Amplitude Modulation of Pulsatile Insulin Secretion by Intrapancreatic Ganglion Neurons

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Neuron activity and insulin release were measured simultaneously from 33 preparations of intrapancreatic canine ganglia and pancreatic parenchyma adjacent to the ganglia. The electrical activity of single neurons of the ganglia was recorded with intracellular microelectrodes, and insulin release from the attached islets was determined with an enzyme-linked immunosorbent assay. Insulin release was 62 ± 18 fmol preparation/min in the presence of 10 mmol/l glucose and pulsatile (3.7 ± 0.4 min/pulse). Corresponding measurements of neuronal electrical activity showed a stable membrane potential of –53.5 ± 0.6 mV. Short, high-frequency (20 Hz) preganglionic nerve stimulation evoked action potentials and, in 46% of the preparations, a threefold rise in the insulin secretory rate associated with increased amplitude of the insulin pulses. The effects were blocked by 10 µmol/l tetrodotoxin (TTX). In other preparations, continuous low-frequency (0.05–0.5 Hz) preganglionic nerve stimulation evoked action potentials and, in 50% of the preparations, a gradual increase of insulin release associated with augmentation of insulin pulse amplitude without alteration of the duration. The effects were blocked by 50 µmol/l hexamethonium (HEX). In the remaining preparations, no change in insulin release was observed during nerve stimulation. In the absence of stimulation, neither TTX nor HEX affected the membrane potential or insulin secretion. These first simultaneous measurements of intrapancreatic ganglion activity and insulin secretion are consistent with amplitude modulation of pulsatile insulin secretion induced by changes in electrical activity in a population of intrapancreatic ganglion neurons. *Diabetes* **50:51–55, 2001**

A rise in plasma glucose concentration increases insulin concentration by modulating the amplitude of the regular variations in the plasma insulin concentrations (1,2). The importance of the increase in the insulin pulse amplitude is illustrated by the hyperglycemia-induced rise in plasma insulin in type 2 diabetic patients (2). In these individuals, the increase is mainly nonpulsatile, with irregular plasma insulin oscillations with small amplitudes. This deranged plasma insulin pattern is believed to be an important factor leading to receptor downregulation (3) and insulin resistance (4). Indeed, pulsatile insulin delivery has greater hypoglycemic effect than nonpulsatile delivery (5,6). Understanding the mechanisms regulating the amplitude of plasma insulin oscillations is therefore crucial in attempts to restore normal oscillatory plasma insulin in diabetic patients.

Amplitude regulation of plasma insulin oscillations depends on the modulation of the insulin pulse amplitude from the isolated islet (7). Glucose and other secretagogues increase insulin release from the isolated islet by increasing the insulin pulse amplitude. However, to obtain pulsatile release from the pancreas (8), coordination of the secretory activities of the islets is required. Although no effect has been observed on plasma insulin oscillations in the presence of drugs affecting the central nervous system (9), amplitude modulation of plasma insulin oscillations does occur in response to cholinergic and adrenergic blockade, thereby indicating a role of the autonomic nervous system (1,10). In this context, it has been suggested that the intrinsic ganglia of the pancreas play a role in the generation of pulsatile insulin release, possibly by synchronizing the secretory activities of the islets (11–13). The role of these ganglia in pulsatile insulin secretion has been difficult to evaluate, because the β-cells receive neuronal input from these ganglia as well as from many other nerves of the autonomic nervous system (14). Furthermore, the secretory activity of the β-cells is also influenced by intestinal peptides released in response to activation of the autonomic nervous system (15).

To determine the role of the intrapancreatic neurons for pulsatile insulin secretion, we isolated individual ganglion together with adjacent islet-containing pancreatic parenchyma. Simultaneous measurements of neuronal activity and insulin release revealed that intrapancreatic ganglia may play a role in amplitude regulation of pulsatile insulin release.

RESEARCH DESIGN AND METHODS

Reagents. Reagents of analytical grade and deionized water were used. Aprotinin, bovine serum albumin (fraction V), hexamethonium (HEX), tetramethylbenzidine, tetrodotoxin (TTX), and insulin peroxidase were purchased from Sigma Chemicals (St. Louis, MO). The rat insulin standard was obtained from Novo Nordisk (Bagsvaerd, Denmark). IgG-certified microtiter plates were purchased from Nunc (Roskilde, Denmark). The antibodies to insulin were raised in guinea pigs.

