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MINI REVIEW

Glucagon-like peptide-1: The missing link in the metabolic clock?

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ABSTRACT

Circadian expression of clock genes in peripheral tissues is critical to the coordinated regulation of intestinal digestive and absorptive functions, insulin secretion, and peripheral tissue nutrient deposition during periods of nutrient ingestion, thereby preventing metabolic dysregulation. As glucagon-like peptide-1 is a key incretin hormone that regulates glucose-dependent insulin secretion, we hypothesized that this intestinal hormone is a player in the peripheral metabolic clock, linking nutrient ingestion to insulin secretion. We have now established that secretion of glucagon-like peptide-1 from the intestinal L cell shows a rhythmic pattern in rats and humans *in vivo* that is altered by circadian disruptors, such as constant light exposure, consumption of a Western diet and feeding at inappropriate times (i.e., during the light period in rodents). Interestingly, the alterations in the rhythm of the glucagon-like peptide-1 secretory responses were found to parallel the changes in the pattern of insulin responses in association with significant impairments in glucose tolerance. Furthermore, we have detected circadian clock gene expression, and showed circadian secretion of glucagon-like peptide-1 from both the murine and human L cell *in vitro*. These findings demonstrate that glucagon-like peptide-1 is a functional component of the peripheral metabolic clock, and suggest that altered release of glucagon-like peptide-1 might play a role in the metabolic perturbations that result from circadian disruption.

INTRODUCTION

Glucagon-like peptide-1$^{7\text{-}36H2}$ (GLP-1) is an intestinal L cell hormone that enhances glucose-dependent insulin secretion after nutrient ingestion[1,2]. As a consequence of its actions as a physiological incretin, GLP-1 receptor (R) agonists and GLP-1 degradation (dipeptidyl peptidase-4 [DPP4]) inhibitors are now used in patients with type 2 diabetes[3–5]. Furthermore, because of the suppression of appetite also induced by GLP-1 and the subsequent bodyweight loss in response to the prolonged administration of the peptide, GLP-1-derivative drugs have also been approved for the treatment of obesity[5–7]. Considering the variety of therapeutic applications of the peptide, identification of novel GLP-1 secretagogues is also generating significant interest as an alternative approach to GLP-1-based therapy[1,8,9]. We have recently shown that the L cell sensitivity to stimuli and the magnitude of the subsequent GLP-1 responses demonstrate a diurnal pattern in rats and humans that is altered by different types of circadian disruption, such as constant light exposure, feeding at inappropriate times (i.e., during the light period in rodents) and consumption of an obesogenic diet[10–12]. As these changes are associated with alterations in the patterns of both insulin release and glucose tolerance, these findings provide a link between GLP-1 secretion and the increased incidence of metabolic syndrome associated with circadian disruption, such as in shift workers[13–15]. The present review will examine the evidence for a role of GLP-1 in linking the rhythmic ingestion of nutrients to the optimization of nutrient disposition through the integrated actions of the peripheral metabolic clock.

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GLP-1 SECRETION

GLP-1 is released by the enteroendocrine L cell, which is found in highest numbers in the epithelium of the distal ileum and colon of rodents and humans[16]. In humans, GLP-1 secretion in response to nutrient ingestion is biphasic, with both an early (30–45 min) and a later (60–90 min) peak[17,18]. Previous studies

in rats have shown that the early peak is mediated indirectly, through activation of the vagus by nutrients in the proximal gut, which then stimulate muscarinic receptors expressed by the L cell[19]; this finding has been at least partially corroborated in humans[17,20]. In contrast, the later peak of GLP-1 secretion is induced by direct L cell sensing of luminal nutrients including glucose, amino acids, long-chain fatty acids and their derivatives, associated bile acids, and short-chain fatty acids[8,9,21–26]. GLP-1 release is also stimulated by a number of nutrient-induced endocrine hormones including, most notably, glucose-dependent insulintropic peptide (GIP; in rodents), cholecystokinin (in humans), leptin and insulin[10,11,27–33]. Because of its regulation by luminal nutrients on the apical surface in addition to neural and circulating factors arising from the basolateral aspect, the polarized L cell represents an unusually multifaceted cell type. The complexity of the mechanisms underlying GLP-1 secretion by the intestinal L cell thus continues to be an area of active exploration, in an effort to identify novel modulators of GLP-1 release for potential therapeutic use.

