Daily rhythm of glucose-induced insulin secretion by isolated islets from intact and pinealectomized rat

Abstract: It is well known that pinealectomy induces in rats a diminished glucose tolerance, insulin resistance, a reduction in GLUT4 content in adipose and muscular tissues, a decrease in hepatic and muscular glycogenesis, impairment of glucagon action and an increase in blood pyruvate concentration. In addition, it has been shown that melatonin suppresses insulin secretion in several experimental conditions. The objective of the present study was to investigate the daily rhythm of glucose-induced insulin secretion and glucose oxidation by isolated pancreatic islets and to investigate the effect of chronic absence of melatonin (30 days of pinealectomy) on this rhythmic process. The data obtained confirmed the presence of a strong 24-hr rhythm of insulin secretion by isolated pancreatic islets. In addition, it was demonstrated that the glucose-metabolizing ability of the B-cell follows a daily rhythm phase locked to insulin secretion rhythm. Most interesting, however, was the demonstration that the daily rhythmic processes of insulin secretion and B-cell – [U-14C]-glucose oxidation by isolated pancreatic islets is completely modified by the chronic absence of the pineal gland. Thus, pinealectomy induced in all groups an increase in 24-hr mean glucose-stimulated insulin secretion and [U-14C]-glucose oxidation, in addition to some alterations in the rhythmic amplitude and a remarkable phase-advancing of the daily curves for 8.3 mM glucose (a condition similar to that observed in fed animals and where the B-cells are supposedly more active). These observations strongly suggest that the presence of the pineal gland may be necessary for the proper synchronization of these metabolic rhythms with other circadian rhythms like activity-rest and feeding.

Introduction

The pineal gland links the cyclic environment and the rhythmic vertebrate organism through the synthesis and release of melatonin [1]. The circadian release of noradrenaline by the sympathetic terminals of the superior cervical ganglia, acting on β_1 - and α_1 -adrenoceptors, leads to the synthesis and activation of the rate-limiting enzyme arylalkylamine serotonin-*N*-acetyltransferase, resulting in the circadian production of melatonin [2].

The functional characteristics of the neuroendocrine system that controls the pineal gland causes the circadian production and secretion of melatonin to be tightly related to the dark phase of the diurnal environmental light–dark cycle. In this way, the plasma concentration curve for melatonin, by following the profile of environmental darkness, is able to send signals to daily and seasonal timing events of the internal milieu [3]. This internal synchronizer role has been already demonstrated for a very wide range of actions of melatonin in the regulation and modulation of several physiological systems [4–9].

Glycemia and insulinemia have a remarkable diurnal rhythm [10–12]. Although blood glucose level is correlated

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with feeding schedule, a diurnal variation of this parameter has been clearly demonstrated in fasted animals. Bizot-Espiard et al. [13] showed a clear diurnal variation in plasma insulin levels in fasted rats kept on a 14:10 hr light– dark cycle. Furthermore, it is well known that both humans [14, 15] and rats [8, 16–18] show a diurnal fluctuation in the oral and intravenous glucose tolerance test and in insulin peripheral sensitivity. These data suggest that the wellknown physiological relationship between blood glucose concentration and insulin secretion is not enough to explain the diurnal oscillation of these variables.

Both humans [19] and rodents present a relationship between pineal gland, melatonin and the regulation of carbohydrate metabolism [8, 12, 20, 21]. Pinealectomy induces diminished glucose tolerance, insulin resistance, decreased hepatic and muscular glycogenesis and an increase in blood pyruvate concentration in rats [22–24]. In addition, pinealectomy also induces a decrease in insulin response and a fall in GLUT4 content in adipose and muscle tissues of rats [8]. Also, it has been shown that melatonin suppresses insulin secretion in several experimental conditions [12, 25–30]. However, the action of melatonin in the process of glucose-induced insulin secretion is not well understood. In order to contribute to the understanding of this subject, the aim of the present investigation was to study the daily rhythm of glucose-induced insulin secretion and glucose oxidation by isolated pancreatic islets and to examine the effect of chronic absence of melatonin (30 days of pinealectomy) on this rhythmic process.

Materials and methods

Animals

Male albino rats weighting 150–200 g (45–60 days old) were obtained from the Institute of Biomedical Sciences, USP, São Paulo. Control rats (Control group) were kept in groups of five at 23°C in a room with a light–dark cycle of 12:12 hr (lights on 7:00 hr) for 30 days with food and water ad libitum. Another group of animals was pinealectomized and kept under the same environmental conditions for 30 days (PINX group). After this period animals were killed by decapitation for islet isolation at several time points along a 24-hr period: 00:00, 03.00, 08.00 and 16.00 hr (respectively, 5 and 8 hr after lights off, and 1 and 9 hr after lights on).

