PACAP contributes to insulin secretion after gastric glucose gavage in mice

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PACAP contributes to insulin secretion after gastric glucose gavage in mice. Am J Physiol Regulatory Integrative Comp Physiol 279: R424–R432, 2000.—Pituitary adenylate cyclase-activating polypeptide (PACAP) is localized to pancreatic ganglia governing the parasympathetic nerves, which contribute to prandial insulin secretion. We hypothesized that this contribution involves PACAP and show here that the PACAP receptor antagonist PACAP-(6–27) (1.5 nmol/kg iv) reduces the 15-min insulin response to gastric glucose (150 mg/kg) by 18% in anesthetized mice (P = 0.041). The reduced insulinemia was not due to inhibited release of the incretin factor glucagon-like peptide 1 (GLP-1) because PACAP-(6–27) enhanced the GLP-1 response to gastric glucose. Furthermore, the GLP-1 antagonist exendin-3-(9–39) (30 nmol/kg) exerted additive inhibitory effect on the insulin response when combined with PACAP-(6–27). The PACAP antagonism was specific because intravenous PACAP-(6–27) inhibited the insulin response to intravenous PACAP-27 plus glucose without affecting the insulin response to intravenous glucose alone (1 g/kg) or glucose together with other insulin secretagogues of potential incretin relevance of intestinal (GLP-1, gastric inhibitory polypeptide, cholecystokinin) and neural (vasoactive intestinal peptide, gastrin-releasing peptide, cholinergic agonism) origin. We conclude that PACAP contributes to the insulin response to gastric glucose in mice and suggest that PACAP is involved in the regulation of prandial insulin secretion.

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PACAP, PACAP-(6–27), intravenously to mice 5 min before glucose was given by gastric gavage. The insulin and glucose responses were then followed for 120 min. The specificity of PACAP-(6–27) was explored by examining its effect on the insulin response to intravenous administration of GLP-1, GIP, the COOH-terminal octapeptide of CCK (CCK-8), VIP, GRP, and the cholinergic agonist carbachol, which all stimulate insulin secretion in vivo in mice (3–5, 21, 36). We also determined the circulatory level of GLP-1 after gastric gavage in the mice to explore whether PACAP antagonism affected the in vivo release of GLP-1 from the stomach. Plasma insulin secretion in vivo in mice (3–5, 21, 36). We also determined the circulatory level of GLP-1 after gastric gavage in the mice to explore whether PACAP antagonism affected the in vivo release of GLP-1 from the stomach. PACAP-(6–27) was found to enhance GLP-1 responses to gastric glucose after intravenous saline, glucose, or PACAP-27. PACAP-(6–27) was administered alone, together with glucose and PACAP-27 (1.5 nmol/kg; a dose known to potentiate glucose-stimulated insulin secretion; 18). PACAP-(6–27) at 0.5 nmol/kg did not affect PACAP-27-induced insulin secretion (data not shown). Figure 1A shows that PACAP-(6–27) at 1.5 nmol/kg slightly increased plasma insulin at 5 min (501 ± 45 vs. baseline 225 ± 35 pmol/l, P = 0.016) and plasma glucose levels at 5 (12.1 ± 1.1 mmol/l) and 20 min (11.1 ± 0.8 mmol/l vs. baseline 9.0 ± 0.9 mmol/l, both P < 0.05) when administered alone. In contrast, the peptide did not affect the increase in plasma insulin or glucose levels induced by intravenous glucose (Fig. 1, A and B). Figure 1, C and D, shows that PACAP-(6–27) (1.5 nmol/kg) markedly reduced the insulinotropic action of PACAP-27 (1.5 nmol/kg) when given together with glucose. The AUC insulin was 37.3 ± 4.1 nmol/l in 50 min in controls and increased by PACAP-27 to 65.3 ± 7.9 nmol/l in 50 min (P < 0.001). PACAP-(6–27) reduced the AUC insulin to 46.1 ± 5.0 nmol/l in 50 min (P < 0.001 vs. administration of PACAP-27 plus glucose). PACAP-27 did not affect the glucose elimination, as known from our previous study (18), and neither did PACAP-(6–27) when administered together with PACAP-27.

