Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1

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Nakabayashi, Hajime, Makoto Nishizawa, Atsushi Nakagawa, Ryouu Takeda, and Akira Niijima. Vagal hepatopancreatic reflex effect evoked by intraportal appearance of tGLP-1. Am. J. Physiol. 271 (Endocrinol. Metab. 34): E808-E813, 1996.—Among proglucagon-derived peptides, the truncated form of glucagon-like peptide-1, GLP-1(7–36) amide (tGLP-1), is known as the most likely physiological humoral incretin. To examine whether there exists any relationship between tGLP-1 levels in the portal vein and activities of the hepatic and pancreatic vagal system, changes of the impulse discharge rate in the hepatic afferent vagus and the pancreatic efferent vagus upon intraportal tGLP-1 injection were measured in situ in rats anesthetized with urethan and chloralose. First, a 1-min bolus tGLP-1 injection at a peripherysiological dose of 0.2 pmol or a pharmacological dose of 4.0 pmol, but not the vehicle injection, significantly facilitated the hepatic vagal afferents for >90 min, showing weaker facilitation at the 0.05 pmol dose. Notably, the injection of noninsulinotropic full-length GLP-1 failed to facilitate the afferents at the 4.0 or 40.0 pmol dose. Second, the intraportal tGLP-1 injections at the 0.05 and 0.2 pmol dose facilitated marginally and significantly the pancreatic vagal efferents in normal rats, respectively, but had no effect on the hepatic vagotomized rats, even at the 40.0 pmol dose. The present results indicate that an intraportal appearance of tGLP-1 is specifically recognized by the hepatic vagal nerve, and this recognition further augments the pancreatic vagal efferent activity in a reflex way, suggesting another nature of tGLP-1 as neuroincretin in the enteroinferior axis.
from the hepatic vagal branch under a dissection microscope (×16 magnification). The distal cut end of the nerve filaments was placed on a pair of silver wire electrodes and was immersed in a mixture of liquid paraffin and Vaseline. To measure efferent activity in the pancreatic vagal nerve, the proximal cut end of the isolated nerve filaments from the pancreatic branch of the dorsal vagal trunk was used in a manner mentioned above in the afferent activity measurement, as previously described (17, 18). In the hepatic vagotomized rats, the hepatic branch of the vagal nerve was selectively sectioned just before preparing the pancreatic nerve filaments. The nerve activity was amplified by a condenser-coupled differential amplifier, monitored by an oscilloscope, and stored on magnetic tape. Analysis of the nerve activities was performed after conversion of raw data to standard pulses by a window discriminator, which distinguished impulse discharges of the afferent or efferent fibers from background noise. The discharge rate was played on a thermal recorder or an oscilloscope by a rate meter with a reset time of 5 s to establish the time course. The injection of tGLP-1, GLP-1, or the vehicle was started after a stable basal discharge rate over at least 30 min in each rat had been confirmed. To calculate the mean discharge rate, the number of spikes per 5 s was averaged over 50 s. The techniques employed had been described elsewhere (17, 18).

The results were expressed as means ± SE. Two-way analysis of variance followed by Scheffé's multiple comparison test for comparing time course data within a group and two-way repeated-measures analysis of variance for comparing variables between groups were employed. Values of \( P < 0.05 \) were considered to be significant.

