The role of metabolism in the generation of plasma insulin oscillations was investigated by simultaneous in vivo recordings of oxygen tension (pO₂) in the endocrine and exocrine pancreas and portal blood insulin concentrations in the anesthetized rat. At the start of the experiment, the blood glucose concentration of seven rats was 6.2 ± 0.1 mmol/l and the arterial blood pressure was 116 ± 5 mmHg. These values did not differ from those obtained at the end of the experiment. Islet pO₂ was measured by impaling superficially located islets with a miniaturized Clark electrode. The pO₂ measurements revealed slow (0.21 ± 0.03 min⁻¹) with superimposed rapid (3.1 ± 0.3 min⁻¹) oscillations. The average pO₂ was 39 ± 5 mmHg. Simultaneous recordings of pO₂ in the exocrine pancreas were significantly lower (16 ± 6 mmHg), but showed a slow and a rapid oscillatory activity with similar frequencies as seen in the endocrine pancreas. Corresponding measurements of portal insulin concentrations revealed insulin oscillations at a frequency of 0.22 ± 0.02 min⁻¹. The results are the first in vivo recordings of an oscillatory islet parameter with a frequency corresponding to that of plasma insulin oscillations; they support a primary role of metabolic oscillations in the induction of plasma insulin oscillations. Diabetes 51:699–703, 2002

The regular variations in blood concentrations of insulin, with a typical duration of 5–10 min (1–4), are critical to the hormone’s effect in lowering blood glucose (5–10). Despite this importance of insulin oscillations, their generation is still unclear. Oscillations of similar duration have been detected in the secretion of insulin from single isolated islets (11,12). Regular plasma insulin variations are therefore believed to be the result of coordinated secretory activities of the islets of Langerhans in the pancreas (10,13–16). In addition, oscillations in the cytoplasmic Ca²⁺ concentration ([Ca²⁺]ᵢ) and oxygen tension (pO₂) have been shown to be correlated with pulsatile insulin release from isolated islets (12,17–21). Therefore, rhythmic changes in ionic fluxes and metabolism are considered to be important for the regulation of plasma insulin oscillations. However, so far these slow in vitro oscillations of [Ca²⁺]ᵢ and pO₂ have not been demonstrated in vivo. In the present study, we performed in vivo measurements of pO₂ in rat pancreatic islets and in adjacent exocrine tissue and simultaneously determined portal vein insulin concentrations.

RESEARCH DESIGN AND METHODS

Materials. Reagents of analytical grade and water purified by a Milli-Q filter (Millipore, Bedford, MA) were used. Tetramethylbenzidine and insulin peroxidase were purchased from Sigma (St. Louis, MO); aprotinin (Trasylo; Bayer (Leverkusen, Germany); and heparin, from LeoLavens (Ballup, Denmark). The rat insulin standard was obtained from Novo Nordisk (Bagvaerd, Denmark). IgG-certified microtiter plates were purchased from Nunc (Roskilde, Denmark). The antibodies to insulin were raised in guinea pigs in our laboratory.

Animals. Male SD rats (n = 7) weighing 300–350 g were obtained from a local breeding colony (Biomedical Center, Uppsala, Sweden). The animals had free access to tap water and pelleted food at all times. The experiments were approved by the local animal ethics committee at Uppsala University.

Surgical preparation. The animals were anesthetized with an intraperitoneal injection of thiobutabarbital sodium (Inactin; Research Biochemicals International, Natick, MA: 120 mg/kg body wt), placed on a heated operating table maintained at body temperature (38°C), heparinized, and tracheostomized. Polyethylene catheters were inserted into the right femoral artery and the right femoral vein, respectively, to monitor the mean arterial blood pressure with a Statham P 23Db pressure transducer (Statham Laboratories, Los Angeles, CA) and to infuse Ringer solution (5 ml·h⁻¹·kg⁻¹) to substitute for body fluid loss. Next the pancreas of each animal was exposed through a midline abdominal incision and a catheter was placed in the portal vein by inserting a sharpened cannula, connected to the catheter, through the vascular wall and in the same direction as the blood flow. The cannula was loosely placed and no ligatures were used that could affect portal blood flow. Only 40 μl blood/min were diverted into the sampling catheter, with the rest of the portal blood being drained, as usual, into the liver. The catheter was connected to a peristaltic pump (Ismatec Reglo 4/12, Glattzüri, Switzerland), which allowed continuous sampling of portal blood. The pancreas was immobilized and superficial islets were visualized in situ by intravenous injection of 0.8-ml sterile-filtered 2% (wt/vol) neutral red (Kebo Greave AB, Stockholm, Sweden) (22). The staining procedure made it possible to insert one oxygen tension electrode into a superficially located islet and one into the adjacent exocrine parenchyma by the use of micromanipulators under a stereo microscope (23). The electrodes were placed ~10 mm apart. The injection with neutral red does not affect blood concentrations of glucose or insulin, blood flow of the whole pancreas or islet, or pancreatic oxygen tension measurements (22). The animals were allowed to rest for at least 30 min after the surgical preparation before recordings of oxygen tension and portal blood sampling were begun. Blood glucose concentration was determined in the beginning and end of each experiment with test reagent strips (Medisense; Baxter-Travenol, Deerfield, IL). Oxygen tension recordings and blood samples from the portal vein were collected continuously (40 μl/min) for ~30 min. At the end of the experiment, blood was also collected from the femoral vein for determination of the hematocrit.

