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Review

Pulsatility of insulin and glucagon release: physiological significance and pharmacological implications

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Numerous endocrine systems are characterised by pulsatile hormone secretion with periodicities ranging from minutes to hours [1]. Such endocrine rhythms have been described among others for growth hormone, prolactin, gonadotropins, parathyroid hormone and corticosterone. The oscillatory pattern of plasma hormone levels may be physiologically important to reduce down regulation of receptors and, consequently, to enhance hormone action. Intermittent administration of hormones is now widely used in therapeutics since pulsatile administration of growth hormone releasing factor [2] or gonadotropin-releasing hormone [3] appears to be more efficient than continuous delivery.

Though circumstantial evidence for the existence of fluctuations in plasma glucose and insulin levels was published earlier, the current interest in the pulsatility of pancreatic hormones secretion dates from the now classical study of Goodner et al. [4]. Ten years after its publication, we will review the available data on the existence of oscillations in peripheral and, when available, portal levels of insulin and glucagon both in animals and in man. In vitro data on pulsatile insulin and glucagon release will be reviewed, with emphasis on the mechanisms involved and possible relationships with oscillations in B-cell-membrane potential. Comparative effects of pulsatile and continuous insulin and glucagon delivery will then be analysed. Finally, the potential importance of the mode of insulin delivery for the management of diabetes mellitus will be briefly considered.

Oscillations in plasma concentrations of insulin and glucagon

In man

The first description of blood glucose oscillations in man was made more than 60 years ago [5] and was confirmed by several subsequent studies [6-8]. Demonstration of oscillations in the circulating levels of pancreatic hormones is only 10 years old. In fact, early studies, performed in three normal subjects submitted to 2 min sampling for 2 h, failed to evidence regular oscillations of plasma glucose, insulin and glucagon; glucose and insulin, however, did display intermittent synchronous fluctuations of small amplitude [4]. In a study reported in 1979 by Lang et al. [9] plasma samples were collected at 1-min intervals from 10 normal subjects for periods lasting from 1-2 h. In five of these subjects, basal plasma insulin levels cycled regularly with a mean period of 13 min, and a mean amplitude of 1.6 $mU \cdot l^{-1}$. A concurrent plasma glucose cycle was demonstrated with a mean amplitude of 0.05 $mmol \cdot l^{-1}$; the average plasma glucose cycle was 2 min in advance of the plasma insulin cycle (Fig. 1). In the subjects with less regular plasma insulin cycles, a similar plasma glucose rise was demonstrated 2 min before the insulin rise. These phase relations suggested the existence of a negative feedback loop between the liver and pancreatic B cells, that might regulate both plasma insulin and glucose concentrations. The possibility that B cells could be stimulated by a cyclic, glucose-independent input was not ruled out, however [9]. The existence of plasma insulin cycles was confirmed by the same group [10] in a larger series of 28 subjects in whom the mean period averaged 10.7 min and the mean amplitude 1.1 $mU \cdot l^{-1}$. Glucagon cycles were also demonstrated with a mean period of 13.7 min and a mean amplitude of 5.5 $ng \cdot l^{-1}$. There was a significant correlation between the amplitude of simultaneous plasma insulin and glucagon cycles and cross-correlation showed that the changes in plasma glucagon levels lagged only 2 min behind the changes in plasma insulin levels. In another study, also performed in fasted volunteers [11], plasma levels of insulin were found to oscillate with a sustained periodicity of 11-13 min. The periodicity was similar (10-13 min) for plasma glucose levels, but ranged from 7 to 26 min for plasma glucagon levels. No consistent relationship could be found by cross-correlation analysis between the periodic fluc-