METHODS

Animals. Nonfasted NMRI mice (Bomholdgaard Breeding and Research Center, Ry, Denmark), weighing 20–25 g, were used throughout the study. The animals were fed a standard pelleted diet and tap water ad libitum. The study was approved by the Animal Ethics Committee at Lund University.

Gastric glucose tolerance test. The mice were fasted overnight and anesthetized with an intraperitoneal injection of midazolam (Dormicum, Hoffman-La-Roche, Basel, Switzerland, 0.4 mg/mouse) and a combination of fentanyl (0.02 mg/mouse; Hypnorm, Janssen, Beerse, Belgium). After induction of anesthesia, synthetic PACAP-(6–27) (1.5 nmol/kg) and/or the GLP-1 receptor antagonist exendin-3-(9–39) (30 nmol/kg; all from Peninsula Laboratories Europe, Merseyside, UK) or saline was injected intravenously in a tail vein (volume load 10 μl/g body wt). After 5 min, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus, after which d-glucose (50 or 150 mg/mouse in 0.5 ml, British Drug Houses, Poole, UK) was administered through a gavage tube (OD 1.2 mm) placed in the stomach. Additional blood samples were taken either after 5, 10, 15, and 30 min or after 15, 30, 45, 60, 90, and 120 min. A total of five or seven blood samples at 75 μl were taken in each animal when determining plasma insulin and glucose concentrations. When plasma GLP-1 levels were determined, five samples at 150 μl were taken, and blood from two mice run together was pooled for each time point. The samples were taken in heparinized tubes and stored on ice. After centrifugation plasma was separated and stored at −20°C until analysis.

Intravenous glucose tolerance test. The mice were anesthetized as described above. Thereafter, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus, after which d-glucose (1 g/kg) was injected rapidly intravenously in a tail vein either alone or together with PACAP-(6–27) (0.5 or 1.5 nmol/kg), with or without addition of synthetic ovine PACAP-27 (1.5 nmol/kg), GLP-1 (10 nmol/kg), synthetic porcine GIP (10 nmol/kg), CCK-8 (6 nmol/kg), carbachol (150 nmol/kg), synthetic porcine VIP (1.5 nmol/kg), or synthetic porcine GRP (4 nmol/kg); peptides from Peninsula Laboratories Europe (Merseyside, UK), carbachol from Sigma (St. Louis, MO). The volume load was 10 μl/g body wt. Additional blood samples were taken after 1, 5, 20, and 50 min. A total of 75 μl blood was taken in each of the five blood samples. After immediate centrifugation at 4°C, plasma was separated and stored at −20°C until analysis.

Analysis. Plasma insulin was determined radioimmunologically with the use of a guinea pig anti-rat insulin antibody, 125I-labeled porcine insulin as tracer, and rat insulin as standard (Linco Research, St. Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l, and the coefficient of variation is less than 3% at both low and high levels. Plasma glucose was determined with the glucose oxidase method. Plasma GLP-1 was measured by a radioimmunoassay after extraction of plasma samples with ethanol. Sodium phosphate buffer, 400 μl 0.05 mol/l, pH 7.5, containing 6% albumin and 0.1 mol/l NaCl was added to 100 μl mouse plasma on ice and mixed well. The mixture was then extracted with 70% ethanol (vol/vol, final dilution), and after vacuum centrifugation the residue was reconstituted in assay buffer and assayed as previously described (35). The antiserum used (code no. 89390) is highly specific for COOH-terminal intestinal GLP-1 and recognizes mouse GLP-1. The sensitivity using this procedure was ~5 pmol/l, and the intra-assay coefficient of variation was about 10%. The recovery of GLP-1 added to mouse plasma was within ±20% of expected values.

Statistics. Means ± SE are shown. Area under the curve for plasma insulin levels (AUC insulin) was calculated by the trapezoidal rule. Statistical analyses were performed with the SPSS for Windows system. Statistical comparisons between groups were performed with Student’s t-test when not otherwise stated. Adjustments for multiple comparisons were made according to Bonferroni.

