Molecular and Cellular Endocrinology xxx (2015) 1–7

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

The role of feeding rhythm, adrenal hormones and neuronal inputs in synchronizing daily clock gene rhythms in the liver

Yan Su [a], Cathy Cailotto [a], Ewout Foppen [b], Remi Jansen [a], Zhi Zhang [b], Ruud Buijs [c], Eric Fliers [b], Andries Kalsbeek [a, b, *]

[a] Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA, Amsterdam, The Netherlands
[b] Department of Endocrinology and Metabolism, Academic Medical Centre (AMC), University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands
[c] Departamento de Biologia Celulary Fisiologia, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Distrito Federal, Mexico

ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form
11 December 2015
Accepted 12 December 2015
Available online xxx

Keywords:
Circadian
Feeding
Adrenalectomy
Denervation
Clock genes
Liver

ABSTRACT

The master clock in the hypothalamic suprachiasmatic nucleus (SCN) is assumed to distribute rhythmic information to the periphery via neural, humoral and/or behavioral connections. Until now, feeding, corticosterone and neural inputs are considered important signals for synchronizing daily rhythms in the liver. In this study, we investigated the necessity of neural inputs as well as of the feeding and adrenal hormone rhythms for maintaining daily hepatic clock gene rhythms. Clock genes kept their daily rhythm when only one of these three signals was disrupted, or when we disrupted hepatic neuronal inputs together with the adrenal hormone rhythm or with the daily feeding rhythm. However, all clock genes studied lost their daily expression rhythm after simultaneous disruption of the feeding and adrenal hormone rhythm. These data indicate that either a daily rhythm of feeding or adrenal hormones should be present to synchronize clock gene rhythms in the liver with the SCN.

1. Introduction

The daily cycle of light and darkness is an important signal for mammals to synchronize their behavioral and physiological rhythms with the environment they are living in. Therefore, mammals have developed a circadian timing system that enables them to adjust their activity to the environmental L/D cycle. In mammals this circadian timing system consists of two main components: 1) the central master clock, located in the suprachiasmatic nucleus of the hypothalamus (SCN) and 2) peripheral clocks, which are found in most organs and tissues (Yamazaki et al. 2000; Yoo et al. 2004). Within individual cells the circadian clock system is driven by two interlocking, regulatory feedback loops. This core loop consists of 7 main clock genes. *Clock* and *Bmal1* (brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)–like) form the positive limb, and *Per1/2/3* (period1/2/3) and *Cry1/2* (cryptochrome 1/2) form the negative limb. The other, accessory loop, consists of *Bmal1*, *Rev-erb* and *Ror* (retinoic acid–related orphan receptor). The interaction between the core loop and the accessory loop results in gene expression rhythms with a period of about 24 h (i.e., circadian) (Bray et al. 2013; Bunger et al. 2000; Van Der Horst et al. 1999; Vitaterna et al. 1994). The SCN uses environmental light information to adjust its circadian rhythm to the exact 24-h period of the environment. Many studies have shown that also the peripheral clocks show daily rhythms, but the coherency of these rhythms seems to depend strongly on the SCN as some studies showed that peripheral tissues lost their daily rhythmicity after an SCN lesion (Akhtar et al. 2002; Sakamoto et al. 1998; Yoo et al. 2004; Yamazaki et al. 2000). However, more recent studies (Husse et al. 2014, 2015; Saini et al. 2013; Tahara et al. 2012) showed that the peripheral clock genes may maintain their daily rhythmicity, although with a different amplitude, after an SCN lesion. These recent findings indicate that the rhythms of peripheral clocks apparently can also be synchronized with the environmental day/night changes through extra-SCN pathways. Nevertheless, the accepted view is that this synchronization occurs via hormonal rhythms, neural connections and/or behavioral

* Corresponding author. Department of Endocrinology and Metabolism, Academic Medical Center (AMC), University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands.
E-mail address: a.kalsbeek@amc.uva.nl (A. Kalsbeek).

http://dx.doi.org/10.1016/j.mce.2015.12.011
0303-7207/

rhythms, such as feeding and body temperature (Mohawk et al. 2012; Schibler et al. 2003).

For the liver, Terazono et al. (Terazono et al. 2003) showed that electrical stimulation of the sympathetic nerves as well as injections of (nor)adrenalin and 6-hydroxydopamine (6-OHDA) modulated the daily rhythm of hepatic *Per1* and *Per2* expression, indicating that sympathetic nervous activity is involved in maintaining daily *Per1* and *Per2* gene expression rhythms in the liver. Balsalobre et al. (Balsalobre et al. 2000) showed that the daily rhythm of albumin D-box binding protein (*Dbp*) expression was delayed for 3–4 h after injecting the glucocorticoid agonist dexamethasone, indicating that also the adrenal hormone corticosterone is involved in modulating daily clock gene expression rhythms in the liver. Finally, several studies (Damiola et al. 2000; Hara et al. 2001; Salgado-Delgado et al. 2013) have shown that also restricted feeding can reset the daily rhythm of clock gene expression in the liver, indicating the daily feeding rhythm as another important signal for entraining clock gene rhythms in the liver.