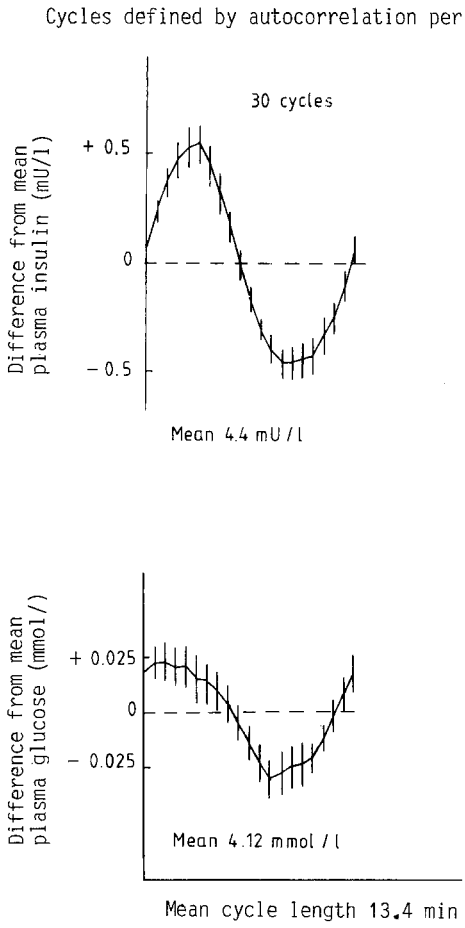

Fig. 1. Insulin and glucose cycles in normal man. Data obtained using the "standard array-averaging technique". The curves show the mean ± SEM plasma insulin and glucose changes in five subjects. The plasma glucose oscillation precedes the insulin by 2 min. The mean plasma insulin amplitude was 1.0 mU/l after averaging the standard array, but 1.6 mU/l when estimated by the differences between troughs and peaks of individual cycle. From Lang et al. [9]. Reproduced with permission of the copyright holder

tuations in insulin, glucose and glucagon. Taken together, these observations made it unlikely that fluctuations in glucose levels were driving pulsatile secretion by A and B cells. A further large series of normal subjects was studied in an attempt to identify the pacemaker mechanisms of these oscillations in hormone release [12]. It was shown that, in the basal state, pulsation frequency of insulin was stable through cholinergic, endorphin, α-adrenergic or β-adrenergic blockade. Small pulses of glucose or insulin also failed to reset the cycles. Moreover, stimulations of insulin secretion with glucose, tolbutamide or sodium salicylate increased the amplitude of insulin oscillations without changing their period [12].

Plasma glucose and pancreatic hormone oscillations were also studied in a few pathological states. In obese subjects, the periodicity of the oscillations in plasma insulin, glucagon and glucose was similar to that observed in the normal weight subjects [11]. In contrast, in Type 2 (non-insulin-dependent) diabetic patients, the oscillations in basal plasma insulin were irregular [13] and the associated glucose swings were about six-fold larger than in normal subjects [12]. Variable results were obtained in six vagotomised patients [12]. Two of them showed regular plasma insulin oscillations in the normal range, while three others had longer periodicities and one subject had irregular changes. Interestingly, the fluctuations in plasma glucose levels were larger and exhibited a longer period in those vagotomised patients than in control subjects. In two patients who had undergone resection of the head of the pancreas (Whipple's operation), plasma insulin levels displayed long-term oscillations with a mean period of 37 min [12]. It should also be noted that besides these "rapid" fluctuations, large concomitant oscillations of insulin and C-peptide levels, with an average period of 80 min, have been described in normal man during continuous enteral infusion [14].

The results of all these studies in man have led to the proposal [13] that the 12-13 min cycle of insulin levels is due to cyclic hormonal release under the control of a pancreatic pacemaker that could be localised in the head of the organ, and that does not involve adrenergic, muscarinic or endorphin receptors. This pacemaker could be under vagal control and function within the context of a feed-back loop (period 30-40 min) between glucose production by the liver and insulin release by the B cells.

In animals

In a single dog, Anderson et al. [15] reported rapid (period of less than 1 min) oscillations in basal concentrations of pancreatic venous insulin and hepatic venous glucose. These oscillations tended to show synchrony for about half of a 6-min period. In a more recent and complete study also performed in the dog [16], frequent sampling from the portal vein revealed basal, spontaneous oscillations of all four pancreatic peptides. Moreover, the pulsatility of C-peptide levels was remarkably parallel to that of insulin, whereas glucose fluctuations were inconsistent. Insulin and glucagon oscillations were the most prominent, were in phase and had a period of 10-14 min. Pulses of pancreatic polypeptide were less frequent, though always associated with insulin pulses. Somatostatin pulses were less consistently associated with those of other peptides [16]. Interestingly, in one of nine dogs, pulsatility of all four hormones included components of both shorter and longer periods. In longer experiments (up to 12 h) carried out in fasted dogs, less frequent blood sampling (every 7.5-15 min) evidenced slow fluctuations in portal and peripheral concentrations of insulin, glucagon and somatostatin, whereas glucose levels did not significantly fluctuate [17]. The period of these oscillations ranged between 32 and 107 min. On the other hand, in 3 pancreatectomized dogs, extrapan-