RESULTS

Effects of PACAP-(6–27) on circulating insulin and glucose after intravenous saline, glucose, or PACAP-27. The first series of experiments was designed to explore whether PACAP-(6–27) exerts PACAP antagonistic action in vivo in the mouse and therefore can be used as a tool for examining the physiological role of PACAP in this species. PACAP-(6–27) at 1.5 or 0.5 nmol/kg was injected intravenously alone, together with glucose (1 g/kg), or together with glucose and PACAP-27 (1.5 nmol/kg; a dose known to potentiate glucose-stimulated insulin secretion; 18). PACAP-(6–27) at 0.5 nmol/kg did not affect PACAP-27-induced insulin secretion (data not shown). Figure 1A shows that PACAP-(6–27) at 1.5 nmol/kg slightly increased plasma insulin at 5 min (501 ± 45 vs. baseline 225 ± 35 pmol/l, P = 0.016) and plasma glucose levels at 5 (12.1 ± 1.1 mmol/l) and 20 min (11.1 ± 0.8 mmol/l vs. baseline 9.0 ± 0.9 mmol/l, both P < 0.05) when administered alone. In contrast, the peptide did not affect the increase in plasma insulin or glucose levels induced by intravenous glucose (Fig. 1, A and B). Figure 1, C and D, shows that PACAP-(6–27) (1.5 nmol/kg) markedly reduced the insulinotropic action of PACAP-27 (1.5 nmol/kg) when given together with glucose. The AUC insulin was 37.3 ± 4.1 nmol/l in 50 min in controls and increased by PACAP-27 to 65.3 ± 7.9 nmol/l in 50 min (P < 0.001). PACAP-(6–27) reduced the AUC insulin to 46.1 ± 5.0 nmol/l in 50 min (P < 0.001 vs. administration of PACAP-27 plus glucose). PACAP-27 did not affect the glucose elimination, as known from our previous study (18), and neither did PACAP-(6–27) when administered together with PACAP-27.
and glucose (Fig. 1D). Therefore, PACAP-(6–27) at 1.5 nmol/kg exhibits PACAP antagonistic action with regard to the insulinotropic action of PACAP-27 without reducing glucose-stimulated insulin secretion. This antagonist was therefore used as a tool for studying the involvement of PACAP for prandial insulin secretion.

Effects of PACAP-(6–27) on circulating insulin and glucose after gastric glucose. To study whether PACAP contributes to the insulin response to gastric presentation of glucose in mice, PACAP-(6–27) was given intravenously (1.5 nmol/kg) 5 min before a gastric glucose gavage. Two series of experiments were performed to analyze the time course of the effects of glucose administration. In the first series, blood was sampled for 120 min after glucose administration, with the first sample at 15 min (Fig. 2, A and B), and in the second series sampling was performed for 30 min with the first sample taken already at 5 min after glucose gavage (Fig. 2, C and D). Gastric administration of glucose increased plasma levels of insulin and glucose with peak levels obtained after 15 and 30 min, respectively. Figure 2, A and B, shows that PACAP-(6–27) reduced the insulin response to gastric glucose gavage. The most pronounced action of PACAP-(6–27) was observed at 15 min after glucose administration. At this time point, plasma insulin was 1,060 ± 118 pmol/l in controls vs. 875 ± 121 pmol/l after administration of PACAP-(6–27) (\(P = 0.041\)). In the experiments designed for the study of the 30-min response (Fig. 2C), the AUC_{insulin} during the 30 min immediately after gastric glucose administration, which was 25.7 ± 2.9 nmol/l in 30 min in controls, was reduced to 19.6 ± 1.7 nmol/l in 30 min by PACAP-(6–27) (\(P = 0.021\)). Furthermore, also after gastric administration of glucose at 50 mg/mouse, PACAP-(6–27) (1.5 nmol/kg iv) inhibited the insulin response, since the 30-min AUC_{insulin} was 10.2 ± 1.9 nmol/l in controls vs. 6.9 ± 1.8 nmol/l after PACAP-(6–27) (\(P = 0.018\); Fig. 2, C and D).
Therefore, the results demonstrate that PACAP-(6–27) reduced the insulin response to gastric glucose in mice.

