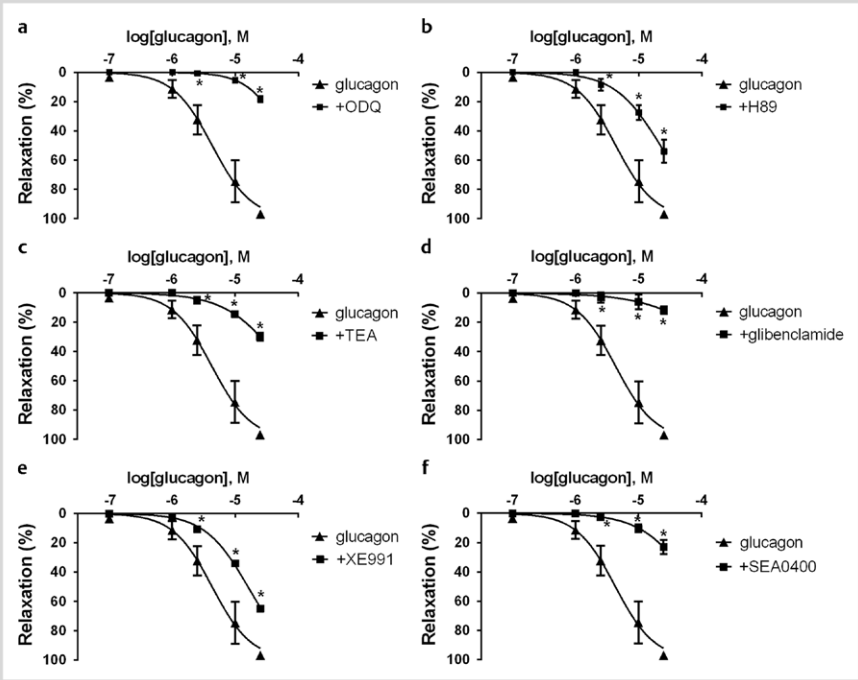

Fig. 3 Effector molecules in the vasodialatation induced by glucagon: Inhibition of soluble guanylyl cyclase with 1*H*-(1,2,4)oxadiazolo [4,3–α] quinoxalin-1-one (ODQ) **a**. cAMP-dependent protein kinase A(PKA) blockade with H89 hydrochloride **b**. Involvement of potassium channels and the Na^+/Ca^{2+}-exchanger in the vasodilator effect of glucagon: Inhibition of the large-conductance calcium-activated potassium channels with tetraethylammonium (TEA) **c**. Blockade of the ATP-sensitive potassium channels with glibenclamide **d**. KCNQ-type Kv channels were blocked by XE991 **e**. Selective inhibition of the Na^+/Ca^{2+}-exchanger with SEA0400 **f**. n = 4, * $p < 0.01$ compared to the relaxation evoked by glucagon only (at respective concentration of glucagon).

gasotransmitters, prostaglandins and free radicals, mainly H_2O_2, thereby activating the NADPH oxidase enzyme and the soluble guanylyl cyclase and PKA, resulting in the activation of potassium channels and finally the NCX, which leads to smooth muscle relaxation, hence vasodilatation (**Fig. 4**).

Decreased vascular resistance and relaxation of hepatic and other, peripheral arteries has formerly been attributed to glucagon [4–8], however, the precise description of the vasodilator mechanism of glucagon has so far not been given.

Metabolic actions of glucagon evolve via the glucagon-receptor [1], however, it has not been verified, whether its vasodilator effect is transmitted by the glucagon receptor. Here we demonstrate that glucagon induces vasodilatation via the activation of both the glucagon- and the glucagon-like peptide-1-receptor (GLP-1R). Moreover, we reveal that the glucagon-receptor is also responsible, at least partially, for the GLP-1-induced vasodilatation. According to our experiments, GLP-1 induces vasodilatation via the glucagon receptor, but at higher concentrations of GLP-1 the vasodilatation does not evoke via the glucagon receptor.

Our findings are in contrast with previous experiments, where in other cell lines GLP-1 did not cross-react with the glucagon-receptor, however, a cross-reactivity of glucagon on the GLP-1 receptor occurred only at 1000-fold higher concentrations than that of GLP-1 [19].

These 2 receptors are homologous G-protein coupled receptors [20]. A study with chimeric glucagon/GLP-1 peptides proved that the major determinant of the glucagon/GLP-1 selectivity of the receptor is the amino-terminal of the extracellular domain of the GLP-1R [20]. The homology of these receptors might be the reason for the cross-talk of the glucagon-and GLP-1-induced vasodilatation. Type 2 diabetes is commonly treated by analogues of native GLP-1 and dipeptidyl peptidase-4 (DPP-4) inhibitors, inhibitors of the enzyme degrading incretin hormones (GLP-1 and GIP), thereby elevating the level of GLP-1 [21]. As a recent study pointed out, GLP-1 agonists might also be used off-label to promote weight loss in obese patients without diabetes [22].