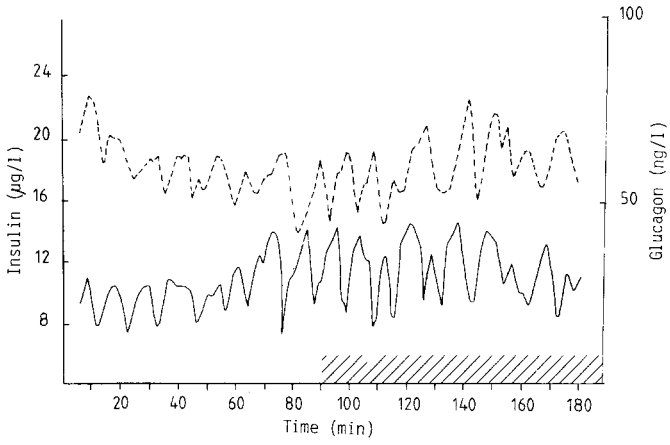

Fig. 2. Comparison of insulin and glucagon cycles in the isolated perfused canine pancreas. Representative cycles of insulin (solid line) and glucagon (broken line) from a single pancreas before and after combined blockade by atropine, propranolol, and dibenziline. Combined blockade (shaded area) was without effect on cycle frequency or the ratio of peak amplitude to the total secretion during each cycle. The shapes of the glucagon curves are exaggerated by the scale which is relative to insulin. From Stagner et al. [24]. Reproduced with permission of the copyright holder

creatic glucagon and somatostatin appeared to be secreted in nonperiodic, randomly occurring pulses [17].

Oscillations in blood glucose and insulin levels were also studied during hyperglycaemia. In 1974, Ookthens et al. [18] reported that, in dogs, plasma glucose and insulin displayed regular, synchronous oscillations with a period of about 50 min for up to 10 h of constant glucose infusion. In another study, constant infusion of glucose into conscious dogs induced oscillations in plasma glucose concentration with an even longer period averaging 112 min [19]. They were accompanied by similar oscillations in plasma insulin levels, lagging 22 min behind glucose cycles. However, a further study showed that clamping plasma glucose concentration at a similar level (by adjusting the infusion rate) did not obliterate but rather augmented the oscillations in plasma insulin levels [20].

In overnight fasted rhesus monkeys, Goodner et al. [4] reported the occurrence of synchronous, regular oscillations in the peripheral plasma concentrations of glucose, insulin and glucagon. The oscillations displayed a mean period of 9 min, and the amplitudes for insulin and glucagon were ten- and five-fold greater than for glucose. Insulin cycled in, and glucagon out, of phase with glucose. Subsequent measurements further showed that plasma C-peptide levels were oscillating in synchrony with glucose [21]. Recently, direct sampling in the portal vein of two baboons confirmed that regular insulin cycles (period 9–10 min) were consistently followed by glucagon pulses 4–5 min later [16]. These latter experiments also revealed that peaks in the profile of pancreatic polypeptide occurred in synchrony with either insulin or glucagon, whereas the fluctuations of somatostatin were erratic, without significant periodicity.

Further studies were performed in the rhesus monkeys to gain insight into the mechanisms underlying these oscillations. Neither the period, nor the relative amplitude of the oscillations in plasma levels of insulin and glucose were affected by muscarinic or α- and β-adrenergic blockade, in spite of changes in mean absolute levels of plasma insulin [22]. General anaesthesia with pentobarbital similarly failed to eliminate the oscillations. Taken together these observations reduced the likelihood that the oscillations originate from a pacemaker in the central nervous system [22]. In 12 rhesus monkeys, both relative and absolute amplitudes of insulin oscillations increased after a mixed meal, but the period of oscillations did not change [23].

Conversely, food deprivation for up to 88 h decreased amplitude and regularity of the oscillations, but, again, did not affect the underlying periodicity of insulin, glucose and glucagon cycles [23].

Pulsatile insulin and glucagon secretion in vitro

From the isolated perfused pancreas

As illustrated by Figure 2, sustained oscillations in the release of insulin, glucagon and somatostatin from the isolated canine pancreas perfused at a constant concentration of glucose were convincingly demonstrated by Stagner and Samols [24, 25]. For insulin cycles a period of about 10 min was estimated from raw data [24], but a shorter period of 7.4 min was computed by mathematical analysis of the results [25]. Insulin and somatostatin cycles were in phase, but glucagon cycles were less regular, shorter and not consistently 90° out of phase with insulin cycles [24]. Similar oscillations in insulin release were observed when the two lobes of the pancreas (respectively rich in glucagon or in pancreatic polypeptide cells) were perfused separately [26]. The period of insulin cycles was unaffected by the concentration of glucose (5 or 11 mmol/l) in the medium perfusing the whole pancreas [24, 25] or the lobe rich in glucagon cells [26], but became shorter when a high glucose medium was used to perfuse the lobe rich in pancreatic polypeptide cells [26].