In order to get a further understanding of the relative importance of these different SCN outputs in the synchronization of daily clock gene rhythms in the liver, we investigated the separate effects of 6-meals-a-day feeding (this feeding schedule effectively removes the day/night rhythm in food intake (Kalsbeek and Strubbe, 1998)), total hepatic denervation, and adrenalectomy, as well as the combined effects of each of the three possible combinations of two of these outputs, on daily gene expression rhythms in the liver. Our results show that the daily rhythm of clock genes was only lost in the animals subjected to the combination of 6-meals-a-day feeding and adrenalectomy. In all other experimental conditions tested, the animals still kept daily clock gene expression rhythmicity.

2. Materials and methods

2.1. Animals

All experiments were performed with adult male Wistar rats (Charles River, Germany). Animals were kept in the animal facility with a 12 h light/12 h dark cycle (lights on at 7:00) under constant conditions of temperature (21 ± 2 °C) and humidity (60 ± 5%). Water was available ad libitum (AL) during the whole experiment. All experiments were approved by the animal care committee of the Royal Netherlands Academy of Arts and Science.

2.2. Six-meals-a-day feeding regimen

Food pellets were available in metal food hoppers, which were attached in front of the perspex cages. Rats could gnaw off pieces of food through vertical stainless steel bars situated in front of the food hopper. Access to food could be prevented by a sliding door situated in front of the food hopper. Door opening and closing were activated by an electrical motor and controlled by a clock. Rats were entrained to a feeding schedule consisting of six meals spread equally over the light/dark cycle (Kalsbeek and Strubbe, 1998). Food was available every 4 h for 12 and 11 min during day-time and night-time meals, respectively. The hopper could be taken off from the cage for weighing so that the amount of food consumed during the day and night could be measured daily. When animals consumed the same amount of food during day and night, adaptation to the feeding schedule was considered completed.

2.3. Surgical procedures

2.3.1. General procedure

Rats were anesthetized with 0.08 ml/100 g body weight (BW) i.m. Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 0.04 ml/100 g BW s. c. Dormicum (Roche, Almere, the Netherlands). During surgery, the abdominal cavity was bathed regular with saline to keep the viscera wet. Post-operative care was provided by subcutaneous injection of the painkiller Buprecare after the operation.

2.3.2. Total hepatic denervation

Hepatic sympathectomy and parasympathectomy was performed as previously described (Kalsbeek et al. 2004). For hepatic sympathectomy, a laparotomy was performed in the midline. The liver lobes were gently pushed up, and ligaments around the liver lobes were severed to free the bile duct and portal vein complex, which were isolated from each other. At the level of the hepatic portal vein, the hepatic artery divides into the hepatic artery proper and the gastroduodenal artery. This cleavage occurs on the ventral surface of the portal vein. At this point, the arteries were separated via blunt dissection from the portal vein. Nerve bundles running along the hepatic artery proper were removed using microsurgical instruments under an operating microscope (25 × magnification). Any connective tissue attachments between the hepatic artery and portal vein were also broken, eliminating any possible nerve crossings. This sympathetic denervation involves an impairment of both efferent and afferent nerves. For hepatic parasympathectomy, the fascia containing the hepatic branch of the vagus nerve were stretched by gently moving the stomach and the esophagus. With the aid of a binocular-operating microscope, the neural tissue was transected between the ventral vagus trunk and liver. Also, small branches running in the fascia between the stomach and the liver were transected.

2.3.3. Adrenalectomy

Adrenalectomy was performed as previously described. In short, in the left and right side a skin incision 2–3 cm long was made just caudal to the rib. A small cut was made through the muscle layer after skin incision. Cotton swabs were inserted through the incision into the peritoneal cavity and used to move the organs and tissue to locate the adrenal gland. Once the gland was located, two forceps were inserted into the peritoneal cavity and used to grasp the periadrenal fat and exteriorized. The vessels at the base of the adrenal gland were clamped with both forceps. The forceps were then used to tear away the gland and its surrounding tissue. The tissue stump was then returned into the abdomen.

2.3.4. Total hepatic denervation plus adrenalectomy

The procedure of total hepatic denervation and adrenalectomy was the same as described above. The animals were subjected to these two surgeries at the same time.

2.4. Experimental set-up

2.4.1. Experiment 1: effects of a six-meals-day feeding schedule on clock gene expression in the liver

Animals (n = 64) were housed in separate cages, 32 animals were entrained to a six-meal feeding schedule (6M group). Rats needed ~10 days to adapt to the six-meal feeding schedule. For the other 32 single-housed rats, food was available ad libitum (AL group). Three weeks after adaptation, animals were sacrificed at 4 time points (ZT2, ZT8, ZT14 and ZT20; ZT0 being the time of lights on).

2.4.2. Experiment 2: effects of adrenalectomy and hepatic denervation on clock gene expression in the liver

Animals (n = 60) were divided into three groups: the first group was adrenalectomized (ADX group) (n = 20), the second group

Please cite this article in press as: Su, Y., et al., The role of feeding rhythm, adrenal hormones and neuronal inputs in synchronizing daily clock gene rhythms in the liver, Molecular and Cellular Endocrinology (2015), http://dx.doi.org/10.1016/j.mce.2015.12.011