The insulin response to gastric glucose is reduced in PAC1 and GRP receptor gene deleted mice (MS#NM/2005/000166)

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Abstract

Background and aims: Islet function is regulated by the islet autonomic nerves. These nerves harbour not only the classical neurotransmitters, acetyl choline and noradrenaline, but also neuropeptides. This study examined whether the neuropeptides, pituitary adenylate cyclase activating polypeptide (PACAP) and gastrin releasing polypeptide (GRP) contribute to the regulation of insulin secretion in model experiments by using receptor gene deleted mice.

Methods: Anesthetized mice with genetic deletion of one of the PACAP receptors (PAC1 receptors) or one of the GRP receptors (GRP receptor) or their wildtype counterparts were given glucose through a gastric gavage (150 mg) or intravenously (0.25, 0.50 or 1g/kg). Blood samples were taken regularly during the following 120 min (after gastric glucose) or at 1 min (after intravenous glucose) for analysis of glucose and insulin.

Results: The insulin response to gastric glucose was suppressed by 66% in PAC1 receptor gene deleted mice in association with impaired glucose elimination, whereas the insulin response to intravenous glucose was impaired by 36% only. The insulin response to glucose was suppressed in GRP receptor gene deleted mice by 24% together with impaired glucose elimination, whereas the insulin response to intravenous glucose was not significantly suppressed.

Conclusions: The insulin responses to gastric versus intravenous glucose in receptor gene deleted mice show that PACAP, and to a lesser extent GRP, contributes to the insulin response to gastric administration of glucose.

A sufficient increase in circulating insulin is a prerequisite for a normal glucose tolerance after meal ingestion. Of particular importance in this respect is the early insulin response, which occurs during the first 30 min after food intake (1). This is explained by the ability of insulin to inhibit hepatic glucose production (2); hence a low or defective early insulin response is associated with defective suppression of hepatic glucose production, which results in enhanced prandial glycemia (3). Consequently, inhibition of the prandial insulin insulin response by somatostatin has been shown to result in glucose intolerance (4). Similarly, the size of the early insulin response to oral glucose correlates to the 2hr glucose level (5).

A mechanism contributing to the early insulin response, besides the absorbed nutrients and the gastrointestinal incretin hormones, is activation of the autonomic nerves (6). The neural pathways may include 1)afferent sensory nerves activated by olfactory, visual, gustatory and oropharyngeal mechanisms and of food content in the gastrointestinal tract, 2)central integrative circuits in the ventro-medial hypothalamus and 3)efferent pathways consisting of parasympathetic nerves innervating the pancreatic islets (6,7). An important neurotransmitter in the efferent islet nerves is acetylcholine. However, also neuropeptides may be involved (6). The contribution by the neuropeptides to the insulin response to meal ingestion is, however, not known.

In this study, we examined possible contribution to the insulin response to gastric administration of glucose by two neuropeptides in model experiments in mice, pituitary adenylate cyclase activating polypeptide (PACAP) and gastrin releasing peptide (GRP). These neuropeptides are localized to parasympathetic nerve endings in the pancreas, released upon nerve activation and stimulate insulin secretion (8,9). There are three different receptor subtypes, which are activated by PACAP (PAC1 receptors, VPAC1 receptors and VPAC2 receptors) of which one, PAC1 receptors, is specific for PACAP (8). This receptor subtype is expressed in pancreatic islets and has been shown to be involved in PACAP-stimulated

insulin secretion (10,11). GRP belongs to the bombesin family of peptides, which activate at least four different receptor subtypes, of which the GRP receptor subtype shows high affinity for GRP and this receptor subtype is expressed in the pancreatic islets (12,13). In this study, to explore the potential role of PACAP and GRP for the insulin response to oral glucose, we examined the insulin response to gastric glucose gavage in mice lacking the PAC1 receptors (PAC1R) or the GRP receptors (GRPR).

Methods.

Animals. PAC1R deficient mice were generated by homologous recombination in embryonic stem cells on a 129/Sv X C57BL/6J background as previously described (11). Heterozygous mice were backcrossed for at least 10 generations with inbred 129T mice; homozygous crosses were then set up to yield homozygous mutations and wild type mice. For generation of GRPR deficient mice, male C57BL/6J mice hemizygous for the GRP receptor gene (which is located on chromosome X, 14), were generated by homologous recombination in embryonic stem cells as previously described (15). The mice and their respective wild types were fed a standard pellet diet and tap water ad libitum. The Ethics Committee at Lund University approved the study.