Persistence of the pulsatility of insulin release in the isolated pancreas suggested that it depends on a pacemaker system present in the gland itself. Several approaches were thus followed to determine whether insulin oscillations are controlled by the intrapancreatic nervous system. First, insulin cycles were disrupted by infusions of various neurotransmitters [24]. This observation means that the mechanisms controlling the oscillations can be overriden by neural influences, possibly acting directly on B cells. Second, and as illustrated by Figure 2, insulin cycles persisted unabated during infusion of atropine, propranolol, and dibenzyline [24]. This shows that muscarinic and adrenergic receptors play no major role in the generation of these oscil-

lations. Third, insulin cycles were altered by tetrodotoxin, a blocker of Na channels [25]. Though this effect may have resulted from the expected nerve blockade, other possibilities cannot be ruled out. Indeed, tetrodotoxin is known to inhibit glucose-induced somatostatin release from the perfused canine pancreas [27] and to alter glucose-induced electrical activity in mouse B cells [28, 29]. Fourth, the period of insulin cycles was decreased by infusion of postsynaptic nicotinic receptor antagonists [30, 31], which are not known to exert direct effects on B cells. These observations are in keeping with the hypothesis that the network of pancreatic ganglia and interconnecting nerves has a role in the control of insulin cycles. However, an alternative interpretation would be that these pharmacological manipulations release a substance or substances (peptidergic?) that override the pacemaker control system and cause the observed increase in insulin secretion. Coordination by a network of ganglia and nerves would also imply the presence of connections across the plane of connective tissue separating the two pancreatic lobes of distinct embryological origin [32, 33], or the existence of two separate but similar pacemakers. To the best of our knowledge, sustained fluctuations of insulin release by the perfused rat or mouse pancreas have not been described. It has only been reported that inhibition of glucose-stimulated insulin release by clonidine disclosed an apparently rhythmic pattern of secretion for a short period of time [34].

From isolated islets

There have been only few attempts to determine whether insulin release by isolated islets is pulsatile or not. A pulsatile pattern was described by using a system in which a single mouse islet was attached to the tip of a micropipet by suction, and dipped into successive small cups [35, 36]. However, this system resulted in the release of enormous amounts of insulin (5–25 μU/15 s for a medium-size islet). This is incomparably more than the rate of insulin release measured in any system of perifusion using groups of islets, or even single large islets [37]. Moreover, the experiments with the dipping technique were performed at 30–31 °C. Since the B-cell membrane is likely to be persistently depolarised at that temperature [38], the attempts to correlate the alleged periodicity of insulin release with oscillations of B-cell-membrane potential [36] are not grounded.

It has also been claimed that insulin release by single mouse islets is pulsatile with a similar periodicity to phases of electrical activity simultaneously recorded in B cells [39]. While it is acknowledged that such experiments are difficult to perform, it is obvious that a more detailed and complete account than an abstract is required to carry conviction. In particular, it is puzzling that insulin release was found to stop after each phase of electrical activity, whereas, in another study, release

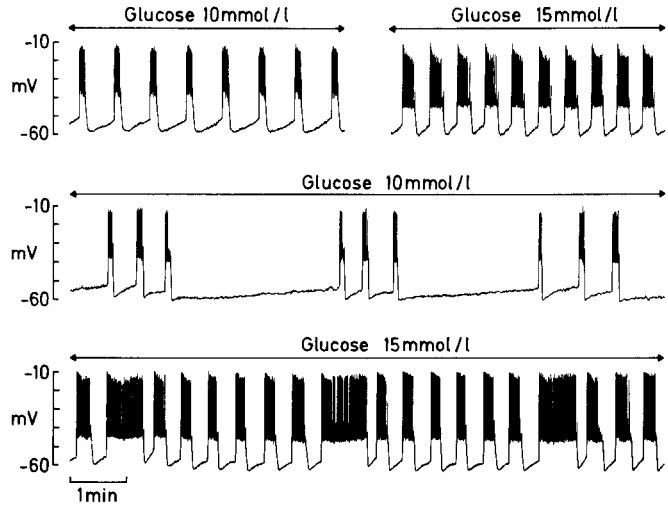

Fig. 3. Effects of 10 and 15 mmol/l glucose on the membrane potential of mouse B cells. The four records were obtained in different mice

continued for quite a while after electrical activity had been stopped by a decrease in glucose concentration [40]. A last short communication reported on the periodicity of insulin release from individual islets of obese rats [41]. These measurements were made just after the change from a glucose-free medium to a medium containing 28 mmol/l glucose. The sampling period (5 min) thus corresponds to the first phase of insulin release and not to steady state release. Furthermore, it is rather surprising that, in these experiments [41], insulin release was stimulated without delay, in spite of the long (45 min) initial period of perifusion with a glucose-free medium. Finally, glucose-stimulated insulin release by groups of perifused rabbit islets was reported to display an irregular, "spiky" pattern, that did not characterise insulin release by groups of rat islets [42]. This peculiarity has neither been confirmed, nor further analysed.