Oxygen tension measurements. The oxygen tension electrodes were modified Clark microelectrodes (24,25), with an outer tip diameter of 2–6 μm and an inner tip diameter of 1–2 μm. The electrodes were polarized at ~0.8 V, which gave a linear response between the oxygen tension and the electrode current. The electrical current was measured by picocamperimeters (Universit y of Aarhus, Aarhus, Denmark). The electrodes were calibrated in water saturated with Na₂SO₃ or air at 37°C before and after the experiments. The drift of the microelectrode recordings was less than 0.5% per hour. The data were acquired at 4 Hz and processed by a MacLab Instrument (AD Instru-
RESULTS

In vivo pO2 of the rat endocrine and exocrine pancreas was monitored simultaneously with determinations of portal blood insulin concentrations in seven animals. The average islet pO2 was 30 ± 5 mmHg at the beginning of the experiment and remained stable throughout the experiment. Simultaneous measurements of pO2 in adjacent exocrine pancreatic parenchyma were significantly lower (16 ± 6 mmHg; P < 0.05) throughout the experiment. Both the endocrine and exocrine pO2 recordings showed a complex pattern of slow (0.21 ± 0.03 and 0.14 ± 0.02 min⁻¹, respectively) and superimposed rapid (3.1 ± 0.3 and 2.7 ± 0.2 min⁻¹, respectively) oscillatory activities (Fig. 1).

The portal plasma insulin concentration was oscillatory with a frequency of 0.22 ± 0.02 min⁻¹ and had an average value of 5.0 ± 0.6 nmol/l (Fig. 2). When analyzing portal insulin determinations and corresponding endocrine measurements of pO2 for oscillatory activity, averages of data points corresponding to 30 s of pO2 recordings were used. The frequency observed in portal insulin measurements was almost identical to that identified in the islet pO2 measurements.

The blood glucose concentration of the portal blood at the start of these experiments was 6.2 ± 0.1 mmol/l and did not differ from that at the end of the experiments (6.6 ± 0.2 mmol/l). Similarly, no change was observed when the mean arterial pressures at the start (116 ± 5 mmHg) and end (110 ± 6 mmHg) of the experiments were compared. The hematocrit was normal (46 ± 1%) at the end of the experiments.

Periodic insulin measurements represent the activity of the whole endocrine pancreas, which consists of islets situated at different distances from the portal sampling point. Synchronous changes in insulin release from islets situated in different parts of the pancreas to the portal sampling point could therefore perturb the pulsatile pattern if there were substantial differences in passage time among islets. To determine the passage time for insulin in the pancreas, d-glucose was injected into the ascending aorta in separate experiments. The glucose concentration was then determined in portal blood samples at 0, 10, and 20 s after the injection (n = 3). We observed that the blood glucose concentration had already risen significantly after 10 s, indicating an intrapancreatic passage time of 10 s or less. In control saline-injected animals (n = 3), portal blood glucose concentrations were not affected (data not shown).

DISCUSSION

The present in vivo measurements recorded oscillations in islet pO2 and plasma insulin with similar frequencies, but also recorded rapid oscillatory activity in pO2, present in the endocrine and exocrine pancreas. The values of pO2 of the endocrine and exocrine pancreas were similar to previously obtained values (23). The significantly lower pO2 in the exocrine tissue is likely to have been related to the lower blood flow in this part of the pancreas (22,26).
Recordings from the endocrine and exocrine pancreas showed a slow and a superimposed rapid oscillatory activity, with approximate frequencies of 0.2 and 3 min⁻¹, respectively. The slower activity had a frequency similar to that of the oscillations in the portal vein insulin, which matched results obtained when portal plasma insulin was analyzed in a canine model (3), but was about double the frequency previously reported for plasma insulin oscillations in the rat (27) and humans (2). The lower frequency observed in the latter studies was most likely attributable to the peripheral sampling and lower sampling rate, which make pulse detection more difficult, as has been shown in the canine model (3). Indeed, when using a sensitive insulin assay and deconvolution, plasma insulin pulses with a period of 4.7 ± 0.1 min have been detected in peripheral blood samples from humans (4). A correlation between oscillations in PO₂ and insulin has already been described in the isolated islet (21) and in groups of islets (17). From this point of view, the present in vivo finding of a slow oscillatory activity in PO₂ was not unexpected. However, from the perspective that slow oscillations in neither [Ca²⁺]ᵢ nor PO₂ have so far been demonstrated in vivo, the finding is important and represents the first in vivo recording of an oscillatory islet parameter with a frequency corresponding to that of plasma insulin oscillations.