Effects of PACAP-(6–27) on circulating GLP-1 after gastric glucose. Enteral glucose causes release of GLP-1 from the gut, and GLP-1 in turn functions as an incretin to stimulate insulin secretion (2). To explore whether PACAP-(6–27) affects GLP-1 secretion, plasma levels of GLP-1 were determined. Two experimental series were undertaken, the difference again being the length of the sampling after glucose administration (120 min shown in Fig. 3A and 30 min shown in Fig. 3B). It should be emphasized that in this series of experiments, plasma from two mice were pooled at each time point to generate sufficient volume of plasma for the GLP-1 determination. Hence, the number of observations shown in Fig. 3 correlates to the number of samples in the analysis, i.e., plasma obtained from twice as many animals. Figure 3 shows that circulating GLP-1 levels increased after gastric glucose presenta-

Fig. 2. Plasma insulin and glucose immediately before and at 10, 30, 45, 60, 90, and 120 min (A and B) or immediately before and at 5, 10, 15, and 30 min (C and D) after gastric administration of glucose (150 or 50 mg/mouse) in anesthetized mice with or without an intravenous injection of PACAP-(6–27) (1.5 nmol/kg) at 5 min before glucose administration. Means ± SE are shown; n, no. of animals in the respective experimental groups. Probability level of random difference of glucose at 150 mg/mouse without vs. with PACAP-(6–27) and of glucose at 50 mg/mouse without vs. with PACAP-(6–27): *P < 0.05.

Fig. 3. Plasma insulin and glucose immediately before and at 10, 30, 45, 60, 90, and 120 min (A and B) or immediately before and at 5, 10, 15, and 30 min (C and D) after gastric administration of glucose (150 or 50 mg/mouse) in anesthetized mice with or without an intravenous injection of PACAP-(6–27) (1.5 nmol/kg) at 5 min before glucose administration. Means ± SE are shown; n, no. of animals in the respective experimental groups. Probability level of random difference of glucose at 150 mg/mouse without vs. with PACAP-(6–27) and of glucose at 50 mg/mouse without vs. with PACAP-(6–27): *P < 0.05.

Fig. 4. Plasma insulin and glucose immediately before and at 10, 30, 45, 60, 90, and 120 min (A and B) or immediately before and at 5, 10, 15, and 30 min (C and D) after gastric administration of glucose (150 or 50 mg/mouse) in anesthetized mice with or without an intravenous injection of PACAP-(6–27) (1.5 nmol/kg) at 5 min before glucose administration. Means ± SE are shown; n, no. of animals in the respective experimental groups. Probability level of random difference of glucose at 150 mg/mouse without vs. with PACAP-(6–27) and of glucose at 50 mg/mouse without vs. with PACAP-(6–27): *P < 0.05.
like PACAP-(6–27), reduced the insulin response to gastric glucose, the 30-min AUC<sub>insulin</sub> being 14.6 ± 1.7 nmol/l in 30 min in controls and 11.1 ± 0.9 nmol/l in 30 min in mice given glucose together with exendin-3-(9–39) (P = 0.037). Figure 4A also shows that when PACAP-(6–27) and exendin-3-(9–39) were combined, the insulin response to gastric glucose was markedly decreased (8.5 ± 0.7 nmol/l in 30 min, P = 0.028) compared with that after pretreatment with exendin-3-(9–39) alone. Figure 4B shows that during the 30-min study period, despite the marked reduction in insulin response, combined treatment with GLP-1 and PACAP antagonism did not have any effect on plasma glucose levels.

**DISCUSSION**

Insulin secretion after oral glucose or food intake is regulated by a multitude of factors. The rise in plasma insulin is more rapid and marked than what can be expected from a stimulation of insulin secretion solely by raising blood glucose (13). Therefore, it is thought that neural factors and intestinal hormones play a crucial role in the early phase of prandial insulin secretion (8, 13). Cholinergic nerves and the gut incretin factors GIP and GLP-1 have been considered of main importance in this respect because cholinergic antagonism (10, 41), GLP-1 antagonism (15, 43), or GIP antagonism (43) reduces the early insulin response to oral glucose in experimental studies in humans and rats. In this study, we have examined the potential contribution of PACAP to the regulation of insulin secretion following gastric presentation of glucose. We used model experiments in mice subjected to gastric glucose gavage and PACAP-(6–27) as a PACAP receptor antagonist. Our model of giving glucose through a gastric...
Table 1. Area under the insulin curve for secretagogue administration with or without PACAP-(6–27) in mice

