Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice

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Ahren, Bo. Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *Am J Physiol Regul Integr Comp Physiol* 286: R269–R272, 2004; 10.1152/ajpregu.00423.2003.—It has been hypothesized that the potent insulino- tropic action of the gut incretin hormone glucagon-like peptide-1 (GLP-1) is exerted not only through a direct action on the beta cells but may be partially dependent on sensory nerves. We therefore examined the influence of GLP-1 in mice rendered sensory denervated by neonatal administration of capsaicin performed at days 2 and 5 (50 mg/kg). Control mice were given vehicle. Results show that at 10–16 wk of age in control mice, intravenous GLP-1 at 0.1 or 10 nmol/kg augmented the insulin response to intravenous glucose (1 g/kg) in association with improved glucose elimination. In contrast, in capsaicin-pretreated mice, GLP-1 at 0.1 nmol/kg could not augment the insulin response to intravenous glucose and no effect on glucose elimination was observed. Nevertheless, at the high dose of 10 nmol/kg, GLP-1 augmented the insulin response to glucose in capsaicin-pretreated mice as efficiently as in control mice. The insulin response to GLP-1 from isolated islets was not affected by neonatal capsaicin, and, furthermore, the in vivo insulin response to glucose was augmented whereas that to arginine was not affected by capsaicin. It is concluded that GLP-1-induced insulin secretion at a low dose in mice is dependent on intact sensory nerves and therefore indirectly mediated and that this distinguishes GLP-1 from other examined insulin secretagogues.

capsaicin; glucose elimination

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is a potent insulino- tropic gut hormone, which is considered to be a main incretin factor (2, 9, 13). GLP-1 is also a potent antidiabetic peptide explored for use in the treatment of type 2 diabetes (2, 7, 12). GLP-1 signals through a classical G protein-coupled seven-transmembrane type of receptor transducing activation mainly through formation of cAMP (8.9). It is well established that the islet beta cells express GLP-1 receptors (20) and that GLP-1 clearly stimulates insulin secretion in vitro from islets (10). However, also an indirect neural effect underlying the insulino- tropic action of GLP-1 has been proposed. Thus it has been demonstrated that the augmentation of glucose-stimulated insulin secretion induced by intraportal administration of GLP-1 is abolished by ganglionic blockade in rats (6) and, furthermore, that intraportal administration of GLP-1 activates electrical activity in hepatic afferents in rats (18). GLP-1 may thus activate afferent nerve terminals, which through a neural effect stimulate insulin secretion, which may be elicited by the autonomic nerves innervating the islets (3). In this study we hypothesized that if indirect neural effects contribute to GLP-1-stimulated insulin secretion, the ability of the hormone to augment glucose-stimulated insulin secretion would be impaired after sensory deactivation because such deactivation would prevent GLP-1 from activating afferents and therefore from inducing neural effects eliciting insulin secretion. To explore this hypothesis, we used the model of neonatal administration of capsaicin to mice. Capsaicin is known to selectively destroy primary afferent nerves of the C-fiber type (14), and in previous studies we have shown that the islet responses to autonomic nerve activation are abolished in capsaicin-treated mice (16). Here, we examined whether GLP-1-induced insulin secretion is affected in such mice.

MATERIALS AND METHODS

Animals and capsaicin treatment. Female mice of the NMRI strain were obtained from Taconic (Ry, Denmark). The animals had free access to standard pellet diet and tap water before and during the experiments. Capsaicin (8-methyl-N-vallyl 6-nonenamide, 50 mg/kg, Sigma Chemical, St. Louis, MO) was given subcutaneously under buprenorphine analgesia (Temgesic, Rieckitt and Colman, Hull, UK, 0.45 mg/kg) to neonatal mice on two occasions at the ages of 2 and 5 days, as previously described (15, 16, 19). Capsaicin was dissolved in vehicle consisting of Tween 80 (10%) and ethanol (10%) in 0.9% NaCl (80%). Control animals were given buprenorphine and vehicle alone. The volume was 10 μl/g body wt. After this treatment, as previously described, body weight is slightly lower in capsaicin-treated animals, but this difference disappears after 6 wk of age (15).

Intravenous glucose tolerance tests. The experiments were performed when the mice were 10–16 wk of age. The mice were anesthetized after a 3-h fast during the late morning hours with an intraperitoneal injection of midazolam (Dormicum, Hoffman-LaRoche, Basel, Switzerland, 0.14 mg/mouse) and a combination of flumison (0.9 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm, Janssen, Beerse, Belgium). After 30 min, a blood sample (75 μl) was taken from the retrobulbar, intraorbital, capillary plexus in a 100-μl pipette that had been prerinised in heparin solution (100 U/ml in 0.9% NaCl; Lövens, Ballerud, Denmark). Thereafter, d-glucose (British Drug Houses, Poole, UK) was injected intravenously over 3 s at the dose of 1 g/kg in a tail vein without flushing of the 27-gauge needle after injection, either alone or together with synthetic GLP-1 (GLP-1, 7–36 amide, i.e., the active form of GLP-1, Peninsula Laboratories Europe, Merseyside, UK) at 0.1 or 10 nmol/kg. These doses were selected from a previous study demonstrating that the threshold level for GLP-1 to augment glucose-stimulated insulin secretion in mice is 0.1 nmol/kg, whereas 10 nmol/kg is a maximal dose (5). In one series of animals, arginine (Sigma) was injected intravenously instead of glucose at a dose of 0.25 g/kg. The volume load was 10 μl/g body wt. Additional blood samples (75 μl each) were taken either at 1, 5, 20, and 50 min. Plasma was immediately separated and stored at −20°C until analyses.