RESULTS

Effect of intraportal administration of tGLP-1, GLP-1, or vehicle on afferent activity of the hepatic vagus in normal rats. Injection of tGLP-1 at a perphysiological dose of 0.2 pmol into the portal vein provoked an increase of the afferent spike discharge rate in the hepatic vagal nerve, not changing the mean amplitude of the spikes in the time course. The increase started within a few minutes after injection and usually lasted for >90 min after injection (Fig. 1). As shown in Fig. 2A, the spike discharge rate significantly rose from 67 ± 4 impulses/5 s before injection to 109 ± 12, 137 ± 17, and 145 ± 13 at 30, 60, and 90 min after injection, respectively (\( P < 0.05 \) at 30 min and \( P < 0.01 \) at 60 and 90 min vs. the value before injection, \( n = 6 \)). Intraportal injection of tGLP-1 at a pharmacological dose of 4.0 pmol also induced a significant increase of the discharge rate from 66 ± 4 impulses/5 s before injection to 99 ± 5, 114 ± 7, and 120 ± 10 at 30, 60, and 90 min after injection, respectively (\( P < 0.01 \) at all time points vs. the value before injection, \( n = 7 \)). The increases with 4.0 pmol injection were not significantly different from those with 0.2 pmol injection at any time point.

To further determine a minimum effective dose of tGLP-1 in eliciting significant rise of the afferent discharge rate, 0.05 pmol of tGLP-1 were injected into the portal vein in five rats. The tGLP-1 administration induced a small and rather short-lasting increase (for 45–60 min) in the activity in three out of five rats tested and induced no change in the others (data not shown). By contrast, intraportal administration of full-length GLP-1 at a dose of 4.0 pmol did not significantly change the discharge rate, although the discharge rate tended to increase slightly in the time course (\( n = 5 \), Fig. 2C). Moreover, even 40.0 pmol GLP-1 again did not induce any significant change of the discharge rate (\( n = 4 \), data not shown). These GLP-1-induced changes of the discharge rates were not significantly different from those induced by the vehicle administration, which also induced a slight but insignificant increase in the discharge rate as shown in Fig. 2D (\( n = 6 \)).

Fig. 1. Afferent impulse discharges before (a) and after (b) intraportal injection of truncated form of glucagon-like peptide-1 (tGLP-1; 0.2 pmol, 200 μl) recorded in situ from hepatic branch of vagal nerve (recordings in a and b correspond to recordings at the time indicated by a and b, respectively, in A) and time course of discharge rate in rat (A). B: discharge rate in vehicle-injected rat (200 μl). Different rat in each time course. Each arrow shows time of intraportal injection.
Fig 2. Effect of intraportal injection of tGLP-1 at a dose of 0.2 pmol (A) or 4.0 pmol (B), tGLP-1 at 4.0 pmol (C), or vehicle (D) on rate of afferent impulse discharges in hepatic branch of rat vagal nerve. Error bars represent means ± SE; n, no. of rats. *P < 0.05 and **P < 0.01 vs. value before injection (0 min).

vagal afferents as noted above, was injected into the portal vein, the pancreatic efferents showed a small and short-lasting increase for ~60 min in three out of six rats tested but showed no change in the others (data not shown).

In hepatic vagotomized rats, however, intraportal injection of 0.2 pmol tGLP-1 did not induce significant changes of the pancreatic efferent discharge rate (n = 5, Figs. 3 and 4), showing apparently smaller changes than those in normal rats at all time points (P < 0.05). Moreover, injection of even 40.0 pmol, 200-fold dose, tGLP-1 also failed to facilitate the efferents (n = 4, data not shown). The responses of the efferents to both doses of tGLP-1 in the vagotomized rats were not significantly different from those to the vehicle in normal rats or hepatic vagotomized rats (n = 4 in each, data not shown).

**DISCUSSION**

As most likely physiological candidates for humoral incretin, tGLP-1 (13, 28, 32) and glucose-dependent insulinotropic polypeptide (also known as gastric inhibitory polypeptide; see Refs. 6, 23) have been listed to date. In fact, both peptides are released into the circulation in response to ingestion of glucose, fatty acids, and a mixed meal, and both stimulate insulin secretion in a physiological range of their plasma concentrations (6, 13, 23, 28, 32). Action of tGLP-1 as incretin has particularly been noted in normal subjects (13) and in patients with non-insulin-dependent diabetes mellitus (NIDDM; see Ref. 24). However, the present electrophysiological results clearly indicate that an appearance of tGLP-1, but not of full-length GLP-1, into the portal vein facilitates the afferent activity in the hepatic vagal nerve. The results also indicate that hepatic afferent activity that is facilitated leads further to facilitation of the efferent activity in the pancreatic vagus, revealing a novel vagal hepatopancreatic reflex pathway. These results suggest that tGLP-1 released into the portal vein upon meal ingestion also behaves as neuroincretin in the enteroinsular axis.

