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Glucagon and GLP-1 exhibit no synergistic enhancement of glucose-stimulated insulin secretion in mice

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ABSTRACT

The combination of glucagon and glucagon-like peptide-1 (GLP-1) has been suggested as an approach to target obesity, since the two hormones have complementary action on body weight. We examined whether complementary action of the two hormones also exist on insulin secretion. Female C57BL/6 mice were injected intravenously with glucose with or without GLP-1, glucagon or the combination of GLP-1 and glucagon at three different dose levels. Furthermore, freshly isolated mouse islets were incubated for 30 min in the presence of 2.8, 11.1 or 16.7 mmol/l glucose or with 11.1 mmol/l glucose in the presence of 100 nmol/l glucagon and/or GLP-1. It was found that at 1 min after glucose injection alone, insulin rose to a peak level and this peak, as well as the 50 min area under the insulin curve (AUC insulin) were dose-dependently augmented by GLP-1 and glucagon. However, peak insulin with the two hormones together (with glucose) was not higher than after either single administration at any of the tested doses, i.e., no additive of synergistic action was observed by the combination on glucose-stimulated insulin secretion. Similar results were observed when calculating insulin for the whole test period. Also in vitro, both glucagon and GLP-1 augmented insulin secretion; however, there was no difference between the combined stimulation of insulin secretion by GLP-1 and glucagon together compared with either hormone alone. Insulin sensitivity did not exhibit significant changes from the glucose only condition.

We conclude that the acute combined administration of the strongly insulinotropic GLP-1 and glucagon, both in vivo and in vitro, did not induce any additive or synergistic action on glucose-stimulated insulin secretion. This shows that the risk of a marked insulinotropic action when the two compounds are given together most likely does not result in increased risk of hypoglycemia.

1. Introduction

The combination of glucagon and glucagon-like peptide-1 (GLP-1) has been suggested as a novel approach to target obesity [1]. The rationale behind this combination is that the two hormones have complementary action on the complex regulation of body weight balance. Thus, whereas glucagon stimulates energy expenditure [2], GLP-1 induces satiety with reduced food intake [3] and when given together there is a strong reduction in food intake and stimulation of energy expenditure [4,5]. In contrast, the two hormones have dissociated effects on circulating glucose, because glucagon stimulates hepatic glucose production through stimulation both of gluconeogenesis and glycogenolysis in the liver, which raises circulating glucose [6]. On the other hand, GLP-1 inhibits hepatic glucose production through inhibition of glucagon secretion from the pancreatic alpha-cells, which reduces circulating glucose [7]. The combination of the two hormones in a therapeutic strategy would therefore be expected to be glucose-neutral since the two hormones may balance the effect of each other. Both glucagon and GLP-1 activate specific G-protein coupled receptors on beta cells, which results in activation of adenylate cyclase and subsequent stimulation of insulin secretion [8,9]. Furthermore, the two hormones have different half-lives which is short for GLP-1 and longer for glucagon [10,11], which might also affect a combination effect. Therefore, a combined effect of the two hormones might be additive or synergistic on insulin secretion, tentatively resulting in hypoglycemia when the two hormones are concomitantly administered. However, it is not known whether there is an additive or synergistic effect on beta cell function when the two hormones are given together. The present study therefore examined the effect

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on insulin secretion and glucose metabolism of their combination both under in vivo and in vitro conditions in model experiments in mice.

2. Materials and methods

2.1. Animals and experimental protocols

A total of 221 experiments were performed. Female C57BL/6 mice (Taconic, Skensved, Denmark) were 4 weeks old on arrival. After 8 weeks of feeding with a standard rodent diet with 10% of energy from fat (D12450B, Research Diets, New Brunswick, NJ, USA), yielding a body weight of 23 ± 2(SD) (g/mouse), they were intravenously injected with glucose (35 mg), glucagon and/or GLP-1 at three different dose combination (termed low, medium and high). The exact doses for the groups of experiments (N = 188) are reported in Table 1. In an additional series of experiments (N = 33), glucagon and GLP-1 were given at the respective high doses alone or in combination, but without glucose.

