

The impact of Metformin on circadian clock genes

Abstract

Circadian clocks are important to maintain the timing in the cycles of physiological processes, cycles of behaviour and metabolism. Having an intrinsic clock to orchestrate rhythms is also important for human health. Circadian desynchrony, a characteristic of shift work, high-fat diet feeding and sleep disruption in humans have been associated with metabolic disorders such as obesity and type 2 diabetes. The main treatment of type 2 diabetes is the drug metformin whose mechanism, in part, is due to activation of adenosine monophosphate-activated protein kinase (AMPK). This mini review summarizes the emerging relationship between molecular clocks, metabolic systems and AMPK and examines evidence that metformin treatment affect the clock machinery in a tissue specific manner.

Keywords: metformin, type 2 diabetes, clock genes, AMPK, bmal1

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Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; SCN, suprachiasmatic nuclei; Clock, *Circadian locomotor output cycles kaput*; Bmal1, *Brain and muscle Arnt-like protein-1*; PER, *Period homolog drosophila*; Cry, *Cryptochrome*; Rev-erb alpha, *Reverse erythroblastosis virus alpha*; Rora, *Retinoic-acid receptor-related orphan receptor alpha*; AICAR, aminoimidazole carboxamide ribonucleoside; CK1, casein kinase 1; Ccgs, clock controlled genes; Nampt, nicotinamide phosphoribosyltransferase; Sirt1, silent mating type information regulation 1 homolog

Introduction

In mammals the 24hours rhythms in metabolism physiology and behavior are driven by cell circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus.^{1,2} These circadian clocks are controlled at the molecular level by a transcriptional/translational feedback loops involving a set of clock genes. The primary loop is composed by the transcription factors *Circadian locomotor output cycles kaput* (Clock) and *Brain and muscle Arnt-like protein-1* (Bmal1) which drive the transcription of many genes and also including genes encoding the *Period homolog drosophila* (Per1, Per2 and Per3) and *Cryptochrome* (Cry1, Cry2) genes. PER and CRY proteins inhibit their own CLOCK: BMAL-1 induced transcription (Figure 1).³ Some transcription factors such as *Reverse erythroblastosis virus alpha* (Rev-erb alpha) can negatively regulate BMAL1 expression whereas *Retinoic-acid receptor-related orphan receptor alpha* (Rora) has a positive regulation on BMAL1 expression. Several lines of evidence indicate that besides the central clock in the SCN, peripheral molecular clocks exist in several organs, such as liver, kidney, heart, adipose tissue and pancreas.⁴⁻⁸ Interestingly, the timing of circadian clocks in peripheral tissues is set by feeding time rather than light and dark signals.⁹ In addition, important genes that regulate metabolism exhibited 24h oscillations⁴ and the circadian clocks have been reported to regulate metabolism. Clock mutant mice and Bmal1^{-/-} mice have circadian dysregulation along with metabolic defects in glucose and lipid homeostasis. For example, clock mutant mice have attenuated diurnal feeding rhythm, are obese, hyperglycemic, hyperlipidemic and hypoinsulinemic.¹⁰ Knockout of the Bmal1 gene leads to profound insulin resistance.¹¹ Interestingly, ablation of the pancreatic clock by deletion of Clock or Bmal1 has been shown to trigger the onset of diabetes mellitus.¹² Rev-

erb alpha, one of the key clock genes, was suggested to link circadian rhythms and metabolism.^{13,14} In the endocrine pancreas, Rev-erb alpha was shown to regulate beta-cell proliferation and insulin secretion in mice.⁷ Thus, peripheral clocks should be well synchronized to maximally and efficiently optimize the timing of metabolic processes for efficient energy storage and utilization.

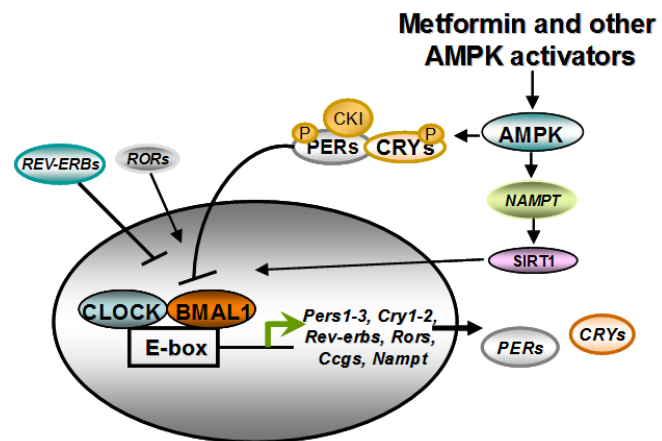


Figure 1 Feedback loop in the core circadian oscillator and the interaction with AMPK.

BMAL1 and CLOCK drive the transcriptional expression of *Per1-3*, *Cry1-2*, *Rev-erbs*, *Rors*, *Nampt* and *Ccgs*. CRY and PER proteins are phosphorylated by the protein casein kinase I (CKI) and inhibit their own CLOCK: BMAL1-induced transcription. BMAL1 and CLOCK also drive the transcriptional expression of *Rev-erbs* and *Rors*, which inhibit and activate BMAL1 expression, respectively.