Chemicals

Collagenase type V and bovine albumin-fraction V were purchased from Sigma Chemical Co., St. Louis, MO, USA, [U-¹⁴C]-Glucose and ¹²⁵I-insulin were obtained from Dupont/NEN. Rat insulin standards and antibody were a gift from Dr Leclercq-Mayer, Université Libre de Bruxelles, Belgium.

Pinealectomy

The animals were anesthetized with pentobarbital (45 mg/kg b.w.) and subjected to surgery according to the method of Hoffman and Reiter [31]. Briefly, the anesthetized animal was placed in a stereotaxic apparatus for small animals and a sagittal opening was made on the scalp. The skin and muscles were pushed aside in order to expose the lambda suture. By means of a circular drill, a disc-shaped perforation was made around the lambda and the disc-shaped piece of bone was delicately removed. Thereafter, the pineal gland (which is located just below the posterior venous sinus confluence) was removed with a fine forceps. After a brief period of hemostasis, the skull was closed by returning the disc-shaped bone and the scalp was sutured with cotton thread.

Insulin secretion during incubation of islets

Rats were killed at 03:00, 08:00, 16:00 and 24:00 hr and pancreatic islets isolated as described by Lacy and Kostianovsky [32]. Incubations of five islets were carried out at 37°C for 60 min in 0.5 mL of Krebs–Henseleit buffer (139 mM Na⁺, 5 mM K⁺, 1 mM Ca²⁺, 1 mM Mg²⁺, 124 mM Cl⁻, 24 mM HCO₃⁻) and 0.2% albumin in the absence and presence of glucose (5.6 or 8.3 mM), equilibrated in a mixture of O₂ (95%) and CO₂ (5%). At the end of the experiment, the medium was collected for insulin assay.

Determination of [U-¹⁴C]-glucose oxidation

Groups of 20 islets from control or pinealectomized rats were incubated in glass tubes containing 100 μ L of Krebs– Henseleit solution, 5.6 or 8.3 mM glucose and [U-¹⁴C]glucose 20 μ Ci/mL (New England Nuclear Co, USA), equilibrated against a mixture of O₂ (95%) and CO₂ (5%). The tubes were transferred to counting vials that were carefully closed with a rubber stopper and incubated for 120 min in a shaker bath at 37°C. After this period, 0.1 mL of HCl (0.5 N) was injected with a syringe into the tubes containing the islets to stop the reaction, without loss of the gas produced during the experiment. The ¹⁴CO₂ produced was exposed for 2 hr to hyamine injected outside the tube containing the islets, in the same manner as for HCl. Scintillation liquid was added and radioactivity of the present ¹⁴CO₂ adsorbed to hyamine was counted.

Statistical analysis

The data, expressed as mean \pm S.E.M., were analysed by two-way analysis of variance for the factors 'time of the day' (hour of the sacrifice) and 'treatment' (control and pinealectomized groups) followed by the Bonferroni post-test (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, CA, USA). If the temporal series showed significance for the 'time of the day' factor, a second mathematical and statistical procedure was applied in order to see if the time series might be represented by a cosine curve (Cosinor method) [33-35]. The fitting of the theoretical cosine curve to the real time series was done using the leastsquares procedure. It was possible to estimate the fitting using the F statistics [36]. The null hypothesis tested was of zero amplitude, i.e. no rhythmicity at the assumed frequency (24 hr). In addition, three parameters of the adjusted curve were calculated: acrophase (time of the maximum value of the adjusted curve), mesor (value of the mean level of the adjusted curve) and amplitude (distance between the mesor and the maximum or minimum value of the adjusted curve). These parameters were compared between the experimental groups using Student's t-test [36].

Results

Two-way analysis of variance showed that the in vitro insulin secretion induced by 5.6 mM glucose was significantly influenced by the two factors considered (hour of the day and pinealectomy, P < 0.0001 and P = 0.0127, respectively). The Bonferroni post-test showed that insulin secretion differed between control and pinealectomized rats at 08:00 and 16:00 hr (P < 0.05 and P < 0.01, respectively) (Fig. 1). Cosinor analysis (Table 1) showed a 24-hr rhythm of insulin secretion induced by 5.6 mM glucose for both the control and PINX groups. Pinealectomy increased the mesor and reduced the amplitude of the daily oscillation of insulin secretion induced by 5.6 mM glucose. The acrophase occurred approximately at 22:00 hr for both the control and the pinealectomized group (5 hr after lights off).