Preparation and perifusion of cells. Adult dogs (20–25 kg) of both sexes were anesthetized with 30 mg/kg body wt sodium pentobarbital i.v. (Fort Dodge Laboratories, Fort Dodge, IA) and killed by exsanguination. Dogs were obtained from a U.S. Department of Agriculture–approved supplier (Triple C, St. Joseph, IL). The use of dogs and the experimental methods used in this study were approved by the Institutional Animal Care and Use Committee (Mayo Clinic). Through a midline abdominal incision, the pancreas was removed and a section of the head region of the pancreas was dissected and pinned to the Sylgard-coated floor of a tissue bath. The tissue was superfused
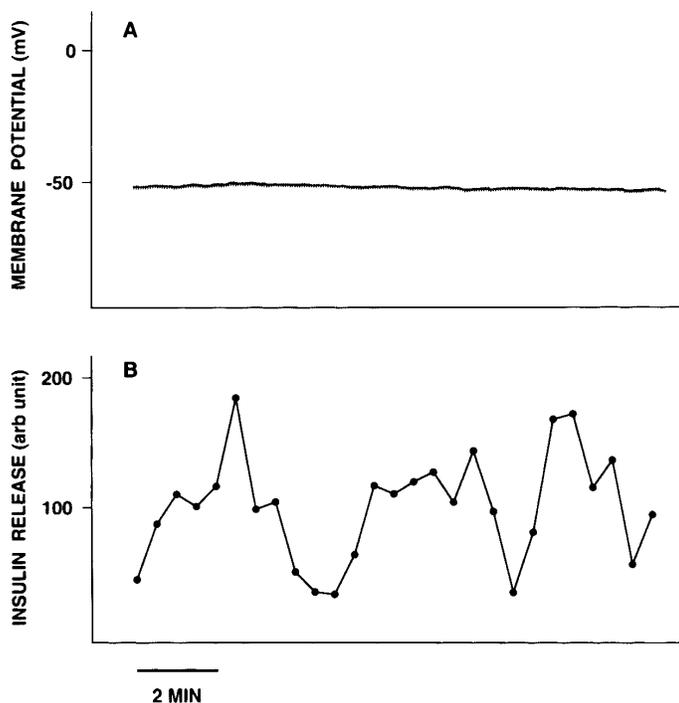
FIG. 1. Simultaneous recordings of membrane potential of a pancreatic ganglion neuron (*A*) and insulin release from an attached piece of pancreatic parenchyma (*B*) in the presence of 10 mmol/l glucose. Intracellular recordings were obtained with a high-resistance glass microelectrode. Insulin release was measured in the perifusate by ELISA. Representative of 27 experiments.

with modified Krebs solution bubbled with 97% O_2 and 3% CO_2 (pH 7.4). The composition of the Krebs solution was as follows (in mmol/l): NaCl 120, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 15.5, NaH_2PO_4 1.2, and glucose 10. Pancreatic ganglia were identified in the interlobular connective tissue with a microscope (magnification $\times 15$). An individual ganglion, attached nerve trunks, and a piece of pancreatic parenchyma attached to the ganglion were dissected together. One such preparation was prepared from each pancreas. A total of 33 pancreata were used for the study. Each piece of pancreas was approximately 1×2 mm in size and 0.5 mm or less in thickness, to limit diffusion-related problems. Nerve trunks were identified as central or peripheral, based on their location relative to the ganglion as it would lie in the pancreas, as previously described (13). The preparation was pinned to the Sylgard-coated floor of a recording chamber (500 µl) and perifused at a flow rate of 800 µl/min using gravity as the driving force with the same buffer as the one used during dissection. After a 2-h perifusion, the buffer was supplemented with albumin (1 mg/ml) and aprotinin (500 kallikrein inhibitor units [KIU]/ml), the flow rate was reduced to 500 µl/min using a peristaltic pump as the driving force, and the preparation was perifused for another hour.