<table>
<thead>
<tr>
<th>Secretagogue</th>
<th>Control Group Without PACAP-(6–27)</th>
<th>Group With PACAP-(6–27)</th>
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</thead>
<tbody>
<tr>
<td>GLP-1</td>
<td>118.7 ± 8.9 (8)</td>
<td>109.7 ± 12.1 (8)</td>
</tr>
<tr>
<td>CCK-8</td>
<td>36.1 ± 4.5 (8)</td>
<td>34.9 ± 4.9 (8)</td>
</tr>
<tr>
<td>GIP</td>
<td>32.8 ± 2.9 (8)</td>
<td>32.0 ± 3.0 (8)</td>
</tr>
<tr>
<td>VIP</td>
<td>23.7 ± 5.3 (6)</td>
<td>27.7 ± 10.8 (6)</td>
</tr>
<tr>
<td>GRP</td>
<td>29.8 ± 3.1 (6)</td>
<td>30.2 ± 3.6 (6)</td>
</tr>
<tr>
<td>Carbachol</td>
<td>45.6 ± 6.1 (6)</td>
<td>40.2 ± 7.6 (6)</td>
</tr>
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Values are means ± SE; numbers in parentheses are animals/group. Area under the 50-min insulin curve (AUCinsulin; nmol/l in 50 min) after intravenous administration of glucose (1 g/kg) together with glucagon-like peptide 1 (GLP-1; 10 nmol/kg), octapeptide of cholecystokinin (CCK-8; 6 nmol/kg), gastric inhibitory polypeptide (GIP; 10 nmol/kg), vasoactive intestinal peptide (VIP; 1.5 nmol/kg), gastrin-releasing peptide (GRP; 4 nmol/kg), or carbachol (150 nmol/kg) with or without concomitant administration of pituitary adenylate cyclase-activating polypeptide [PACAP–(6–27); 1.5 nmol/kg] in anesthetized mice. Samples were taken at 0, 1, 5, 20, and 50 min, and the substances were injected immediately after the 0 sample had been taken. There were no significant differences between respective groups. A control group injected with glucose alone was included in each experiment. Mean AUCinsulin for this group was 5.5 ± 1.8 nmol/l in 50 min (n = 42).

tube has been used previously for examination of glucose tolerance in mice overexpressing human islet amyloid polypeptide (6). The technique allows serial blood sampling under standardized conditions in nonstressed (anesthetized) mice. A concern is, however, that in some series up to seven blood samples are taken, which could induce hemodynamic changes resulting in sympathoadrenal reflexes influencing glucose tolerance. We have demonstrated previously that taking seven samples over 50 min did not affect circulating levels of catecholamines after injection of glucose or the circulating concentration of glucose or insulin after injection of saline (18). Therefore the hemodynamic changes induced by the sampling strategy do not seem to affect insulin or glucose levels per se.

We found that PACAP-(6–27) inhibited the insulin response to gastric glucose. This suggests that PACAP is involved in the insulin response to oral glucose. At the same time, the GLP-1 response to gastric glucose was potentiated by PACAP-(6–27), suggesting a compensation in the GLP-1 secretion when PACAP effects are antagonized. Therefore the inhibition of insulin release by PACAP antagonism seems not to be mediated by reduced GLP-1 secretion. On the contrary, the exaggerated GLP-1 response and insulinotropic action of GLP-1 might have resulted in an underestimation of the importance of PACAP for the insulin response to gastric glucose. When PACAP antagonism was combined with GLP-1 antagonism, the insulin response to gastric glucose was strikingly reduced, showing the importance of both PACAP and GLP-1.