Two-way analysis of variance showed that the in vitro insulin secretion induced by 8.3 mM glucose was also significantly influenced by the factors 'hour of the day' Picinato et al.



Fig. 1. Daily profile of glucose-stimulated insulin secretion by pancreatic Langerhans islets isolated from rats sacrificed at different times during the 12:12 hr light–dark cycle (indicated by the black and white bar above the *x*-axis). The experiments were done using two different concentrations of glucose: 5.6 and 8.3 mM. It should be noticed that in some data points the error bar is small enough to be included in the symbol. Two-way ANOVA showed a significant effect of time of sacrifice and of pinealectomy (PINX). *Significant difference between the values obtained for the Control and PINX groups at each of the indicated time points (Bonferroni post-test).

and pinealectomy (P < 0.0001 for each factor). The Bonferroni post-test again showed that the two groups had significantly different secretion values at 08:00 and 16:00 hr (P < 0.001) (Fig. 1). Cosinor analysis (Table 1) showed a 24-hr rhythm of insulin secretion by isolated pancreatic islets induced by 8.3 mM glucose for both the control and PINX groups (P = 0.03 and P = 0.005, respectively). Moreover, pinealectomy induced a significant phase-advance of the daily curve of insulin secretion, and an elevation of the mesor without changing the amplitude of the curve.

The statistical analysis of the process of glucose oxidation induced by 5.6 mM glucose also depends on the two factors: hour of the day when the animal is sacrificed (P < 0.0001) and pinealectomy (P < 0.0001). The posttest showed that the two groups differed at 16:00 hr (P < 0.01) (Fig. 2). Cosinor analysis showed a 24-hr rhythm of glucose oxidation in pancreatic islets isolated from control (P = 0.01) or pinealectomized (P = 0.009)rats. The rhythmic analysis also showed that pinealectomy induced a mesor elevation without changing either amplitude or acrophase of the daily curve (Table 1).

0.005 h** 0.02 0.03(pmol/islets/120 min) 368.55 ± 119.45 $(\mu U/islets/min)$ $(\mu U/islets/min)$ Mesor 829.30 ± 86.03 $1.53 \pm 1.90*$ 8.3 mM Glucose (pmol/islets/120 min) Amplitude $(\mu U/islets/min)$ 543.49 ± 184.66 $(\mu U/islets/min)$ 723.35 ± 121.30 11.62 ± 2.92 $20:06 \pm 00:43 \text{ hr}^*$ $21:26 \pm 01:04 \text{ hr}^*$ $17:51 \pm 00:48 \text{ hr}$ Acrophase 0.0001 P** 0.02 0.01 (pmol/islets/120 min) $(\mu U/islets/min)$ $(\mu U/islets/min)$ Mesor $44.41 \pm 1.49*$ 75.52 ± 3.48 $2.80 \pm 0.80^{*}$ 5.6 mM Glucose (pmol/islets/120 min) Amplitude $(\mu U/islets/min)$ $(\mu U/islets/min)$ $57.72 \pm 2.14^*$ 21.87 ± 5.33 3.22 ± 1.22 hr hr $21:57 \pm 01:13 hr$ 00:47 $22:40 \pm 00:08$ Acrophase 22:03 ± Control Control PINX Glucose [U-¹⁴C] oxidation secretion Insulin

Table 1. Cosinor parameters of insulin secretion and glucose oxidation by rat isolated islets of Langerhans

All parameters were estimated by fitting a cosine curve for the period of 24 hr to real time series data with at least 10 animals for each time point. PINX: Pinealectomized animals studied 30 days 0.05 Student's *t*-test for comparison with the PINX group in the same condition. ** P ≤ 0.05 indicates the presence of significant 24-hr rhythmicity. VI after surgery. *P

0.03

(pmol/islets/120 min)

(pmol/islets/120 min)

 12.27 ± 3.37

 $17:12 \pm 01:18 hr$

0.009

(pmol/islets/120 min)

(pmol/islets/120 min)

 3.80 ± 0.73

 \pm 00:38 hr

19:39

PINX

 5.05 ± 0.48

 18.15 ± 2.49



Fig. 2. Daily profile of glucose-stimulated B-cell glucose oxidation in islets isolated from rats sacrificed at different times during the 12:12 hr light–dark cycle (indicated by the black and white bar above the x-axis). The experiments were done using two different concentrations of glucose: 5.6 and 8.3 mM. It should be noticed that in some data points the error bar is small enough to be included in the symbol. Two-way ANOVA showed a significant difference between the values obtained for the Control and PINX groups at each of the indicated time points (Bonferroni post-test).