In summary, conclusive evidence for or against pulsatility of insulin release by isolated islets is still lacking. Demonstration of its presence (or absence) also requires precise characterisation of the immunoassay and careful mathematical analysis of the data, as has been done in in vivo studies.

Oscillations of B-cell-membrane potential

It is doubtful that classical methods will ever be able to demonstrate whether insulin release from a single B cell is pulsatile or not. This might perhaps become possible by monitoring changes in membrane capacitance [43], if these latter prove to reflect adequately exocytosis-endocytosis processes.

On the other hand, it has long been recognised that the membrane potential of mouse B cells oscillates rhythmically during stimulation by glucose concentrations which induce insulin release [44, 45]. These oscil-

lations (Fig. 3) are characterised by phases of depolarisation (slow waves) with bursts of spikes superimposed on the plateau, alternating with phases of silent repolarisation (intervals). The ionic currents underlying these electrical events, and the mechanisms whereby glucose controls them are not yet fully understood (for a review see 46). Nevertheless, strong evidence supports a causal role of these phenomena in glucose-induced insulin release.

In many B cells, slow waves and intervals remain very regular as long as the concentration of glucose remains constant (Fig. 3, upper records). However, they can vary in frequency, amplitude or duration from islet to islet. Smaller differences may also exist between B cells within the same islet, probably because not all B cells of the islet are (permanently) electrically coupled [47, 48]. In certain cells, slow waves and intervals themselves also display fluctuations in their duration. These fluctuations were first recognised during steady-state stimulation with a mixture of glucose and leucine [49]. Thereafter, it became evident that they are sometimes present during stimulation with glucose alone [50, 51]. Figure 3 shows examples of these fluctuations in two B cells stimulated by 10 or 15 mmol/l glucose.

Studies of these oscillations is fraught with the difficulty that they may spontaneously disappear with time. From retrospective analysis of the recordings, it appeared that their incidence was higher (45 vs 20% of B cells) and their period slightly longer (3.9 vs 3.4 min) in the presence of 15 vs 10 mmol/l glucose [50]. Their presence also seemed to be unrelated to islet size or to the season, and did not require connection of the islet to extrainsular tissue [51]. Attempts to disrupt these oscillations showed that they were insensitive to blockade of muscarinic, or α- and β-adrenergic receptors [50]. Preliminary experiments also suggest that they are unaffected by blockade of opiate and nicotinic receptors (J.C. Henquin and W. Schmeer, unpublished data). A *reversible* suppression of these oscillations could only be produced by increasing cAMP levels in B cells. Thus, upon addition of a low concentration of forskolin, a potent activator of the adenylate cyclase, the electrical activity became very regular, with slow waves and intervals of constant duration [52]. In summary, the mechanisms and significance of the slow fluctuations of glucose-induced electrical activity present in certain B cells, and their possible relationship with pulsatile insulin secretion are still unknown.

Nature of the pacemaker

It is now generally agreed that the fluctuations in plasma insulin levels measured in vivo reflect similar fluctuations of insulin release, and that the pacemaker of these oscillations is localised in the pancreas itself. It might be premature, however, to dismiss a possible *modulatory* role of extrapancreatic factors. Thus, the period of insulin cycles measured in the portal vein of conscious dogs averaged 12 min [16], as compared to 7.6 min in the isolated canine pancreas [25].

There is no experimental evidence that paracrine influences play a role in the generation of insulin cycles, but no decisive argument permits one to exclude it formally either. As already emphasised, the hypothesis currently in favour is that the ensemble of connected ganglia and associated nerves of the pancreas synchronizes islet hormone oscillations [30, 31]. Whether this synchronization is exerted directly on B cells or employs less direct pathways is unknown. Final proof that pulsatility of insulin release is under neural control may require its blockade by a specific antagonist that is without direct effect on islets freed from all neural connections.

An alternative possibility is that pulsatility of release is an intrinsic property of each islet or of each B cell. Fluctuations measured in portal or peripheral blood could thus be a beat oscillation of secretory events occurring with a more rapid, but not necessarily synchronous periodicity in all islets, and damped by diffusion and dilution [50, 53]. Strong support for this possibility was recently provided by a study performed in dogs with intrasplenic islet autografts [54]. These animals exhibited oscillations in basal portal insulin levels which were similar to those measured in control dogs. Obviously, both confirmation of this new observation and experiments using single isolated islets are necessary to assess this hypothesis. In this perspective a prerequisite is the demonstration that oscillations in portal insulin levels exist (in vivo and in vitro) in the species from which the islets are isolated. It would be unwise to assume, a priori, that they must exist and have the same periodicity in every species as has been reported in man, monkeys or dogs.