Electrical neuronal recordings. Intracellular recordings were made with 3 mol/l KCl-filled microelectrodes (40–80 MΩ resistance) connected to an electrometer with an active bridge circuit that allowed passage of depolarizing or hyperpolarizing current through the recording electrode. The membrane potential and intracellular current injections were displayed on an oscilloscope (Tektronix 513), and permanent records were made on a chart recorder (Gould Brush 220) and an FM tape recorder (Hewlett Packard 3968A). Satisfactory impalements resulted in a stable resting membrane potential of –40 mV or a more negative potential. Central nerve trunks were stimulated by bipolar platinum wire electrodes connected to a stimulator (Grass S88) and a stimulus isolation unit (Grass SIU5). A high-frequency stimulation (20 Hz, 100 V), with a pulse duration of 0.5 ms and a train duration of 30 s and a sustained low-frequency stimulation (0.05–0.5 Hz, 60 V) with a pulse duration of 0.5 ms were used to evoke action potentials in the impaled ganglion neurons. The duration of the high-frequency stimulation was restricted to 30 s, because a prolonged stimulation period would cause damage to the nerve trunk.

Insulin release measurements. Insulin release was measured in the perifusate, which was collected in 30-s fractions and immediately cooled on ice. Determinations of insulin were made by a competitive enzyme-linked immuno-

sorbent assay (ELISA) with the insulin-capturing antibody immobilized directly in the solid phase and with inter- and intra-assay variation <10% (7). The amounts of insulin were calculated from linear standard curves in semilogarithmic plots. Insulin release in the presence of 10 mmol/l glucose varied between 9 and 211 fmol per preparation and minute, corresponding to the secretory rate from 3 to 70 isolated islets, respectively (7). Because of this variability in endocrine secretion from the pancreatic preparations, insulin release during the first 5 min of perifusion of each preparation was normalized to 100 arbitrary units. The cross-reactivity of canine insulin with the insulin assay was 70%.

Data and statistical analysis. The occurrence and duration of insulin pulses were assessed from power spectra obtained by Fourier transformation of the original data. Average secretory rates were calculated using moving averages of three consecutive sample points. Analyses of the frequencies and average rates were performed with Igor software (Wave Metrics, Lake Oswego, OR). Data are presented as means ± SE. Differences in secretory rates were evaluated with analysis of variance.

RESULTS

Insulin release and neuronal activity were recorded simultaneously from 33 preparations consisting of an intrapancreatic ganglion and pancreatic parenchyma adjacent to the ganglion. In the presence of 10 mmol/l glucose, insulin release was 62 ± 18 fmol preparation/min and was pulsatile with a pulse duration of 3.7 ± 0.4 min/pulse. The corresponding measurements of neuronal electrical activity showed a stable membrane potential of –53.5 ± 0.6 mV with no spontaneous electrical activity. Only occasional excitatory postsynaptic potentials (EPSPs) or action potentials were observed (1 EPSP/3.1 ± 0.4 min). An example of simultaneous recording of insulin release and neuronal activity is shown in Fig. 1.

Neuronal activity and insulin release in the presence of 10 mmol/l glucose were recorded simultaneously during high-frequency (20 Hz) preganglionic stimulation of the centrally attached nerve trunk in the absence and presence of 10 µmol/l of the Na^+ channel blocker TTX (16) in 13 preparations. In the absence of TTX, nerve stimulation induced a train of action potentials and a more than threefold augmentation of insulin release associated with increased amplitude of the insulin pulses in six preparations (Fig. 2; Table 1). In the presence of TTX, both the neuronal response and the associated increase in the amplitude of insulin release observed in the absence of TTX were blocked in these preparations. In the remaining seven preparations, no change in insulin release was observed during high-frequency stimulation in the absence of TTX. In all 13 preparations, in the absence of nerve stimulation, TTX had no effect on the membrane potential of the ganglion neurons and no effect on the amplitude or duration of the insulin pulses (Table 1).