Experiments. In late morning after removal of food from the cages 16 hours earlier, the animals were anesthetized with an intraperitoneal injection of midazolam (Dormicum®, Hoffman-La-Roche, Basel, Switzerland, 0.2 mg/mouse) and a combination of fluanison (0.4 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm®, Janssen, Beerse, Belgium). Thirty minutes later, a gastric tube (outer diameter 1.2 mm) was inserted in the anesthetized mice, and glucose (150 mg) was instilled into the stomach. In one series of experiments, D-glucose (0.25, 0.50 or 1.0 g/kg) was injected intravenously in a tail vein. Blood samples were taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes before and after

glucose administration. Blood was kept in heparinized tubes and immediately centrifuged whereupon plasma was separated and stored at –20°C until analysis for insulin (radioimmunoassay, Linco Research, St Charles, MO) and glucose (the glucose oxidase technique).

Statistics. Data are reported as means±SEM. Insulinogenic index in the gastric glucose tolerance test was calculated by dividing the suprabasal 15 min insulin levels with the 15 min glucose levels. Insulinogenic index in the intravenous glucose tolerance test was calculated by dividing the suprabasal acute insulin response (i.e., the mean of suprabasal 1 and 5 min values) with the 1 min glucose levels. Student´s t-test was used for statistical evaluation.

Results

PACAP. The insulin response to gastric glucose was markedly suppressed in PAC1 receptor gene deleted mice, in association with impaired glucose elimination (Fig. 1). The insulinogenic index was 82.6±10 (pmol insulin/(mmol glucose) in wildtype mice versus only 28.1±4.1 (pmol insulin)/(mmol glucose) in PAC1 receptor gene deleted mice, corresponding to a reduction by 66% ($P<0.001$). Also the insulin response to iv glucose was impaired in PAC1 receptor gene deleted mice (Fig. 2). After 0.25g/kg glucose administration, the 1 min glucose levels were ≈15 mmol/l, i.e., similar to the 15 min glucose level after gastric glucose. The insulinogenic index after iv glucose at 0.25g/kg was 28.1±1.9 (pmol insulin)/(mmol glucose) in wildtype mice versus 18.2±0.9 (pmol insulin)/(mmol glucose) in PAC1 receptor gene deleted mice, corresponding to a reduction by 36% ($P<0.001$).

GRP. The insulin response to gastric glucose was slightly suppressed in GRP receptor gene deleted mice, in association with slight impairment of glucose elimination (Fig. 1). The insulinogenic index was 118.6±9.8 (pmol insulin/(mmol glucose) in wildtype mice versus

90.5±8.6 (pmol insulin)/(mmol glucose) in GRP receptor gene deleted mice, corresponding to a reduction by 24% (P=0.018). The insulin response to iv glucose was significantly enhanced in GRP receptor gene deleted mice after 1g/kg glucose administration, but not affected at lower glucose levels (Fig. 2). The insulinogenic index after iv glucose at 0.25g/kg was 38.5±2.9 (pmol insulin)/(mmol glucose) in wildtype mice versus 41.8±3.6 (pmol insulin)/(mmol glucose) in GRP receptor gene deleted mice, corresponding to a non-significant augmentation by 8.5%.

Discussion

Previous studies have reported that the insulin response to gastric glucose is impaired in mice with genetic deletion of PAC1 receptors and GRP receptors (11,16,17). This has suggested that these two neuropeptides are involved in the neural contribution of the insulin response to food ingestion (6). However, a problem with this interpretation has been that also insulin secretion directly from the beta cells is perturbed in these genetically deleted mice. Thus, both in vivo after intravenous glucose administration and in vitro in isolated islets glucose-stimulated insulin secretion is reduced in PAC1 receptor gene deleted mice (11) and augmented in GRP receptor gene deleted mice (13). The reason for these perturbations is not established. One hypothesis is that long-standing deficiency of PAC1 receptors down-regulates signalling pathways of importance for glucose to stimulate insulin secretion in the beta cells, such as cyclic AMP (11). Regarding the upregulation of insulin secretion in GRP receptor gene deleted mice, an increased sensitivity to cholinergic stimuli has been hypothesized (13). Nevertheless, and for the sake of this investigation, the perturbed islet responses after these gene deletions has complicated interpretations of results after oral glucose, since also the direct effect of glucose is altered in these mice. The present study therefore re-examined the relative contribution of the neuropeptides PACAP and GRP for the

insulin response to gastric glucose administration in mice by comparing the insulin responses to gastric glucose with that of intravenous glucose at matched glucose levels.