Comparative effects of continuous and pulsatile hormone delivery

In vivo studies in fasted rhesus monkeys demonstrated that the oscillations in the concentration of plasma glucose reflect fluctuations in hepatic glucose production, and that these fluctuations were synchronous with the oscillations in insulin and glucagon concentrations [55]. This synchrony suggests that hepatic glucose metabolism is entrained to the islet cycle. Though the physiologic and physiopathologic significance of these observations remains to be established, several studies were performed to assess the possible biologic advantages of cyclic delivery of pancreatic hormones.

Insulin

Matthews et al. [56] were the first to suggest that pulsatile insulin might have greater biologic effects than continuous delivery: in six normal subjects in whom

pancreatic insulin output was suppressed by somatostatin, they showed that intermittently delivered insulin (2 min pulses separated by gaps of 11 min) had greater hypoglycaemic effects than the same amount of insulin delivered continuously. Such differences were significant only after 7 h of insulin infusion. A recent study by Schmitz et al. [57] confirmed these findings: in 8 healthy subjects soluble insulin was given intravenously either at a constant rate or in identical amounts in pulses of 1.5 to 2.25 min separated by intervals of 10.5 to 9.75 min. The amounts of insulin infused completely inhibited endogenous glucose production, as estimated by the [3-^{3}H] glucose infusion technique. Glucose uptake, expressed as metabolic clearance rate (MCR), was significantly increased as compared with continuous administration. Here again the superior efficacy of pulsatile insulin delivery in glucose uptake was not consistently found until after 210 min of insulin administration. In experiments of shorter duration, as those of Verdin et al. [58] or Paolisso et al. [59], such superior efficacy of pulsatile insulin was not found.

Several factors may be critical for the demonstration of the superior efficacy of pulsatile insulin. First, insulin is infused in a peripheral vein and not in the portal vein where the largest spontaneous fluctuations occur. Second, the greater efficacy could be lost if insulin pulses are not accompanied with glucagon pulses or are asynchronous with endogenous glucagon cycles. Third, and as illustrated by Figure 4, slight hyperglucagonaemia, in the range of about 200 $ng \cdot l^{-1}$ is sufficient to abolish the higher efficacy of pulsatile insulin [60].

In vitro studies by Komjati et al. [61] have confirmed the superior efficacy of pulsatile versus continuous insulin exposure on the isolated perfused rat liver. In this system, insulin (100 $mU \cdot l^{-1}$) given continuously and intermittently (3 min on/off intervals) inhibited glucagon-stimulated hepatic glucose production to the same extent (37.4 and 41.1% respectively). Doubling the off-period to 6 min, and thereby reducing the total insulin dose to 33%, did not significantly diminish the suppressive effect of insulin on glucagon-stimulated hepatic glucose release (34.6%).

It is also possible that the fluctuations in plasma insulin concentration are of importance for the regulation of islet-cell function itself. In fasted anaesthetized rats [62], the rise in plasma insulin levels induced by an intravenous glucose challenge was attenuated by previous intraportal infusion of insulin in a continuous mode, but was augmented if the same amount of insulin had been given in pulses. In normal human subjects (Paolisso et al., submitted for publication), whose blood glucose was maintained constant, insulin infusion resulted in a significant decline in basal plasma glucagon and C-peptide, and in a clearcut decrease in the glucagon response to subsequent arginine stimulation. All these changes were more pronounced when identical amounts of insulin were delivered in a pulsatile rather than in a continuous manner. Obviously these in vivo studies do not indicate the site of islet-cell control by insulin. It could well be intrainsular for the A-cell [63] and extrapancreatic (neurally mediated) for the B cell [64].

Glucagon

In contrast to insulin, the functional consequences of pulsatile glucagon delivery have been less investigated. Weigle et al. [65] have shown that administering glucagon as a series of brief pulses to perifused rat hepatocytes resulted in the production of a greater total amount of glucose than was obtained when the same amount of glucagon was administered as a continuous infusion. The response augmentation by pulsatile glucagon administration was interpreted as a delayed relaxation in hepatocyte glucose production after termination of each hormone pulse. Using a model based on the waveform of the hepatocyte response to a transient glucagon stimulus, the same authors [66] demonstrated that the time constant for response decay was an important determinant of the relative efficacy of continuous and intermittent hormone delivery. In further studies using the same in vitro system, Weigle and Goodner [67] reported that the enhancement of hepatic glucose production by glucagon-pulses is a frequency-dependent phenomenon and that hepatic glucose production is optimised for interpulse intervals of 10–20 min, a period close to the physiological secretory period of 10 min observed in non-human primates [4, 22, 23] and of 14–20 min reported in humans [10–12]. Recently, Komjati et al. [61] have investigated the effect of pulsatile versus continuous glucagon exposure on glucose production from the isolated perfused rat liver. They observed that continuous exposure to glucagon (35 $pmol \cdot l^{-1}$) induced a two-fold increase in hepatic glucose production, while intermittent exposure (3 min on/off intervals; total dose 50%) to the same glucagon concentration elicited an almost identical increase in hepatic glucose output. Therefore, all these in vitro studies concur to demonstrate that pulsatile delivery of glucagon is more efficient that continuous exposure to stimulate hepatic glucose production. The only study on the respective effects of continuous and intermittent glucagon infusion in vivo performed in man has been by Paolisso et al. [68]. Six male volunteers were submitted to a 260 min glucose-controlled glucose intravenous administration using the Biostator. The endogenous secretion of the pancreatic hormones was inhibited by somatostatin. Basal insulin secretion was replaced by a continuous insulin infusion resulting in steady plasma insulin levels averaging 8–11 mU/l. Glucagon was infused intravenously in two conditions at random: either continuously or intermittently with a switching on/off length of 2/11 min. During continuous glucagon infusion, glucagon plasma levels averaged 189 ± 38 ng/l; during pul-