Two-way ANOVA showed that the daily glucose oxidation by isolated pancreatic islets induced by 8.3 mM glucose was significantly influenced by the hour of sacrifice (P < 0.0001) and by pinealectomy (P < 0.0001). The Bonferroni post-test showed that the two groups of animals differed at 03:00, 08:00 and 16:00 hr (P < 0.001 for each time point) (Fig. 2). Cosinor analysis showed a significant 24-hr rhythm of oxidation in both groups (P = 0.02 for control and P = 0.03 for pinealectomized rats). In addition, in the presence of 8.3 mM glucose, pinealectomy induced a phase-advance of the process of oxidation, and an increase of the mesor without changing the amplitude of the adjusted curve (Table 1).

Discussion

Studies conducted on normal rats have shown a circadian rhythm of plasma insulin concentration and an increase of the hormone release at the beginning of the night coincidentally with the onset of the circadian phase of activity and food intake [10, 12, 29, 30, 37]. The circadian oscillation of insulin is associated with an improvement in glucose tolerance, a rise in insulin sensitivity and B-cell responsiveness, and secretion of counter-regulatory hormones [11, 12, 21, 38–40]. Lima et al. [8] demonstrated that 30 days after surgery pinealectomized rats developed glu-

cose intolerance, decreased adipose cell responsiveness to insulin, reduced GLUT4 content in muscle and adipose tissue, as well as a modification in the daily rhythm of in vivo insulin secretion induced by glucose overload.

The present data confirmed the existence of a strong 24-hr rhythm of insulin secretion by isolated pancreatic islets as previously demonstrated [12, 40]. In addition, it was documented that the glucose-metabolizing ability of the B-cell follows a daily rhythm phase locked to the insulin secretion rhythm.

Most interesting, however, was the demonstration that the daily rhythmic processes of insulin secretion and B-cell [U-¹⁴C]-glucose oxidation by isolated pancreatic islets is completely modified by the chronic absence of the pineal gland. In this way, 30-days pinealectomy induced in all groups an increase in mean 24-hr glucose-stimulated insulin secretion and [U-14C]-glucose oxidation, provoking some alterations in the rhythmic amplitude and a remarkable phase-advancing of the daily curves for 8.3 mM glucose, a condition similar to that observed in fed animals and where the B-cells are supposedly more active. As the main differences between control and pinealectomized animals are concentrated in time points sampled during the lights on period, it is possible to conclude that the absence of the pineal gland eliminates the clear daily fluctuation of production of insulin and metabolization of glucose by pancreatic B-cells.

These observations strongly suggest that the presence of the pineal gland is necessary for keeping the acrophase of the pancreatic rhythms of these B-cells during the first half of the dark period, thus contributing to the synchronization of these metabolic rhythms with other circadian rhythms like activity-rest and feeding.

It should be noticed, in addition, that the physiological processes of insulin secretion and glucose oxidation were in phase with each other in both control and PINX rats (although both processes were phase-advanced in PINX compared with control animals). This relationship between the variation rate of glucose-induced insulin secretion and the B-cell glucose metabolism would be expected, considering the importance of sugar metabolism in the process of insulin secretion.

One point that deserves discussion is how the pineal gland controls several rhythmic processes involved in carbohydrate metabolism. In spite of the existence of data in the literature showing that some other putative pineal secretory products might influence glucose metabolism [28, 41], melatonin is, by far, the best candidate as the main pineal secretory product regulating carbohydrate metabolism [26–28, 41–46].

There are solid grounds to postulate that melatonin may act peripherally by regulating insulin secretion [28, 44] and insulin action on sensitive tissues [8, 42], by influencing glucagon secretion and action [23, 47], and by acting on hepatocytes regulating glucose metabolism and secretion [46]. In addition, some of these peripheral actions of melatonin were shown to influence directly rhythmic processes of insulin secretion [12] or action [8].

Taking into account these data, it is possible to propose that melatonin acts on the regulation of insulin secretion both as an internal synchronizer [in this case synchronizing the pancreatic peripheral oscillators [12]] and as a masking agent, directly regulating the ability of B-cells to produce and release insulin [44, 50]. On the other hand, melatonin might, alternatively or concomitantly, modulate the neuroendocrine system that participates in the complex control of carbohydrate metabolism acting either centrally [39, 43] or peripherally [48, 49].

In summary, our findings emphasize the important role played by the pineal gland in the control of circadian rhythms involved in the regulation of glucose homeostasis.

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