It was found that the early (15 min) insulin response to gastric glucose (when glucose was 15 mmol/l) was reduced by ≈75% in PAC1 receptor gene deleted mice. At a comparable glucose level after iv glucose, glucose-stimulated insulin secretion was reduced by only ≈35%. Hence, the insulin response to gastric glucose was more severely suppressed in PAC1 receptor gene deleted mice than the insulin response to intravenous glucose. This would suggest that PACAP is of larger importance for the insulin response after gut presentation of glucose than after intravenous glucose. Several mechanisms may explain this, because after gut presentation of glucose, both gut hormones and neural activity contributes to the insulin response, and PACAP nerves may be influential both for gut hormone release as well as for islet function. The detailed mechanism for this contribution by PACAP needs therefore to be established. In any case, PACAP nerve activity in the islets and/or in the gut may be suggested as important mechanisms.

Also in GRP receptor gene deleted mice the insulin response to gastric glucose was impaired. Previously, we showed that GRP receptor gene deleted mice have a reduced release of the intestinal incretin glucagon-like peptide-1 (GLP-1) (17). The rather modest suppression of the insulin response to gastric glucose in mice together with this previously reported results suggest that the intraislet nerve contribution of GRP is of lesser importance for insulin secretion under these conditions.

In conclusion, by comparing the insulin responses to gastric versus intravenous glucose in receptor gene deleted mice we suggest that PACAP, and to a lesser extent GRP, contributes to the insulin response to gastric administration of glucose.

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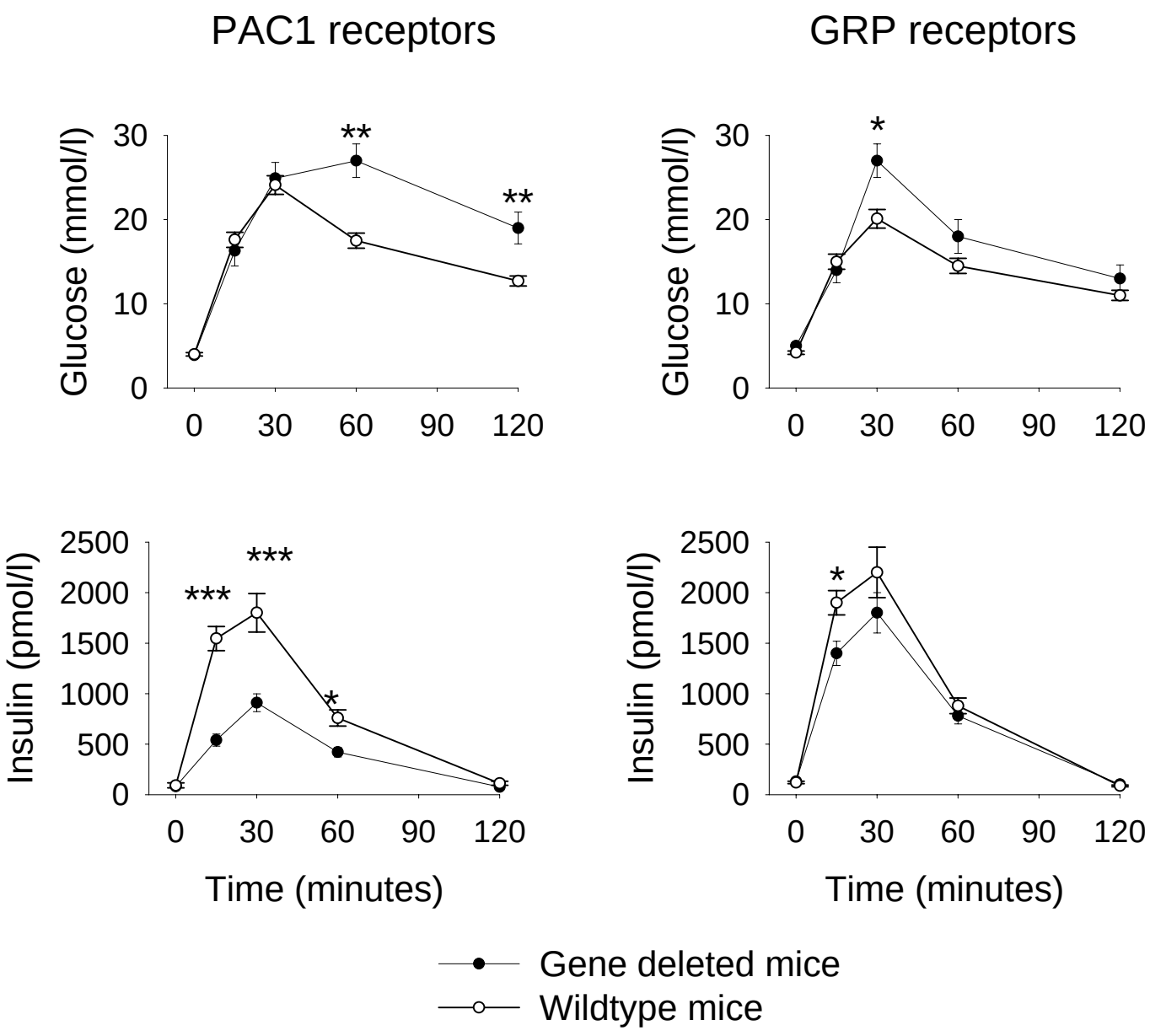
Legends to the Figures.