The mice were anaesthetized with an intraperitoneal injection of midazolam (0.4 mg/mouse, Dormicum; Hoffman–La Roche, Basel, Switzerland) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (0.02 mg/mouse, Hypnorm; Janssen, Beerse, Belgium). A basal blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinised tubes containing the protease inhibitor aprotinin (Trasylol, 500 KIE/ml; Bayer, Leverkusen, Germany), followed by rapid intravenous injection (3 sec) of the compounds into a tail vein (total volume load 10 μl/g of body weight). Additional samples were taken at 1, 5, 10, 20 and 50 min. Serial blood samples (75 μl each) were taken from the retrobulbar plexus. Plasma samples were immediately obtained by separation with centrifugation and stored at −20 °C until analysis.

2.2. Islet studies

Mouse islets were isolated by collagenase digestion and hand-picked in an inverted microscope. Batches of freshly isolated islets were pre-incubated in HEPES balanced salt solution containing 125 mmol/l $MgCl_2$, 25 mmol/l HEPES (pH 7.4), 5.6 mmol/l glucose and 0.1% fatty acid free BSA (Boehringer Mannheim, Germany) for 60 min. Thereafter, islets in groups of three were incubation for 30 min in 200 μl of the buffer described above with 2.8, 11.1 or 16.7 mmol/l glucose or with 11.1 mmol/l glucose in the presence of 100 nmol/l glucagon or GLP-1.

2.3. Assays

Insulin in plasma or medium was measured by ELISA (Mercodia, Uppsala, Sweden). The intra-assay CV of the method is 4% at both low and high levels, while the inter-assay CV is 5% at both low and high levels. The lower limit of quantification of the assay is 6 pmol/l. Plasma glucose concentrations were determined using the glucose oxidase method. The animal studies were approved by the regional ethics committee in Lund, Sweden.

2.4. Data analysis

Insulin peak, evaluated as the hormone concentration at the 1-min sample, represents the early phase insulin response, while the total area under the insulin curve ($AUC_{insulin}$) describes the total insulin release. Incremental AUC (ΔAUC) is the area over the basal level. Beta cell function was evaluated as $AUC_{insulin}$ divided by $AUC_{glucose}$.

The net glucose elimination rate after the glucose injection (K_G, the glucose tolerance index) was calculated as the slope for the

Table 1
Insulin peak concentration (at 1 min sample), suprabasal AUC for insulin and glucose concentrations and beta cell function (BCF) when GLP-1 and glucagon are given together with glucose at low, medium and high doses. Glucose dose is in every experiment of 35 mg.

	Peptide dose nmol/kg	N	Peak insulin pmol/l	p[a]	$\Delta AUC_{insulin}$ min pmol/l	p[a]	$\Delta AUC_{glucose}$ min mmol/l	p[a]	BCF $mmol_{insulin}/mol_{glucose}$	p[a]
Glucose only		54	967 ± 57		2893 ± 600		127.2 ± 11.8		0.023 ± 0.001	
Glucose + GLP-1	0.01	22	1591 ± 93	<0.0001	1284 ± 1145	0.18	38.3 ± 14.3	0.0001	0.029 ± 0.002	0.007
Glucose + glucagon	0.1	20	987 ± 132	0.876	5117 ± 1017	0.06	106.8 ± 17.0	0.36	0.025 ± 0.002	0.410
Glucose + GLP-1 + glucagon	0.01 + 0.1	8	1361 ± 93	0.013	5298 ± 1241	0.147	113.3 ± 9.8	0.66	0.035 ± 0.002	0.002
Glucose + GLP-1	0.3	6	2070 ± 281	<0.0001	7826 ± 1995	0.013	119.4 ± 35.8	0.84	0.030 ± 0.002	0.106
Glucose + glucagon	1.0	5	1609 ± 135	0.0015	3370 ± 1171	0.81	207.9 ± 41.4	0.053	0.014 ± 0.001	0.092
Glucose + GLP-1 + glucagon	0.3 + 1.0	12	2260 ± 136	<0.0001	6194 ± 801	0.016	80.0 ± 15.2	0.076	0.036 ± 0.002	<0.0001
Glucose + GLP-1	3.0	31	1853 ± 213	<0.0001	8449 ± 1078	<0.0001	62.8 ± 13.7	0.0009	0.034 ± 0.004	0.001
Glucose + glucagon	10.0	14	2723 ± 348	<0.0001	11118 ± 1616	<0.0001	176.2 ± 15.6	0.051	0.029 ± 0.003	0.065
Glucose + GLP-1 + glucagon	3.0 + 10.0	16	2427 ± 465	<0.0001	9286 ± 2768	0.0009	161.9 ± 21.3	0.163	0.033 ± 0.007	0.015

[a] Comparison with glucose only.