In previous studies, NH₂-terminal truncated PACAP fragments have been shown to meet the structural requirements of the PACAP receptors without activating the receptors (37). We show here that PACAP-(6–27) abolishes the PACAP-27-induced response in insulin secretion, verifying its PACAP antagonistic action also in vivo in mice. Because a number of gut hormones and neurotransmitters might contribute to the prandial insulin response, a conclusion that PACAP is important in the regulation of insulin secretion after food intake requires that PACAP-(6–27) does not inhibit these secretagogues. Therefore, we studied the potential inhibitory effect of PACAP-(6–27) on the insulinotropic response of secretagogues of relevance for prandial insulin secretion. We studied GLP-1, CCK-8, and GIP because they are intestinal hormones released after food intake (12, 30, 32). VIP was studied because of its structural homology to PACAP (33) and because of its function as a transmitter in the parasympathetic nervous system (27). Lastly, GRP (28) and carbachol were used because GRP and acetylcholine act as neurotransmitters in the parasympathetic nervous system and are therefore important for mediating insulin release during the early phase of prandial insulin secretion. Our results show that PACAP-(6–27) fails to affect insulin secretion stimulated by these insulinotropic agents. Thus PACAP-(6–27) is a selective tool for studies of the impact of PACAP in mice. Of special interest is the failure of PACAP-(6–27) to inhibit the insulinotropic action of VIP because VIP and PACAP share two types of receptors (9), called VPAC₁ and VPAC₂. In addition, PACAP exerts effect through a third subtype of PACAP receptors, called PAC₁ receptors. The finding that PACAP-(6–27) inhibits the insulinotropic response to PACAP-27 but not that to VIP might therefore indicate that PACAP exerts its insulinotropic action in mice by activation of PAC₁ receptors. This is supported by studies demonstrating expression of this type of receptor in the endocrine pancreas (19, 44).

Under basal conditions PACAP-(6–27) seems to increase insulin secretion per se because plasma insulin levels at 5 min after administration of PACAP-(6–27) were elevated. However, because plasma glucose was elevated by PACAP-(6–27) per se, it is possible that the increase of insulinemia under these conditions is not a direct action of the antagonist on B cell function but rather an indirect action mediated by hyperglycemia, which in turn might be induced by stimulation of glucagon secretion (18).

PACAP has been localized to pancreatic nerves (19, 22). In the pig pancreas PACAP is mainly localized to parasympathetic nerves in the pancreas, and vagal nerve stimulation of the pig pancreas has been shown to stimulate the release of PACAP (42). This implies that PACAP is a neurotransmitter in the parasympathetic nervous system. The parasympathetic nervous system has been shown to mediate the early phase of insulin secretion after food intake (10). Therefore, the results in this study might suggest that neural PACAP located in parasympathetic nerve terminals is of importance for the early insulin response after food intake. However, PACAP also has been shown to be localized to capsaicin-sensitive sensory nerves both...
centrally in the superficial layer of the dorsal horn of the spinal cord (34) and peripherally throughout the digestive tract, including the pancreas (17). These nerves might also contribute to the insulinotropic action of PACAP prandially. This would imply that PACAP both as a parasympathetic and as a sensory neurotransmitter may be of importance for prandial insulin secretion. However, we cannot from our results draw any conclusion regarding the relative importance of PACAP in the parasympathetic nervous system vs. PACAP in the sensory nerves in the regulation of prandial insulin secretion.

Besides its localization to pancreatic nerves, PACAP also has been localized to nerves throughout the gastrointestinal tract (26) and has been suggested to mediate relaxation in the smooth muscles of several parts of the intestine (16, 25). Therefore, it is possible that PACAP-(6–27) inhibits the intestinal relaxation, which would lead to a stimulated bowel emptying, thus fastening glucose uptake and increasing insulin secretion. However, this is unlikely as judged from the values of plasma glucose, which suggest that the rate of increase in circulating glucose after gastric glucose is not affected by PACAP-(6–27). Instead, the present study suggests that glucose entry rate into the bloodstream does not differ between controls and after PACAP-(6–27) administration. This, in turn, implies that the direct effect on the B cells is more important for insulin secretion than any effects of PACAP via gastric emptying and glucose absorption rate.