Insulin secretion from isolated islets. Pancreatic islets were isolated by collagenase digestion in Hank’s balanced salt solution (HBSS;...
Kebo Laboratory, Spnga, Sweden) at 37°C in 5% CO2 air. The islets were handpicked into a HEPES medium (pH 7.36) supplemented with glucose at 3.3 or 10 mmol/l or glucose at 10 mmol/l with GLP-1 at 100 nmol/l. Following incubation at 37°C for 60 min, 25 μl of the medium were collected from each chamber and stored at -20°C until analysis.

Analyses. Insulin concentration was determined by a double-antibody radioimmunoassay using guinea pig anti rat insulin antibodies, 125I-labeled human insulin, and, as standard, rat insulin (Linco Research, St. Charles, MO). Glucose was measured by the glucose oxidase technique.

Calculation and statistics. The acute insulin response (AIR) to intravenous glucose with or without GLP-1 was calculated as the suprabasal 1-min value. The glucose elimination was quantified as the KE, i.e., the glucose elimination constant, as the reduction in circulating glucose between minute 1 and minute 20 after intravenous administration after logarithmic transformation of the individual plasma glucose values and expressed as percent elimination of glucose per minute. Data and results are reported as means ± SE. Statistical comparisons between two groups were performed with ANOVA followed by Bonferroni post hoc analysis.

RESULTS

The experiments were undertaken when the mice were 10–16 wk of age. At 10 wk of age, body weight was 22.8 ± 0.9 g in control mice (n = 18) vs. 22.7 ± 1.0 g in capsaicin-treated mice (n = 18). Similarly, at 16 wk of age, no significant difference was observed between the groups. Figure 1 shows that in control, vehicle-treated mice, GLP-1 at both 0.1 and 10 nmol/kg augmented the insulin response to the intravenous glucose as evident by higher insulin levels after 1 and 5 min (P < 0.001). In contrast, in capsaicin-treated mice, only GLP-1 at the higher dose of 10 nmol/kg could augment glucose-stimulated insulin secretion (P < 0.001), whereas at 0.1 nmol/kg, insulin levels were not increased compared with administration of glucose alone. This is also evident from Fig. 2, where the acute suprabasal 1-min insulin response (AIR) is shown. Again, GLP-1 at 0.1 nmol/kg augmented the AIR in vehicle-treated mice (P < 0.001) but not in capsaicin-treated mice. What is also evident, and which confirms a previous report (15), is that the insulin response to glucose was higher in capsaicin-treated than in vehicle-treated mice. Thus the AIR after glucose alone was 1,459 ± 120 pmol/l in vehicle-treated mice vs. 2,150 ± 150 pmol/l in capsaicin-treated mice (P < 0.001).

The augmented insulin response to glucose by GLP-1 was associated with increased glucose elimination. This is evident by inspection of Fig. 1 but also when calculating the glucose elimination constant, KE. In vehicle-treated animals, KE after injection of glucose alone was 2.37 ± 0.25%/min and this was increased by GLP-1 at 0.1 nmol/kg to 3.41 ± 0.38%/min (P < 0.001) and at 10 nmol/kg to 4.44 ± 0.51%/min (P < 0.001). Conversely, in capsaicin-treated animals, KE after injection of glucose alone was 2.96 ± 0.31%/min, after glucose plus GLP-1 at 0.1 nmol/kg was 2.76 ± 0.36%/min (NS), and after glucose plus GLP-1 at 10 nmol/kg was 4.59 ± 0.51%/min (P < 0.001).