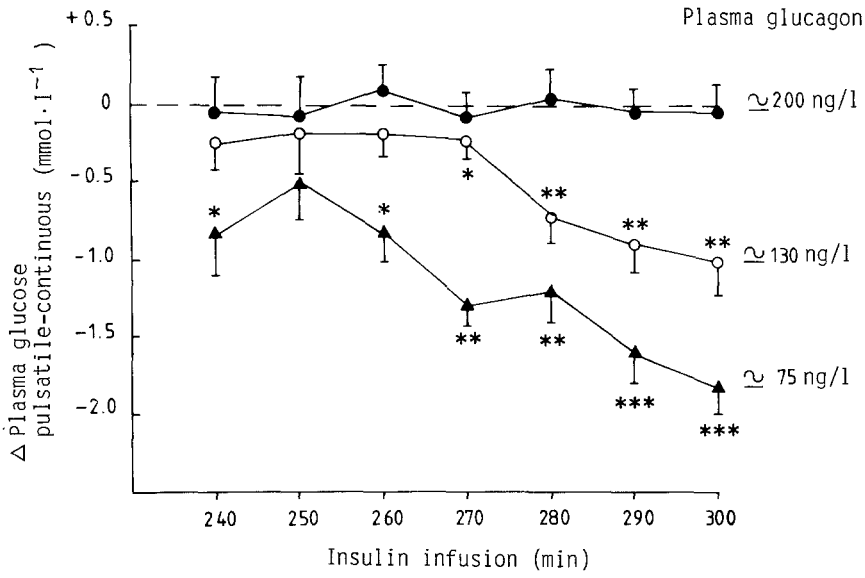

Fig. 4. Differences (Δ) in plasma glucose concentration between pulsatile and continuous modes of insulin delivery. The same six Type 1 diabetic patients were investigated at three different plasma glucagon levels, as indicated. During the fifth hour of infusions, there was no difference between the two modes of insulin administration when somatostatin-suppressed endogenous glucagon was replaced by exogenous infusion maintaining circulating glucagon levels at about 200 ng/l. The superior efficacy of pulsatile over continuous insulin infusion appears for glucagon levels averaging 130 ng/l and is more marked when glucagon levels are around 75 ng/l. From Paolisso et al. [60]. Reproduced with permission of the copyright holder

satile infusion they varied between 95 and 501 ng/l. When compared with continuous delivery, pulsatile glucagon infusion: (a) initially induced a similar increase in endogenous (hepatic) glucose production and blood glucose, (b) did not prevent the so-called "evanescent" effect of glucagon on blood glucose, and (c) after 3 h tended to reduce rather than increase hepatic glucose production [68]. The reasons for the discrepancy between in vitro and in vivo experiments using glucagon are still unclear. The relatively high circulating glucagon levels achieved and the characteristics of glucagon kinetics in vivo in man are likely to be involved. We have speculated [68] that in the conditions achieved in our experimental protocol in vivo, the hepatocytes remain exposed to circulating glucagon concentrations which, despite the intermittent mode of administration, may still be high enough to desensitise the hepatocytes even more than during continuous administration of the hormone. As reviewed by Rodbell [69], the intimate mechanism of such a desensitisation, already reported in various systems [70, 71], is still not understood.

Pulsatility of insulin delivery and diabetes management

In Type 1 (insulin-dependent) diabetes

Bratusch-Marrain et al. [72] recently compared the effects of continuous and pulsatile insulin administration on hepatic glucose production-utilisation in Type 1 diabetic patients submitted to a euglycaemic insulin clamp procedure. The total amount of insulin infused in the pulsatile manner was 40% less than when it was infused continuously.