Fig. 1. Plasma levels of glucose and insulin before and after gastric administration of 150mg glucose to anesthetized PAC1 gene deleted mice (n=8) or GRP receptor gene deleted mice (n=8) and their respective wildtype controls (n=8 in each group). Means±SEM are shown. Asterisks indicate the probability level of random difference between the groups. *P<0.05, **P<0.01, ***P<0.001.

Fig. 2. Acute insulin response (suprabasal mean 1 and 5 min) versus the 1 min glucose level after intravenous administration of glucose (0.1, 0.25, 0.5 and 1g/kg, respectively in anesthetized PAC1 gene deleted mice or GRP receptor gene deleted mice and their respective wildtype controls (n=4-8 in each group). Means±SEM are shown.


PAC1 receptors
GRP receptors
Glucose (mmol/l)
Insulin (pmol/l)
Time (minutes)
0
10
20
30
500
1000
1500
2000
2500
0
30
60
90
120
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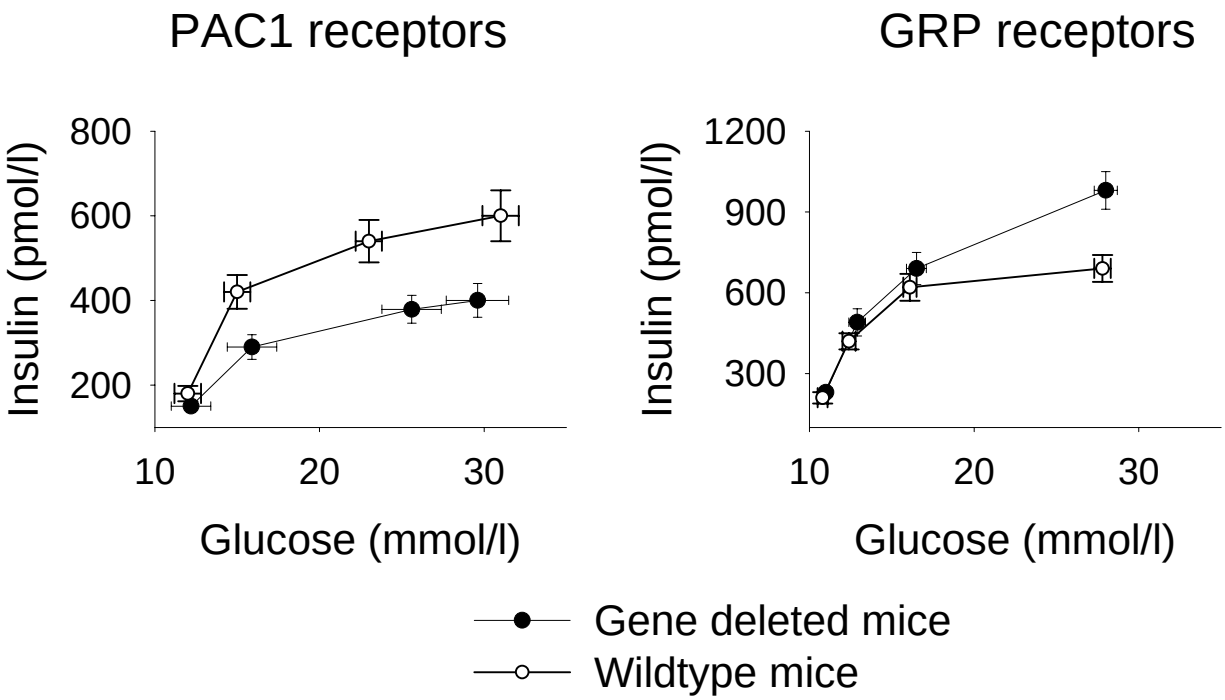
Gene deleted mice
Wildtype mice


PAC1 receptors
GRP receptors
Insulin (pmol/l)
800
600
400
200
10
20
30
Glucose (mmol/l)
1200
900
600
300
Gene deleted mice
Wildtype mice