The hepatic vagus seems to be very sensitive in recognizing an intraportal appearance of tGLP-1. As to plasma tGLP-1 levels, it has been reported that tGLP-1 levels in the peripheral venous plasma increase from 1–10 pmol/l to 20–50 in response to ingestion of a mixed meal or glucose in humans (7, 13, 29) and that the levels in postprandial arterial plasma are ~20 pmol/l in rat (32), using different assays among laboratories. Because intraportal tGLP-1 levels observed in a physiological situation such as meal ingestion have not been reported previously in rats and knowing its levels is prerequisite for evaluating the sensitivity in the vagal recognition, we attempted to obtain the portal blood samples in conscious rats but hardly succeeded. Alternatively, we assumed by calculation that intraportal tGLP-1 administration at a dose of 0.2 or 4.0 pmol achieves systemic tGLP-1 levels within a perphysiologi-
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Fig. 3. Efferent impulse discharges before (a) and after (b) intraportal injection of tGLP-1 (0.2 pmol, 200 µl) recorded in situ from pancreatic branch of vagal nerve (a and b correspond to a and b, respectively, in A) and time course of discharge rate in rat (A). B: discharge rate in hepatic vagotomized rat injected with tGLP-1 (0.2 pmol, 200 µl). Different rat in each time course. Each arrow shows time of intraportal injection.

Fig. 4. Effect of intraportal injection of tGLP-1 at a dose of 0.2 pmol on rate of efferent impulse discharges in pancreatic branch of vagal nerve in normal (A) and hepatic vagotomized (B) rats. Error bars represent means ± SE, n, no. of rats. **P < 0.01 vs. value before injection (0 min).

We have recently obtained a line of unique evidence for vagal chemoreception to another brain gut hormone, somatostatin, in the hepatoportal area (16, 18, 19). First, the hepatic vagus is receptive to somatostatin circulating in the portal vein (18, 19). The receptivity to somatostatin is completely abolished with a monoclonal antibody against somatostatin receptor that binds to the binding site of the receptor (19, 21). The results strongly suggest that the reception of somatostatin is mediated by the specific receptor system. Second, as the relevant histological structure in the chemoreception to somatostatin, we have further discovered the neural bodies that are located beneath the endothelium of the large branches of the intrahepatic portal vein in rat and that preferentially trap somatostatin (18; the neural body had been termed as the neural corpuscle in Ref. 18). Each neural body contained many nerve fiber arborizations with terminal nodular swellings like the afferent nerve endings in its core (16, 18). Moreover, when the neural body is immunostained with the monoclonal antibody to somatostatin receptor, presence of the receptor on the terminal tip of the nerve fiber arborizations was clearly visualized (16). By analogy of these electrophysiological and histological evidence on the specific receptor-mediated chemoreception of somatostatin, we can hypothesize that a specific receptor system for tGLP-1 could also be involved in the present observation, by which humoral information (circulating tGLP-1) is converted to neural information. As to tGLP-1 receptor in the liver, it has been reported that the specific receptor is not present either on

cal or a pharmacological range, respectively, in a previous report (32). In this case, actual systemic tGLP-1 levels seem to become lower than those expected in the intraportal administrations, since the liver extracts tGLP-1 to some extent. Besides, the intraportal administration of tGLP-1, even at a dose as small as 0.05 pmol, has elicited some increases of the hepatic vagal afferent activity (data not shown). This stresses that the vagal recognition system is exquisitely sensitive to tGLP-1. Taken together, it is conceivable that the maximal response to tGLP-1 would already be evident at a supposed periphysiological dose of 0.2 pmol, because the dose of 4.0 pmol induced no further augmented responses.