CENTRAL CONTROL OF CIRCADIAN RHYTHMS

Most physiological processes follow circadian rhythms in all organisms, generated by cell-autonomous, self-regulating clock genes expressed in the suprachiasmatic nuclei (SCN) that integrate light input from the external world with sleep–wake patterns and periods of feeding/fasting[13–15]. The major components of this 'body clock' include the 'positive' arm, aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1) and circadian locomotor output cycles kaput (Clock), and the 'negative' feedback branch, consisting of cryptochrome (Cry) and Period (Per; Figure 1). Thus, heterodimers of Bmal1 and Clock bind to E-boxes in the Cry and Per promoters, enhancing the expression of the respective proteins, which in turn dimerize and inhibit the expression of Bmal1 and Clock. Without the stimulatory effect on Per and Cry expression provided by Bmal1/Clock heterodimers, intracellular Per and Cry levels eventually decrease, no longer repressing Bmal1 and Clock expression and, thus, allowing the feedback loop to start again approximately every 24 h[13–15]. Additional regulators of this clock include, but are not limited to, feedback from several nuclear receptors, most notably reverse-erb receptor α and retinoic acid orphan receptor α. Of note, this feedback loop regulates up to 15% of the genes expressed by the mammalian genome, the so-called 'clock controlled genes'[15]. The activity of the central clock in the SCN, also known as the 'master clock' in mammals, is synchronized by light input through neurons coming from the retina. This master clock coordinates the circadian patterns in body temperature, immune function, and the release of cortisol, growth hormone and T3, in addition to regulating whole-body metabolism by dictating the timing of feeding and fasting.

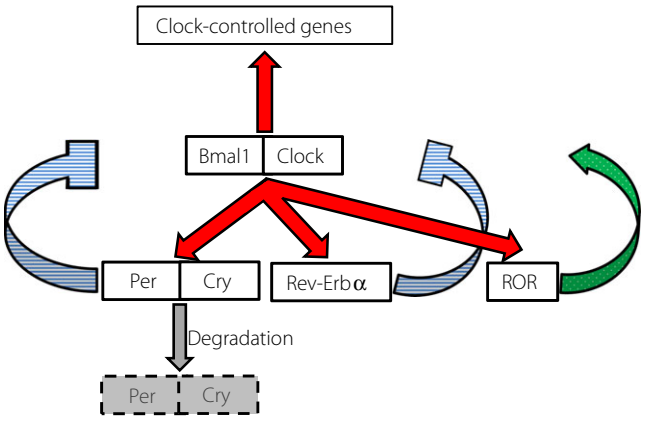

Figure 1 | Schematic of the main components of the circadian clock and their regulatory interactions. Bmal1; aryl hydrocarbon receptor nuclear translocator-like protein 1; Cry, cryptochrome; Per, Period; Rev-Erbα, reverse-erb receptor α; ROR, retinoic acid orphan receptor α.

METABOLIC CLOCK

Recent studies have shown that functional clock genes are expressed by most cells in the body, including the major regulators of peripheral metabolism, the intestinal epithelium, pancreatic β-cells, hepatocytes, skeletal muscle and adipocytes (Figure 2)[34–36]. Importantly, the metabolic activities of these tissues appear to be temporally coordinated with the feeding cycle, such that nutrient ingestion is associated with optimal nutrient assimilation, thereby ensuring metabolic homeostasis. The importance of the clock genes in regulating these circadian rhythms is nicely demonstrated by findings of disruptions in mice harboring defective Clock/Bmal1 heterodimers such as in $Clock^{\Delta 19/\delta 19}$ mutant and $Bmal1^{-/-}$ mutant mice. Hence, rhythmic expression of intestinal nutrient transporters (i.e., sodium-glucose transporter-1, peptide transporter-1, β-cell exocytotic proteins (i.e., vesicle-associated membrane protein-3), hepatic enzymes (i.e., glucokinase and phosphoenolpyruvate carboxykinase), the muscle glucose-transporter-4 and adipokines (i.e., adiponectin and leptin) are dampened or abrogated by the loss of functional Clock/Bmal1 dimers[34–36]. Importantly, expression of all of these metabolic genes peaks in association with the normal feeding period, suggests that, just as light entrains the SCN clock, nutrient ingestion serves as a zeitgeber for the metabolic clock. As GLP-1 is a key hormone that directly links dietary nutrients to the insulin secretion, we therefore hypothesized that GLP-1 might also serve a role in the synchronization of the peripheral metabolic clock with nutrient intake.