These drugs are also known to decrease glucagon level [21]. Alike GLP-1, its analogues also cause vasodilatation [13]. Speculatively, based on our novel findings, the drugs that increase GLP-1 level, might also induce vasodilatation via the glucagon receptor. Moreover, glucagon and its receptors have been suggested to be potential targets for the treatment of type 2 diabetes and its complications [23].

Also GLP-1 and other related peptides are known to induce vasodilatation in central as well as peripheral vessels, but the mechanism of action differs in the different parts of the arterial tree [13,24–28]. Native GLP-1 dilates rat thoracic aorta and femoral artery in an endothelium-independent manner, and at the same time independent of nitric oxide production in vitro [24,25], while in the rat pulmonary arteries the vasodilatation induced by GLP-1 is endothelium-dependent [26].

Both GLP-1R-dependent and -independent vasodilator mechanisms of GLP-1 mimetics have been described [13,28]. In $Glp1r^{-/-}$ mice, native GLP-1 reduced the ischemic damage after ischemia-reperfusion and also increased the production of cGMP, thereby leading to vasodilatation, and increased coronary flow [28]. However, the same study reported GLP-1R-dependent cardioprotective and glycemic effects of native GLP-1 amide [28]. It has also been suggested that GLP-1 peptides induce vascular relaxation in a GLP-1R-independent manner, at least in the rat aorta, independently of its well-known metabolic actions [24].

Although the presence of glucagon receptors on hepatocytes is well known, their density is increased following exercise in fasting in rats [29], and the presence of GLP-1 receptors on hepatocytes is not so evident [1,30]. Despite this fact, increasing number of evidences indicate, that among their pleotropic effects, GLP-1 analogues have a beneficial effect on liver function [31–33]. Liraglutide, a long acting GLP-1 analogue, decreases lipotoxicity as well as increases hepatic insulin sensitivity in nonalcoholic steatohepatitis (NASH) [30]. Another study also demonstrated, that liraglutide significantly improved liver function and histological features in NASH patients with glucose intolerance [32]. Liraglutide and exenatide, another GLP-1 ago-