Neuronal activity and insulin release in the presence of 10 mmol/l glucose were also recorded during continuous low-frequency (0.05–0.5 Hz) stimulation of the centrally attached nerve trunk in the absence and presence of 50 µmol/l of the nicotinic receptor blocker HEX (17) in 14 preparations. Low-frequency nerve stimulation evoked action potentials and an increase in insulin release in seven preparations (Fig. 3; Table 2). Based on control experiments, this increase could not be explained by an increase in nonpulsatile insulin release, but was at least partially related to augmentation of the insulin pulse amplitude. In these control experiments, individual islets were perifused under the same conditions used in this study (500 µl chamber and a flow rate of 500 µl/min). Similar insulin release patterns to those observed in Fig. 3 before stimulation were obtained from these islets. When a constant amount of insulin was added to each fraction of
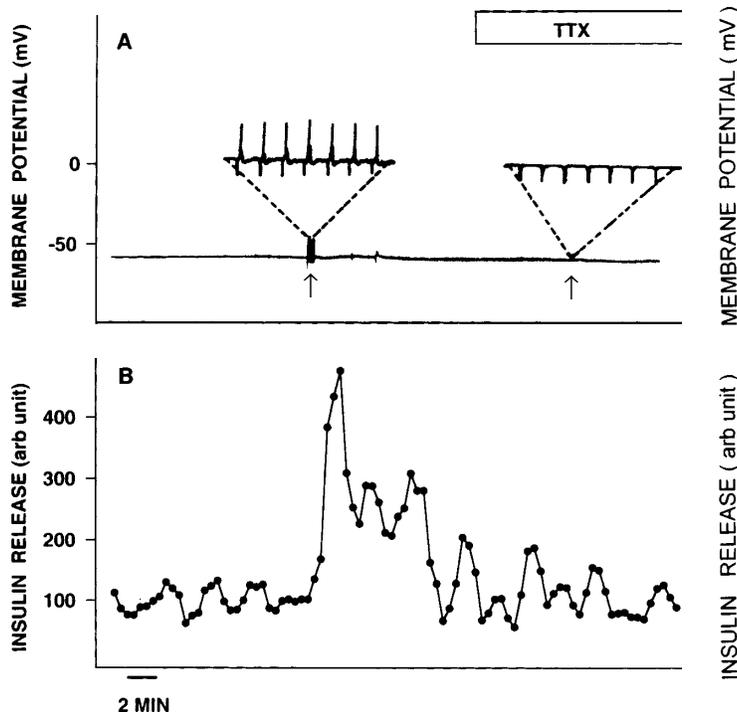
FIG. 2. Simultaneous recordings of membrane potential of a pancreatic ganglion neuron (*A*) and insulin release from an attached piece of pancreatic parenchyma (*B*) at 10 mmol/l glucose in the absence or presence of TTX. Arrows indicate periods of high-frequency (20 Hz) preganglionic electrical stimulation of a central nerve trunk attached to the ganglion; the bar indicates addition of 10 μmol/l TTX to the perifusion medium (TTX). Membrane potential recordings during electrical stimulation are expanded in the absence and presence of TTX. Data are representative of six experiments.
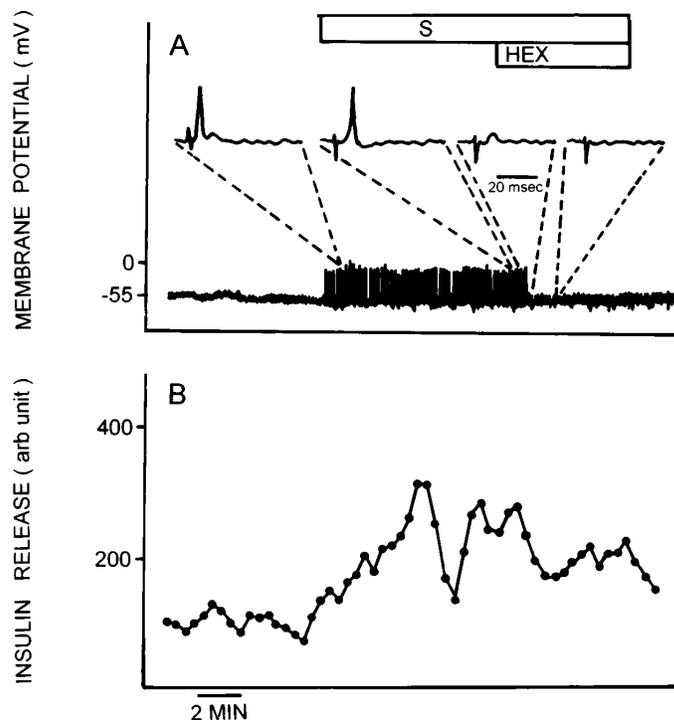