CIRCADIAN REGULATION OF GLP-1 SECRETION

We have recently shown circadian expression of Bmal1, period-2 (Per2) and reverse-erb receptor α in a well-established *in vitro* model of the murine (m) intestinal L cell, the murine proglucagon-SV40 large T antigen (mGLUTag) cell line[10,12]. Importantly, the pattern in Bmal1 is antiphasic to that of Per2, with periods of ~23–29 h and amplitudes that wane over time as the cells lose synchrony, consistent with findings on Per2 expression in murine β-cells[35]. Furthermore, secretion of GLP-1

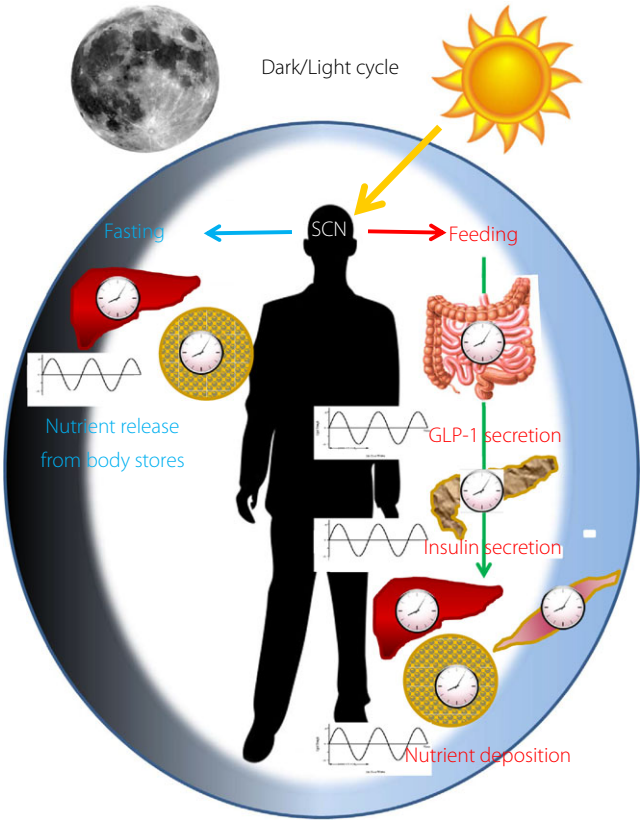

Figure 2 | Schematic of the peripheral metabolic clock, which integrates suprachiasmatic nuclei (SCN)-regulated feeding periods with clocks in the gut, L cell, β-cell, liver, skeletal muscle and adipose tissue. Coordinated release of glucagon-like peptide-1$^{7\text{-}36H2}$ (GLP-1) and insulin optimizes disposition of ingested nutrients.

by the mGLUTag cells in response to the known secretagogue, bethanechol, shows a circadian periodicity that parallels the rhythm in Bmal1 expression, with similar differential responses to both GIP and insulin observed at the peak and trough time-points[10]. Rhythmic expression of Bmal1 and Per2, as well as cyclic GLP-1 secretion in response to bethanechol and GIP, has also been detected in the human (h) hNCI-716 L cell line[11]. Collectively, these *in vitro* studies suggest the existence of a cell-autonomous clock in the intestinal L cell that regulates responsiveness to GLP-1 secretagogues in a circadian manner.