Slow in vitro oscillations in [Ca²⁺]ᵢ (12,18,19,28,29) as well as in metabolism (17,21,30–35), with a frequency similar to that of the plasma insulin oscillations, have been recorded in the isolated islet. The key role of [Ca²⁺]ᵢ (36) and metabolism (37) for exocytosis of insulin and the similar frequency of the slow [Ca²⁺]ᵢ and metabolic oscillations and those in plasma insulin have promoted the notion that regular variations in plasma insulin may reflect either [Ca²⁺]ᵢ, or metabolic oscillations of the β-cell, or a combination of the two. More direct support for this concept was obtained by parallel measurements of [Ca²⁺] and insulin release (12,18,19,29) and PO₂ and insulin release (17,21) from individual islets. In these studies, slow oscillations in [Ca²⁺]ᵢ, and PO₂ were always synchronous with pulses of insulin release. However, when islet [Ca²⁺]ᵢ was nonoscillatory after exposure to low glucose concentrations or depolarizing conditions, the corresponding insulin release was still pulsatile (38,39). In contrast, when PO₂ was measured under the same conditions, oscillations were recorded that were synchronized with pulsatile release of insulin (21). Oscillations in metabolism have been proposed to be the result of spontaneous glycolytic oscillations through allosteric regulation of phosphofructokinase-1-M (30). In support of such a role of the enzyme, impaired plasma insulin oscillations have been observed in individuals with mutations in the gene for the enzyme (40). The present report of in vivo PO₂ oscillations that are synchronous with plasma insulin oscillations further supports a primary role of metabolic oscillations in the induction of plasma insulin oscillations.

Until now, [Ca²⁺]ᵢ and membrane potential were the only islet parameters besides insulin that had been monitored in vivo (41–43). In vivo measurements of [Ca²⁺]ᵢ, and membrane potential have demonstrated oscillatory activity that was faster (2–5 min⁻¹) than the frequency of the plasma insulin oscillations. The rapid oscillations in membrane potential consist of “slow waves,” which are periods of depolarization, when accumulations of action potentials (or “bursts”) promote influx of Ca²⁺, interspersed with periods of hyperpolarization when no Ca²⁺ influx is present. These “slow waves” give rise to the rapid oscillations in [Ca²⁺]ᵢ, which have been extensively characterized in the isolated islet (28,44–47). The observed superimposed, rapid oscillatory in vivo activity of PO₂ has shown a similar frequency. Such rapid changes in PO₂ have been previously observed in vitro and have been related to rapid oscillations in [Ca²⁺]ᵢ (34,35). The relation between the rapid oscillatory activity in metabolism and ionic movements is complex; two explanations that have been suggested are that ionic changes drive metabolism by activating mitochondrial dehydrogenases (33,48–50) and that oscillatory metabolic changes precede the ionic movements (31,51). Irrespective of causality, it is reasonable to assume that these rapid ionic and metabolic events have
their secretory counterpart in individual islets in vivo, as has been demonstrated in the isolated islet in vitro (19,47,52–56). With the presently found estimated passage time of insulin in the pancreas of \(<10 \text{ s}\), these rapid secretory events would theoretically be detectable in the portal vein. However, given that no synchronization of the rapid oscillations in electrical activity of different islets from the same pancreas in vivo has been observed (57), it is less plausible that the rapid secretory events are present in plasma insulin. Another consequence of the rapid passage time of the blood through the pancreas is that blood-borne factors could play a role in coordinating the secretory activities of the islets in the pancreas (10,58). It has been suggested that the intrinsic nervous system of the pancreas is responsible for interislet coordination (13–16,59), or that the relaying of membrane potential changes among islets via the exocrine tissue is a possible mechanism for synchronizing the islets in the pancreas (10).

In conclusion, the recorded oscillations in \(\text{pO}_2\), with a frequency similar to that of plasma insulin oscillations, support the presence of insulin oscillations in vivo in each individual islet, as well as in groups of islets, and that these oscillations are dynamic entities that may contribute to the pulsatile secretion of insulin.

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