FIG. 3. Simultaneous recordings of membrane potential of a pancreatic ganglion neuron (*A*) and insulin release from an attached piece of pancreatic parenchyma (*B*) at 10 mmol/l glucose in the absence and presence of HEX. Bars indicate low-frequency (0.05–0.5 Hz) preganglionic electrical stimulation (S) of a central nerve trunk attached to the ganglion and addition of 50 μmol/l HEX to the perifusion medium (HEX). Membrane potential recordings during electrical stimulation are expanded in the absence (first two stimulations) and presence (last two stimulations) of HEX. Data are representative of seven experiments.

perifusate, the pulsatility of insulin release was almost completely obscured. The addition of insulin approximately doubled the amount of insulin in the fraction. When HEX was present, nerve stimulation failed to evoke postsynaptic

TABLE 1
Neuronal stimulation of insulin release from pancreatic parenchyma attached to a pancreatic ganglion in the absence or presence of TTX

Electrical stimulation (Hz)	TTX (μmol/l)	Insulin release	
		%	min/pulse
—	—	100	4.3 ± 0.5
20	—	351 ± 100*	—
—	10	124 ± 33	6.2 ± 0.9
20	10	136 ± 21†	—

The preparation was perifused at 10 mmol/l glucose for 180 min, after which a central nerve trunk attached to the ganglion was stimulated electrically (20 Hz) for 30 s in the absence and presence of 10 μmol/l TTX. The average secretory rate and pulse duration of the pulsatile insulin release were calculated. The secretory rate obtained in the absence of stimulation and TTX was set to 100%. Data are means ± SE for six experiments in which electrical stimulation in the absence of TTX evoked an increase in insulin release. *$P < 0.05$ versus control; †$P < 0.01$ versus electrical stimulation in the absence of TTX.

action potentials in these preparations (Fig. 3). There was a concomitant gradual decline of insulin release associated with a decrease in the insulin pulse amplitude, but with no change in the pulse duration. After a 10-min perifusion in the presence of HEX, insulin release in the presence of electrical stimulation was no longer different from that of control (Table 2). In the remaining seven preparations, no change in insulin release was observed when low-frequency stimulation was applied in the absence of HEX. The effect that adding HEX to the perifusion medium had on the membrane potential of the neuron and insulin release when no electrical stimulation was applied was investigated in six preparations (Table 2). Neither the membrane potential nor the amplitude or duration of the insulin pulses was affected by HEX under these conditions.

DISCUSSION

The influence of the parasympathetic and sympathetic nervous systems on blood insulin concentration has been studied extensively (18–22). Apart from these two branches of the autonomic nervous system, the enteric system of the gastrointestinal tract may also play an important role in regulating insulin release. A population of enteric ganglion neurons project from the distal stomach and proximal duodenum to the pancreas, where they synapse with islet cells directly or via intrapancreatic ganglia (23). Although the intrapancreatic neurons are likely to influence the secretory activity of the

TABLE 2
Neuronal stimulation of insulin release from pancreatic parenchyma attached to a pancreatic ganglion in the absence or presence of HEX

Electrical stimulation (Hz)	HEX (μmol/l)	Insulin release	
		%	min/pulse
—	—	100	3.8 ± 0.4
0.5	—	159 ± 10*	3.7 ± 0.9
—	50	104 ± 5	4.7 ± 0.5
0.5	50	114 ± 8†	5.5 ± 0.5

The preparation was perifused at 10 mmol/l glucose for 180 min, after which a central nerve trunk attached to the ganglion was stimulated electrically (0.05–0.5 Hz) for up to 30 min in the absence and presence of 50 μmol/l HEX. The average secretory rate and pulse duration of the pulsatile insulin release were calculated. The secretory rate obtained in the absence of stimulation and HEX was set to 100%. Insulin release in the presence of electrical stimulation was calculated from data obtained 5 min after introduction of stimulation. Insulin release in the presence of electrical stimulation and HEX was calculated from data obtained 10 min after introduction of HEX. Data are means ± SE for seven experiments in which electrical stimulation in the absence of HEX evoked a rise in insulin release and six experiments performed in the absence of electrical stimulation. *$P < 0.01$ versus control; †$P < 0.01$ versus electrical stimulation in the absence of HEX.

islet cells, very little is known about how they affect the plasma insulin oscillations.