To examine the specificity of the action of capsaicin on glucose- and GLP-1-stimulated insulin secretion, mice were also injected with arginine, another insulin secretagogue. It was found that the insulin response to arginine was not different in the two groups. Thus the 1-min suprabasal insulin response to arginine was 430 ± 39 pmol/l in vehicle-treated mice (n = 8)
Capsaicin is a fairly specific tool for sensory deactivation, as suggested by previous reports that GLP-1 is a potent insulinotropic peptide markedly augmenting glucose-stimulated insulin secretion in mice (1, 5, 11), which is similar as the potent effect of the peptide in other species, including humans (2, 7, 8, 9, 12, 13). The novel observation in this study is that this effect of GLP-1 is not seen when the peptide is administered at a low dose in capsaicin-treated mice. Neonatal administration of capsaicin is a model that previously has been introduced to examine the function of sensory nerves in mice. Capsaicin is a drug that destroys primary afferent nerves of the C fiber type (14) and therefore causes a sensory deactivation. To verify the sensory deactivation by capsaicin in islets, a previous study has examined the immunocytochemical islet distribution of neuropeptides, which are confined to sensory nerves. Thus fairly numerous nerves containing CGRP and substance P are normally localized both around blood vessels and within islets in the pancreas of normal mice (16). After neonatal capsaicin, a substantial reduction of the number of substance P-containing nerves is reduced, although few nerve fibers remain (16). In contrast, nerves immunostained for galanin, which is confined to adrenergic nerve terminals (3), remain virtually unaffected by capsaicin (16). This shows that in mice, capsaicin markedly reduces the number of sensory nerves leaving the adrenergic nerves unaffected, verifying that capsaicin is a fairly specific tool for sensory deactivation, as judged by immunocytochemistry. Our finding that capsaicin reduces the insulinotropic action of GLP-1, therefore, shows that intact sensory nerves are required for the insulinotropic action of GLP-1 at a low dose level. In contrast, at the maximal dose of 10 nmol/kg, GLP-1 augmented glucose-stimulated insulin secretion by similar efficiency in vehicle- and capsaicin-treated animals, showing that at this high dose level, the direct effect of GLP-1 on beta cells seems sufficient.

In contrast to the impaired augmentation of glucose-stimulated insulin secretion by GLP-1 in capsaicin-treated mice in vivo, the insulinotropic action of GLP-1 from isolated islets was retained after capsaicin. This suggests that it is not the islet sensitivity to GLP-1 that is perturbed by capsaicin but rather that sensory activation is required by GLP-1 for its action. This in turn suggests that GLP-1 augments insulin secretion by an effect initiated at the level of afferent nerves. It has previously been hypothesized that sensory nerves in the portal vein may sense GLP-1, thereby initiating a neural circuit involving efferents to the islets (6). Since GLP-1 is released from the gut, the activation of a portal signal by GLP-1 may be a mechanism by which the hormone augments insulin secretion. Our results may also suggest that GLP-1-sensitive receptors initiating efferent impulses may be located outside the portal vein because in our study GLP-1 was administered through a peripheral vein. The idea that GLP-1 activates autonomic nerves to the pancreas, which may contribute to the insulin response after meal ingestion, may also partially explain findings that neuronal ganglionic blockade reduces the insulin response to meal intake (4). Such an indirect action of GLP-1 is, however, not explaining the entire action of the hormone on insulin secretion, because at a high dose level, the insulinotropic action of GLP-1 was not reduced by capsaicin. Hence, it may be hypothesized that sensory nerves are important for the action of GLP-1 at low dose levels, whereas at high dose levels, the direct beta cell action of the hormone is more important.

A previous study has shown that insulin secretion stimulated by glucose is augmented in capsaicin-treated mice (15). It has also been demonstrated that the first phase of glucose-stimulated insulin secretion in the perfused rat pancreas is augmented by capsaicin (21). In this study we confirmed that glucose-stimulated insulin secretion is augmented after capsaicin treatment in mice. In contrast, we found that the insulinotropic action of another secretagogue, arginine, was not affected by capsaicin, and previously it has also been demonstrated that the insulin secretion induced by the cholinergic agonist carbachol likewise is not perturbed by capsaicin (15). This suggests that glucose, carbachol, and arginine do not seem to depend on sensory nerves for their insulinotropic action and therefore that the impaired action of GLP-1 is rather specific. Why glucose-stimulated insulin secretion is augmented after capsaicin is yet to be explained. The augmentation is not seen in isolated islets, as shown previously both in statically incubated and dynamically perfused islets (15) and confirmed here in statically incubated islets. Previously we suggested that it may be explained by impairment of an adrenergic reflex initiated by sensory activation, because the insulinotropic action of phentolamine was impaired in capsaicin-treated mice in vivo but not in vitro (15). Hence, sensory nerves might be activated by glucose initiating an adrenergic response restraining the glucose-stimulated insulin secretion. This would be supported by a previous result that the glucagon response to the glucose analog 2-deoxy-glucose, which is partially dependent on adrenergic nerves, is diminished after capsaicin (16). However, the present study does not add further information on the
mechanism underlying the intriguing observation that sensory deactivation augments glucose-stimulated insulin secretion.

Also the glucose elimination after intravenous glucose was augmented after capsaicin. This may be explained by the increased insulin secretion, but it may also be explained by improved insulin sensitivity, which is an effect seen after sensory deactivation in neonatal rats (17). This confirms the importance of the sensory nerves for glucose homeostasis. The improved overall glucose tolerance after sensory deactivation might in addition be tentatively discussed in the context of targets for treatment of impaired glucose tolerance and Type 2 diabetes.

In conclusion, this study in mice shows that neonatal capsaicin diminishes the augmentation of glucose-induced insulin secretion by GLP-1 in vivo. This suggests that sensory nerves contributed to the insulinotropic action of GLP-1.

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GRANTS

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