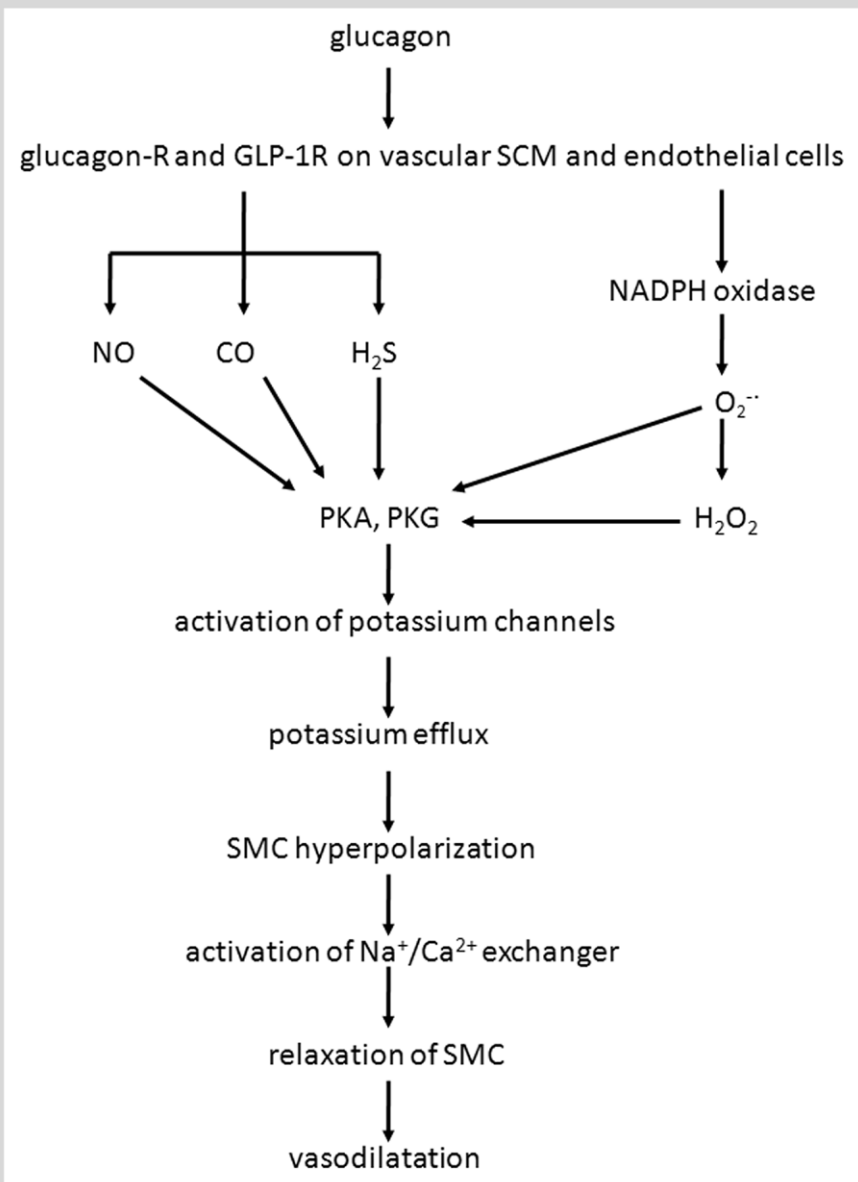

Fig. 4 Hypothetical mechanism of the vasodilation induced by glucagon. NO: Nitric oxide; H_2S: Hydrogen sulfide; CO: Carbon monoxide; $O_2^{-\bullet}$: Superoxide anion; H_2O_2: Hydrogen peroxide, PKA: cAMP-dependent protein kinase; PKG: cGMP-dependent protein kinase, SMC: Smooth muscle cell.

nist, were shown to improve transaminase levels as well as histology in patients with NASH [33]. Sitagliptin, an inhibitor of the DPP-4 enzyme, also showed improvement in transaminases [33]. The possibility that GLP-1 receptor agonists may cross-activate glucagon receptors, for example, on the hepatocytes, could explain this beneficial effect of the GLP-1 agonist drugs.

Our novel finding might be one of the underlying mechanisms explaining the pleotropic effects of GLP-1, as we have demonstrated that GLP-1 activates the glucagon-receptors as well, moreover, glucagon also acts on GLP-1 receptors. For instance, a dissociation of endocrine and metabolic effects of the Roux-en-Y gastric bypass operation was found in a mildly obese patients with type 2 diabetes, namely, when compared to the preoperative meal tolerance test, after the operation, no increase of GLP-1 and insulin secretion, but improved hepatic and peripheral insulin sensitivity were found [34].

Another pleotropic effect of GLP-1 may be the recently described stimulation of the expression of a novel insulin-mimetic adipocytokine, visfatin, via the PKA pathway, which might also influence glucose metabolism [35].

Insulin was also previously shown to induce vasodilatation via increasing NO production through the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway [36, 37]. However, insulin was also shown to cause vasoconstriction via the MAPK pathway via inducing the production of endothelin-1 [38]. A pathway-specific impairment in phosphatidylinositol 3-kinase-dependent signaling is present in insulin resistance, thereby contributing to the endothelial dysfunction [38].

Similar to the signal transduction of insulin, PI3K/Akt is also involved in the effect of glucagon together with the cAMP/PKA, PLC/PKC and ERK, MAPK pathways [3]. We demonstrated the role of PKA and the sGC-cGMP-protein kinase G pathway in the vasodilatation induced by glucagon.

The role of NO and prostaglandins in the glucagon-induced vasodilatation have previously been presented [5], but we went further and reveal that all of the 3 gasotransmitters, NO, hydrogen sulfide (H_2S), and carbon monoxide (CO), prostaglandins and reactive oxygen species (ROS)-superoxide anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) are parts of the vasodilatation induced by glucagon. Production of ROS is mediated by the NADPH oxi-

dase, while these products may activate the sGC-cGMP-PKG pathway, leading to vasodilatation.

Potassium channels are frequently targets of gasotransmitters, thereby leading to vasodilatation [17]. Glucagon activates the ATP-sensitive – the large-conductance calcium –activated and the KCNQ-type voltage-gated potassium channels and causes vasorelaxation.

The terminal effector of the glucagon-induced vasodilatation according to our experiments is the Na^+/Ca^{2+}-exchanger, which is a transmembrane protein, regularly extruding calcium with a simultaneous entry of sodium into the cell upon repolarization [18].

Conclusions

Glucagon may activate both the glucagon- and the GLP-1-receptor, thereby leading to dose-dependent, endothelium-independent vasodilatation with the contribution of the NADPH oxidase enzyme, free radicals, gasotrasmitters, prostaglandins, PKA, sGC, potassium channels, and finally the NCX.

Limitations of our study are the use of a single methodology (myography) and the absence of in vivo experiments, and the specificity of the inhibitors.

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Conflict of Interest

The authors declare no conflict of interest.

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