Plasma insulin is oscillatory not only in normal subjects (1,24), but also in individuals with a transplanted pancreas (25). The latter observation, together with the pulsatile insulin release from the isolated perfused pancreas (8), indicates that the intrinsic intrapancreatic ganglion neurons may play a role as coordinators of the secretory activities of the islets in the pancreas. The rhythmic occurrence of spontaneous electrical activity in neurons of interconnected intrapancreatic ganglia, with similar frequency as in insulin release, further supports a role of the ganglia in the synchronization of the islets (13). When ganglionic activity was inhibited by TTX, the duration of the insulin oscillations from the perfused isolated pancreas was altered (11). Indeed, after prolonged perfusion with the neurotoxin, insulin release from the isolated pancreas was essentially nonoscillatory and sustained (26). These observations indicate that frequency modulation of pulsatile insulin release could occur via the intrapancreatic ganglia. However, when agents affecting the central (9) or peripheral nervous system (1,10) were administered in healthy individuals, no changes in the duration of the plasma insulin oscillations were observed. Instead, alterations in the amplitude of the regular variations in plasma insulin concentration were recorded. This preferred modulation of the amplitude of insulin oscillations has been observed in response to different secretagogues in the isolated perfused pancreas (8) and isolated islets (7).

Insulin release from islets of Langerhans connected to an intrapancreatic ganglion in the present study was pulsatile when the neurons showed a steady membrane potential, and the amplitude was modulated when the ganglion was electrically stimulated. The results demonstrate that pulsatile insulin release from individual islets does not require intermittent neural input from the intrapancreatic ganglia. Also, the denervated isolated islet has pulsatile insulin release (7). Although it has been suggested that pulsatile insulin from the isolated islet is controlled by an intrinsic islet nervous system that is sensitive to TTX (27), this was not confirmed in a more recent study (26). Furthermore, in the present study, neither TTX nor HEX had any effect on insulin release by themselves. However, TTX and HEX did block the increase in insulin release during orthodromic stimulation, which strongly suggests that the increase was mediated by the pancreatic ganglion. In some of the experiments in our study, there was no change in secretory activity during orthodromic nerve stimulation. This was interpreted as indicating that the islet cells of these experiments were not innervated by the particular pancreatic ganglion neurons under study.

The property of the isolated islet to release insulin intermittently may be related to an oscillatory metabolism. Regular variations in the ATP/ADP ratio (28), with a frequency similar to the insulin oscillations (1,7,8), support this notion. Because exocytosis of insulin granules is an energy-requiring process (29), it has been suggested that the oscillations in the ATP/ADP ratio could be responsible for the insulin oscillations (28,30). Indeed, we recently demonstrated that the regular variations in insulin release are synchronous with oscillations in oxygen tension in the isolated islet (31). The intrapancreatic ganglia may serve as coordinators of these intrinsic rhythmic secretory activities of the individual islets of Langerhans in the pancreas and explain the pulsatile release of insulin from the pancreas (8,11,12).

The stimulation of preganglionic nerves of extrinsic origin and perhaps also nerve fibers of intrapancreatic ganglion neurons is mediated by nicotinic transmission in the intrapancreatic ganglia (17), leading to transmitter release from the intrapancreatic ganglion neurons. Although intrapancreatic ganglion cells have been reported to contain peptides that inhibit insulin release (32), the observed increase in the amplitude of insulin release indicates the significance of acetylcholine in the transmitter release. Acetylcholine causes an inositol triphosphate–induced release of Ca^{2+} from intracellular stores via the cholinergic receptors, which leads to an elevation of the cytoplasmic calcium concentration ($[Ca^{2+}]_i$) in the pancreatic β-cells (33). Although this elevation is not oscillatory, it may increase the amplitude of the insulin pulses in the same way as secretagogues, which also induce nonpulsatile elevation in $[Ca^{2+}]_i$, cause rises in the amplitude of the pulsatile insulin release from isolated islets (34–36).

The present results are the first simultaneous measurements of electrical activity of intrapancreatic ganglion neurons and insulin release from attached islets and are consistent with a role of the ganglia in the amplitude regulation of insulin pulses. It can be speculated that intrapancreatic neuronal discharge could be an important factor for the normalization of blood glucose levels by increasing the amplitude of plasma insulin oscillations.

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