This recognition system for tGLP-1 in the hepatoporal area is very specific as well as sensitive. Intraportal administration of full-length GLP-1 known to have no incretin effect did not change significantly the afferent activity of the hepatic vagus, even at a dose of as large as 40.0 pmol, showing a slight but insignificant increase in the time course that is similar to an unexplained and insignificant change in the present vehicle administration. The results indicate that the vagal recognition system can distinguish NH2-terminal difference of only six amino acids between tGLP-1 and GLP-1-(1—37), suggesting an involvement of specific receptor for tGLP-1 in the recognition. In this regard,
hepatocytes or in rat liver membranes (2), but the tGLP-1 receptor mRNA is present in the liver of the mice (3). It is thus intriguing whether tGLP 1 receptor is present in the nervous system associated with the portal vein in the liver, particularly on the nerve fiber arborizations within the neural bodies, as observed in the case of somatostatin reception (18).

The time course of tGLP-1-induced changes of the afferent discharge rate in the hepatic vagus is noteworthy; the vagal afferent activity started to increase within a few minutes after tGLP-1 injection and lasted for >90 min, even after the 1-min bolus injection. This time course seems to represent a characteristic feature of the vagal chemoreception, which involves a specific receptor system for a peptide hormone secreted from the splanchnic organs, since a similar electrophysiological event was also observed in the neural chemoreception of somatostatin (18, 19). This vagal reception of somatostatin was completely canceled when the monoclonal antibody to somatostatin receptor was administered intraportally before intraportal somatostatin injection (19). By contrast, the reception was evident without any modification when the antibody was administered after somatostatin injection (19). This shows that the afferent activity, once stimulated by the hormone, cannot be hampered any more by modulating the ligand binding to the receptor with the antibody. These observations suggest that a unique postreceptor mechanism is involved in the reception and that the mechanism offers a base for explaining the long-lasting effect of somatostatin (probably also of tGLP-1) on the afferent discharge rate. At the same time, the observation on this unique postreceptor mechanism implies that the receptor-mediated reception might have a nature suitable for detecting an initial increase in intraportal concentrations of both of these hormones raised usually for 3–4 h after a mixed meal. It may also be relevant to this hypothesis that an additional administration of a larger dose of somatostatin in the increasing phase of the pancreatic vagal afferent activity induced by somatostatin failed to stimulate further increases (17). An additional interesting question of how the afferent event such as stimulation of the pancreatic vagal afferent activity and suppression of the activities in the gastric vagus and the adrenal sympathetic nerve (25) and further leads to divergent efferent events such as stimulation of the pancreatic vagal activity and suppression of the activities in the gastric vagus and the adrenal sympathetic nerve (26). In addition to our previous observation on the somatostatin-induced facilitation of the hepatic vagal afferents (18, 19), we have recently observed that intraportal somatostatin administration causes suppression of the small intestinal vagal efferents and facilitation of the efferents in the intestinal sympathetic nerve in rats (unpublished observation). These results seem to be pertinent to our previous observation, which showed that an intraportal somatostatin administration suppressed the entry of fat from the intestine to blood circulation under intact, but not under disrupted, vagal (extrinsic) innervation in dogs (20). Thus it is tempting to speculate that the efferent parasympathetic and/or sympathetic limbs of the present reflex pathway triggered by intraportal tGLP-1 appearance reach the stomach, liver, and muscle. If so, tGLP-1 might change gastric emptying, hepatic glucose metabolism, and insulin-independent glucose disposal, and these factors might be relevant to the tGLP-1-induced reduction of postprandial glucose excursions observed clinically.

Finally, the present results will provide a new insight into the role of tGLP-1 in the enteroinosal axis,
implying its additional physiological roles related to nutrient homeostasis.

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