The signaling pathways that link GLP-1 secretion to the molecular clock have not been explored in detail. However, microarray analysis and quantitative reverse transcription polymerase chain reaction of the mGLUTag cells at the peak and trough time-points of the GLP-1 secretory rhythm showed that not only the canonical clock genes, but also a number of other genes, are differentially expressed at these two time-points. Two genes were specifically identified that showed an antiphasic pattern of expression[10]: *Ptp4a1* (phosphatase of the regenerating liver-1), a modulator of the extracellular signal-regulated kinase-1/2 signaling pathway[37] that regulates GLP-1 secretion[33], and thyrotroph embryonic factor, involved in the circadian regulation of insulin release[38]. Interestingly, knockdown of phosphatase of the regenerating liver-1 (expressed at high levels when Bmal1 and GLP-1 secretion were at their peaks) decreased GLP-1 secretion in response to known secretagogues, whereas knockdown of thyrotroph embryonic factor (expressed at high levels when Bmal1 and GLP-1 secretion were at their troughs) increased the release of GLP-1[10], indicating that genes with a daily pattern of expression in the L cell can regulate GLP-1 secretion. Whether phosphatase of the regenerating liver-1 and thyrotroph embryonic factor expression are directly regulated by the clock genes remains to be determined, as do the exact mechanisms by which these and, possibly, other rhythmically-expressed proteins regulate rhythmic release of GLP-1 release.

To determine the physiological relevance of our *in vitro* findings, the diurnal variation in GLP-1 secretory responses was also examined in both rats and healthy human subjects, under normal conditions and after circadian disruption. Although several other studies have reported circadian-like patterns of GLP-1 levels in normal humans, as well as a dampening of the amplitude in subjects with obesity and type 2 diabetes[39–41], these studies did not separately analyze basal as compared with nutrient-induced GLP-1 secretion, thus showing a rhythm in GLP-1 levels that was heavily dependent on the time-period between meals and/or snacks and the different caloric loads administered throughout the day, as expected for an incretin hormone, but distinct from effects of the actual time of feeding. In contrast, our studies in both rats and humans utilized a consistent fasting period in association with a constant caloric load; rats were thus administered an identical oral glucose tolerance test every 4 h over 24 h, each following a 4-h fast, and the humans were followed over 22 h with identical meals given at two time-points separated by 12 h, each after a 5-h fast. Collectively, the results of these studies show that the GLP-1 response to nutrient ingestion *in vivo* demonstrates a diurnal pattern in both rats and humans[10–12].

Although no significant variations in basal GLP-1 levels were found in rats, peak responses to the oral glucose tolerance test were observed at zeitgeber time 10 (10 h after the beginning of the light period, corresponding with 17.00 hours under the normal light–dark conditions of our animals), immediately before the normal feeding period, whereas the trough response occurred 12 h later, at zeitgeber time 22 (05.00 hours)[10]. Furthermore, the peak in GLP-1 appears to be entrained by the timing of normal nutrient ingestion, as feeding of the animals only during the daylight hours, as compared with only during the night-time, resulted in a complete, 12-h reversal of the timing of the peak and trough responses. Importantly, the rhythm in GLP-1 release was paralleled by identical peak and trough insulin responses by the β-cell. In contrast, circadian disruption through exposure of the animals to constant light completely abrogated the rhythms in both GLP-1 and insulin secretion, in association with the development of hyperglycemia. To determine whether these changes occurred as a result of

light-induced stress, rats were administered continuous-release corticosterone implants to both elevate circulating levels and abolish the normal circadian pattern of glucocorticoids[12], as commonly found under stress conditions[42]. Although glucocorticoids are known to modulate several peripheral oscillating systems[43], this treatment did not prevent the normal rhythmic pattern, but increased the amplitude of the GLP-1 responses, suggesting that the alterations in GLP-1 secretory rhythm induced by circadian disruption are not produced by changes in the stress hormones. Furthermore, these findings also show that, as opposed to other peripheral clocks, corticosterone does not play a major role as a zeitgeber for the intestinal L cell. Finally, just as circadian disruption alters metabolism, changes in metabolism can affect circadian patterns. Hence, rats were fed a high-fat, high-sucrose 'Western' diet, an established circadian disruptor[44]. Examination of the peak and trough GLP-1 and insulin responses in these obese animals showed, again, a complete loss of the normal rhythmic patterns in these hormones, as well as significantly impaired glucose tolerance[12]. Pretreatment of the mGLUTag cells with the saturated fatty acid, palmitate, a major component of the high-fat diet, similarly dampened both *Bmal1* and *Per2* expression, as well as GLP-1 release at the normal peak time of the response. These studies thus show that, in rats, GLP-1 secretion in response to an oral glucose load follows a daily pattern that is tightly linked to the secretion of insulin, and that disruption of these patterns is associated with altered L cell clock gene expression *in vitro* and hyperglycemia *in vivo*.