Despite this reduction, insulin given in the pulsatile manner was equally potent in reducing hepatic glucose production and stimulating glucose utilisation. The authors suggested that intravenous pulsatile administration of insulin might reduce systemic hyperinsulinaemia and, in the long run, attenuate insulin resistance by reversing down-regulation of insulin receptors. Paolisso et al. [60] confirmed the greater efficacy of pulsatile insulin in reducing plasma glucose in Type 1 diabetic patients (Fig. 4) but demonstrated that this effect was critically dependent on plasma glucagon levels: completely absent at plasma glucagon concentrations averaging 200 ng/l, it was observed at glucagon levels averaging 130 ng/l; and occurred earlier, and was more pronounced when plasma glucagon concentrations were about 75 ng/l. Another beneficial effect of pulsatile insulin administration in diabetic patients could be a reduction of hyperglucagonaemia, a universally found feature of diabetes [73]. Thus, the exaggerated response of the pancreatic A-cell to intravenous arginine observed in patients with diabetes was significantly reduced when small amounts of insulin were delivered in a pulsatile manner while identical amounts delivered continuously were without significant effect (Paolisso et al., submitted for publication). However, attempts to achieve better control of Type 1 diabetes by pulsed insulin given *subcutaneously* have failed. In the study performed by Levy-Marchal et al. [74] in 6 Type 1 diabetic patients, overnight metabolic control was similar when a given amount of insulin was delivered subcutaneously either in a continuous manner or intermittently as pulses spaced at 30-, 60- and 120-min intervals. Under those conditions, however, no oscillations in plasma insulin were achieved. In contrast to what has been reported above with intravenous insulin administration [72], a reduction of the dose of insulin infused subcutaneously was not compensated for by the intermittent administration of the hormone [75].

In Type 2 (non-insulin-dependent) diabetes

In Type 2 diabetic patients the short-term oscillations of plasma insulin are more rapid and generally less

regular than in normal subjects [13]. These brief, irregular oscillations with a mean period of 8.8 min are superimposed on longer time fluctuations (> 30 min). The importance of the deviation of these oscillations from the normal pattern in the pathophysiology of Type 2 diabetes is entirely unknown. It is possible that such disturbances of the normal oscillatory secretory pattern of insulin contribute to the hyperglucagonaemia of Type 2 diabetes, a classically recognised but still poorly understood phenomenon [73]. In such patients, glibenclamide increased plasma insulin levels but failed to restore normal insulin pulsatility [76].

Conclusions

Numerous studies performed in animals (dog, baboon, rhesus monkey) and in man have depicted the existence of peripheral plasma insulin and glucagon oscillations. Such oscillations were also reported in the portal blood of dogs and monkeys. The frequency of these oscillations is remarkably stable but their amplitude is increased after a meal and decreased by food deprivation. Sustained oscillations in the release of insulin, glucagon and somatostatin from the *isolated perfused canine pancreas* have been repeatedly reported. Systematic experiments have shown that these oscillations are not affected by exposure to various pharmacological compounds including atropine, propranolol, or dibenziline. The suggestion has been made that the pulsatility of pancreatic hormone release depends on a pacemaker system present in the gland itself. In contrast, conclusive evidence for or against pulsatility of hormone release by *isolated islets* is still lacking. Similarly, the possible relationship of the slow fluctuations of glucose-induced electrical activity present in certain B cells with pulsatile insulin secretion is still unknown.

Several in vivo and in vitro studies have shown that pulsatile insulin has greater biological effects than continuous delivery. However, various factors are critical for the demonstration of the superior efficacy of pulsatile insulin; these include duration of hormonal exposure, circulating levels of insulin achieved and, most importantly, coexisting concentrations of circulating glucagon. In fact, slight hyperglucagonaemia (in the range of 200 $ng \cdot l^{-1}$) is sufficient to abolish the higher efficacy of pulsatile insulin in man. In vitro studies have shown that pulsatile delivery of glucagon is more efficient than continuous exposure to stimulate hepatic glucose protection. Until now, attempts to confirm such effects in man have failed. Finally, recent reports have indicated that pulsatile *intravenous* insulin infusion in diabetic patients is more efficient than continuous delivery in reducing hepatic glucose production, stimulating glucose utilisation and inhibiting A-cell glucagon release. In contrast, attempts to achieve better control of Type 1 diabetes by pulsed insulin given *subcutaneously* have failed. In Type 2 diabetic patients, preliminary data have shown that the short-term oscillations of plasma insulin are more rapid and generally less regular than in normal subjects; it has been suggested that such disturbances of the normal oscillatory secretory pattern of insulin may contribute to the hyperglucagonaemia of Type 2 diabetes. Until now, attempts to restore normal insulin pulsatility in these patients have failed.

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