Although gastrointestinal PACAP seems to be involved mainly in the regulation of motility, PACAP in the gastrointestinal tract might also affect the release of gastrointestinal hormones such as GLP-1, because GLP-1 secretion is known to be affected by different gastrointestinal hormones and neurotransmitters (2). Alternatively, PACAP could influence GLP-1 secretion directly. In any case, such an effect would influence insulin secretion after food intake, because GLP-1 stimulates insulin secretion (2). Under normal conditions it has been shown that GLP-1 is released into the circulation after food intake in humans (2). We show here that plasma GLP-1 levels are increased after gastric glucose presentation in mice. For the measurements, we used a COOH-terminally directed GLP-1 assay, which determines both GLP-1 and its degradation product, GLP-1-(9–36) amide (35). We also examined the GLP-1 levels after gastric glucose in the presence of PACAP-(6–27) and found that the increase in plasma GLP-1 levels after gastric glucose was potentiated by PACAP-(6–27). This suggests that PACAP may inhibit intestinal GLP-1 release and/or that GLP-1 may compensate the reduced insulinotropic action of PACAP after PACAP-(6–27). This also suggests that a compensation of the reduced insulinotropic action of PACAP after treatment with PACAP-(6–27) is an increased intestinal release of GLP-1. Hence, the contribution of PACAP to the insulinotropic action of gastric glucose might have been underestimated due to the compensatory increase in GLP-1. To examine this possibility further, we used the GLP-1 antagonist exendin-3-(9–39). Exendin-3-(9–39) is a peptide isolated from the venom of the reptile Heloderma horridum and has been found to be a potent antagonist for the GLP-1 receptor (23). The peptide has been shown previously to antagonize GLP-1-induced actions on insulin secretion in vivo in mice (7). It also has been shown previously that the peptide inhibits the insulin response to oral glucose in humans (15), which supports the role of GLP-1 as an incretin hormone. Here we verify that GLP-1 is an incretin hormone also in mice, since exendin-3-(9–39) inhibited the insulin response to gastric glucose presentation. This is in accordance with studies performed in mice lacking the GLP-1 receptor (40). We also found that exendin-3-(9–39) and PACAP-(6–27) additively inhibited the insulin response to gastric glucose. This might imply that PACAP and GLP-1 are the two major incretin factors in mice. However, previous studies have shown that the B cells require a threshold level of cAMP for normal responsiveness to glucose (39). Blockage of both PACAP and GLP-1 receptors might lower the cellular cAMP content because both peptides act through increasing intracellular cAMP (1, 14). This might also aggravate insulin secretion stimulated by glucose and perhaps also other secretagogues. The actual extent of the importance of PACAP as a mediator of prandial insulin secretion therefore cannot be established by this study.

Although insulin secretion after oral glucose was markedly reduced when PACAP and GLP-1 receptors were blocked, this did not lead to a reduced glucose tolerance within the 30-min study period because plasma glucose levels were not affected by PACAP-(6–27) together with exendin-3-(9–39) and, furthermore, the glucose tolerance was not impaired by PACAP-(6–27) alone. This may be surprising but can be explained by findings that insulin is not the only responsible factor for glucose tolerance after acute rise of circulating glucose in rodents (31).

In conclusion, we have shown that PACAP antagonism reduces the insulin response to gastric presentation of glucose in mice. This suggests that PACAP is involved in the physiological regulation of insulin secretion after food intake.

**Perspectives**

After food intake, insulin secretion is stimulated to augment the uptake of glucose in peripheral tissues. The stimulation of the B cell is elicited by nerves, hormones, and nutrients. Nerves are activated both by sensory stimuli in the oral cavity, yielding the so-called cephalic phase of prandial insulin secretion, and by food presentation in the stomach and the small intestine. These neural reflexes are executed by the parasympathetic nerves. Furthermore, gut hormones, mainly GLP-1 and GIP, are released during food intake and they potentiate glucose-stimulated insulin secretion. Finally, absorbed nutrients, for example glucose and amino acids, further stimulate the secretion of insulin. The relative contribution of these mechanisms for prandial insulin secretion has not been established.
Furthermore, the relative contribution of the parasympathetic neurotransmitters for the neural activation of insulin secretion during food intake is not known. The results of the present study suggest that PACAP is involved in the prandial insulin secretion, since a specific PACAP receptor antagonist, PACAP-(6–27), inhibited the insulin response to gastric glucose in mice. Because PACAP is a neurotransmitter in parasympathetic nerves in the pancreas, which is known to partly mediate prandial insulin secretion, the results infer involvement of these PACAP-containing nerve fibers in the prandial regulation of islet function. Now studies are required to examine the relative contribution of PACAP vs. other neurotransmitters in the islet parasympathetic nerves, i.e., VIP and acetylcholine, for prandial insulin secretion. Furthermore, studies are also required to examine the possible importance of PACAP for prandial insulin secretion in humans.

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