Activation of AMPK by metformin or other AMPK activators modulates clock genes directly or via Nampt-Sirt1 pathways. Ccgs, clock controlled genes; Nampt, nicotinamide phosphoribosyltransferase; Sirt1, silent mating type information regulation 1 homolog.

The role of AMPK on clock genes

AMPK is a critical regulator of energy homeostasis that is activated by cellular ATP depletion. AMPK is a heterotrimeric serine/threonine protein kinase composed of a catalytic alpha-subunit and non-catalytic beta and gamma subunits. The link between AMPK and clock genes has been demonstrated in several tissues. In skeletal muscle,

pharmacological activation of AMPK by intraperitoneal injection of AICAR (5-aminoimidazole-4-carboxamide ribonucleoside) caused changes in the expression of clock genes including *Bhlhb2* (alias *Dec-1*), *Nr1d1* (alias *Rev-erb alpha*) and *Cry2*. These changes were prevented in the muscle from AMPK gamma 3-subunit knock-out mice indicating that AMPK heterotrimeric complexes containing the AMPK gamma 3-subunit may play a specific role in linking circadian oscillators and energy metabolism in skeletal muscle.⁸ The link between AMPK and clock genes was confirmed in fibroblasts and liver. Stimulation of AMPK destabilized cryptochromes which allow the transduction of nutrient signals to circadian clocks in mammalian peripheral organs. In addition, AMPK activation shifts the phase of entrainment in mouse fibroblasts and mouse livers.¹⁵ Studies with AMPK alpha 1 and alpha 2 knockout mice demonstrated that the catalytic activity of AMPK regulates circadian rhythm of behavior, energy metabolism and gene expression in isoform- and tissue-specific manners.¹⁶ Casein kinases are known to regulate the clock function by phosphorylating serine residues in the *Per2* gene. On the other hand, AMPK can phosphorylate casein kinase I epsilon thereby increasing phosphorylation-mediated PER degradation.¹⁷ Thus, animal studies indicate an important role of AMPK and its isoforms in the control of the clock machinery in a tissue specific manner.

The effect of metformin on circadian clocks

Metformin is one of the most commonly drugs for type 2 diabetes. Metformin mechanism of action is linked, in part, to the activation of AMPK which lowers serum glucose levels and maintain energy homeostasis by directly sensing the AMP/ATP ratio. The effect of metformin on circadian clocks has been studied in different tissues in mice. In young lean healthy mice, AMPK activation by metformin phase advanced the mRNA expression of *Per1*, *Clock*, *Bmail1* and *Ror alpha* in the liver and phase delayed in the *Bmal1* and *Rev-erb alpha* gene expression in the skeletal muscle showing a differential effects of metformin in the liver and muscle and a important role of circadian clock in metabolic processes.¹⁸ Interestingly, injection of metformin increased degradation of *Per2* and changes the circadian expression of clock genes in mouse heart, skeletal muscle and fat.¹⁷

The effects of metformin on clock genes were also shown in the endocrine pancreas and white adipose tissue. In db/db mice the expression pattern of clock genes were downregulated in white adipose tissue and metformin treatment restored the levels of clock genes in this tissue via an AMPK-Nampt-Sirt1 pathway.¹⁹ The measurements of clock genes in this study were done at one time point of the day and future studies are necessary to check whether metformin can restore the 24h pattern of clock genes in white adipose tissue. In the endocrine pancreas, AMPK has been shown to be regulated by glucose levels and control glucagon secretion.²⁰ Recently, activation of AMPK by metformin was shown to change the pattern of clock genes in a glucagon secreting cell line. It was demonstrated that glucose by regulating AMPK can modulate Nampt-Sirt1 signalling that control *Rev-erb alpha* gene and thus glucagon secretion.²¹ The authors suggested that strategies to target the Nampt-Sirt1-Rev-erb alpha in pancreatic alpha cells can be useful for the treatment of hyperglucagonemia present in diabetes. Thus, accumulating evidence suggest the link between AMPK and clock genes in animal studies. Studies in humans are needed to confirm this interaction mainly through the use of drugs such as metformin to check whether this type of treatment is altering the human biological clock.

Conclusion

Obesity and type 2diabetes has been linked with disturbances

in circadian rhythms. Indeed, animal models of obesity and type 2diabetes exhibited alterations in circadian rhythms clock gene expression in peripheral tissues. The ability of metformin to activate AMPK and the clock machinery open new pharmacological pathways to treat disturbances in circadian rhythms. One interesting question to be answered is whether AMPK links metabolism and clock genes in humans. In addition, it would be interesting to check whether patients with type 2 diabetes treated with metformin exhibited improved in the circadian clock machinery.

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None.

Conflict of interest

Author declares that there is no conflict of interest.

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