Human volunteers kept under standard light–dark cycle conditions showed rhythmic patterns in basal GLP-1 and insulin levels, with highest levels at 06.00 hours, the beginning of the normal feeding period[11]. Diurnal variations in the postprandial levels of both hormones, after ingestion of a mixed meal at 23.00 and 11.00 hours, were also observed in these participants[11]. However, unlike the findings in rats, in which GLP-1 and insulin responses changed in parallel[10,12], the responses of these hormones in humans appeared to be antiphasic, with higher GLP-1 secretion observed at 23.00 hours as compared with the insulin response, which was higher at 11.00 hours. The mechanism underlying this finding remains to be established. However, in rats, the β-cell has been shown to exhibit greater sensitivity to GLP-1 immediately before, as compared with the end of the normal feeding period[10]. Accordingly, exposure of human islets to melatonin for 12 h, which occurs during the normal circadian pattern of melatonin secretion, increases the sensitivity of the β-cell to the stimulatory effects of GLP-1[45]. Thus, it appears likely that the human β-cell shows a circadian pattern in its response to GLP-1. Finally, as in rats, exposure of normal humans to constant light for 22 h was found to dampen the patterns in both GLP-1 and insulin release in association with a remarkable increase in insulin resistance. These changes were not observed in participants maintained under the same sleep-deprivation protocol but with the normal light–dark period. This effect was also independent of changes in circulating cortisol levels, suggesting that light *per se* plays a role in the hormonal responses to nutrient ingestion in humans. However, as the hNCI-H716 cells do not express either of the known human melatonin receptors[11], a direct effect of melatonin in the human L cell seems unlikely. It therefore remains unclear as to how this effect is modulated.

INTEGRATION OF THE METABOLIC CLOCK

Collectively, our studies show that GLP-1 is a novel component of the peripheral metabolic clock, whereby the central clock in the SCN coordinates the sleep–wake cycle permitting diurnal intake of nutrients, whereas the peripheral clock synchronizes the patterns in nutrient absorption, GLP-1 and insulin secretion, and peripheral tissue metabolism, in order to optimize the disposition of ingested nutrients (Figure 2). Our findings further suggest that nutrient ingestion is a major zeitgeber for GLP-1 release, whereupon GLP-1 acts not only as an incretin hormone, but also plays a role in temporally coordinating the magnitude of the insulin response. Furthermore, we also found that alterations in the patterns of GLP-1 responses, such as that induced by constant light exposure, inversion of the feeding pattern and ingestion of a Western diet, are consistently associated with changes in the pattern of insulin responses and with significant impairments in glucose tolerance. Interestingly, previous studies have clearly shown that insulin release by the β-cell is similarly coordinated by clock genes. Hence, β-cells express several of the known clock genes in a circadian rhythm, and knockout or mutation of either *Bmal1* or *Clock* results in impaired insulin release in response to a variety of secretagogues, including both glucose and the GLP-1 receptor agonist, exendin-4[35]. Furthermore, exposure of rats to constant light alone or in combination with a high-fat diet results in altered islet clock gene expression as well as disrupted insulin release[46,47]. When taken with our studies on the intestinal L cell, these findings suggest that the clock genes play a role in the fine-tuning of nutrient-induced release of GLP-1 and insulin, as well as in the ensuing β-cell response to GLP-1. Further studies to establish whether clock gene expression is coordinately regulated in the L cell and the β-cell are clearly warranted. Furthermore, as previous reports show that not only the genetic manipulation of clock components, but also the existence of naturally-occurring clock gene polymorphisms[48–51] are associated with alterations in glucose homeostasis, it is important that we continue to elucidate the intracellular mechanisms underlying the rhythmic secretion of both hormones, in physiology as well as in patients with obesity and type 2 diabetes.

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DISCLOSURE

The authors declare no conflict of interest.

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