Role of neuropeptide RFRP-3 in circadian rhythm

Circadian rhythms play a fundamental role in the effective functioning of complex organisms by allowing the body to anticipate changing environment that enhances survival[1]. Conversely, defective rhythmicity places organisms at a disadvantage, e.g. the debilitating effects of jet lag, shift working and genetic sleep disorders on behaviour and physiology[2]. In recent years, a basic description of the molecular clock that drives the endogenous circadian rhythm is available. Although most work initially assumed that the hypothalamic suprachiasmatic nuclei (SCN) was the locus of the endogenous clock, it has now become clear that the same or very similar molecular mechanisms function in most cells and tissues of the body[3] and that these clocks can continue to run in the absence of SCN[4]. The role of SCN is now seen as coordinating multiple clocks of the body through a variety of neural and humoral mechanisms[5] to keep them in an appropriate phase relationship with one another, such that whole-body function is effectively integrated[6]. The role of the SCN clock is seen as important because it provides the major route by which external environmental circadian changes, principally the light/dark cycle, can be used to gain synchronization of the phase and period of the endogenous body clocks with solar and calendrical time. However, this hierarchical model may underestimate the capacity of the peripheral clocks to respond to environmental inputs independent of the influence of SCN[7].

Timing and rhythm are important during reproduction and development[8]. The circadian rhythm and SCN are intimately involved in timing of the leuteinizing hormone (LH) surge during the estrous cycle[9], the initiation of fertility[10], and also via the pineal, influence seasonal changes in fertility[11]. Moreover, seasonal changes have been reported in the circadian rhythm of serotonin and dopamine concentration in the SCN of Syrian hamster[12]. The coordination of maternal circadian rhythmicity with that of foetus and neonate may be important for parturition and early post-natal survival[13].

It is obvious that several circadian rhythms of the body (neural, hormonal, physiological, metabolic, etc.) have different acrophase/timing of peak activity, and hence their activity phase may or may not coincide with one other establishing different temporal interactions of a given time. This provides the basis for the role of temporal synergism of circadian oscillations in the regulation of reproduction in both continuous and seasonal breeders. The experimental basis was that hormones/neurotransmitters have different activities (as a function of time of the day) and the phase of these circadian rhythms changes seasonally. In this communication, resetting of the annual cycle (photo/scotorefractory and photo/scotosensitive conditions) has been reported in some seasonally breeding species with timed daily injections of 5-hydroxytryptophan (5-HTP, serotonin precursor) and L-dihydroxyphenyalanine (L-DOPA, dopamine precursor)[14,15]. This treatment (5-HTP and L-DOPA given at specific time intervals), in addition to modulating the gonadal activity, is also reported to alter the phase relation of the circadian hypothalamic content of serotonin and dopamine in quail[16].

In spite of a large number of experimental evidences in seasonal breeders in support of this hypothesis, so far no attempt has been made to test this (hypothesis) in those species having different breeding strategies, i.e. in continuous breeders, especially in terms of the role of temporal synergism of neural oscillations on gonadal growth. Here we focus on the role of temporal synergism of neural oscillation and the neuropeptide – Rfamide-related peptide-3 (RFRP-3), the mammalian ortholog of avian gonadotropin-inhibitory hormone (GnIH)[17].

Three-week-old prepubertal mice (12–14 g) were randomly divided into three groups ($n = 5$ per group). The mice were housed under hygienic conditions in a well-ventilated photoperiodically controlled room (light : dark 12 : 12; lights on at 0800 h) at temperature 22 ± 2°C, and were provided with commercial food and tap water ad libitum. Mice of the control group received two daily injections of normal saline at 0800 and 2000 h. The two precursor drugs 5-HTP and L-DOPA, were prepared daily and injected intraperitoneally (5 mg/100 g body wt/day) in 0.1 ml solution over a period of 13 days in experimental mice. Mice of both the experimental groups received serotonin precursor (5-HTP) at 0800 h; and the dopamine precursor (L-DOPA) at different time intervals in the two groups, i.e. at 1600 and 2000 h, so as to establish 8 or 12 h phase relations respectively, between the two injections. Twenty-four days after the last injection, mice were anaesthetized. Brain and testes were dissected out and post-fixed to be processed for the immunohistochemistry of RFRP-3 (in brain) and for routine histology (in testis). Results showed degenerated structures in the testicular histology of the 8 h mice compared to the control. Some seminiferous tubules showed complete absence of a defined structure, while some tubules appeared atrophic showing intraepithelial vaculoation. Other tubules were apparently still normal but with reduced layers of the seminiferous epithelium containing pycnotic nuclei. Presence of desquamation in the enlarged lumen and enlarged interstitial spaces was also apparent in the testis of 8 h mice. The seminiferous tubules of the 12 h mice showed full breeding condition, with all the successive stages of transformation of spermatogonia into spermatozoa. Coronal sections of the brain showed increased immunostaining in the dorsomedian nucleus of the hypothalamus (DMH) neurons of 8 h mice, which decreased in the 12 h mice compared to the control. The total number of immunoreactive RFRP-3 cell bodies using a 20× objective lens and MacBiophotonics ImageJ software and RFRP-3 neuronal area with image analyser software (Motic Images 2000 version 1.3) were analysed in the DMH. Significant increase in the neuronal area and number was observed in the 8 h mice, while these parameters decreased in the 12 h mice compared to the control[18].

Different effects of 5-HTP and L-DOPA if given at different time intervals, appear to be physiological effects and not pharmacological because: (i) in spite of receiving the same dose of neurotransmitter precursors, the response was different in the two groups of mice as a function of their time relation, and (ii) in addition to affecting testicular function, 8 h and 12 h relation also induced opposite effects on the RFRP-3 neurons. Combining these two responses together, it is clear that decreased gonadal activity in mice correlates with

increased RFRP-3 neuronal activity in DMH nuclei and vice versa.

This work opens up a new avenue of research to support the role of circadian system to comprehend gonadal development during the maturation of reproductive axis in mice, suggesting the possibility of a common mechanism in the reproductive regulation of both seasonal and continuous breeders. This communication also indicates that temporal phase relation between the circadian serotoninergic and dopaminergic oscillations may modulate gonadal development during the process of sexual maturity in the laboratory mice. Further, there would be many inhibitory neuroendocrine mechanisms involved in the prepubertal and drug-induced (8 h relation) reproductive suppression, and RFRP-3 appears to be one of these factors. Additional studies involving administration of RFRP-3 in adult or 12 h mice and its antagonist in prepubertal or 8 h mice may strengthen the evidence supporting this suggestion.

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Global influence of Cancer Statistics articles

The contribution or impact of a research article can be assessed based on the number of times the article is cited by other research articles. Each year, *CA – A Cancer Journal for Clinicians* publishes a series of articles called Cancer Statistics, which receives an extremely high number of citations. Over the years, Cancer Statistics articles have provided important information for cancer researchers and clinicians throughout the world. Here we report the global influence of Cancer Statistic articles and demonstrate the shift in their influence toward East Asia, where researchers have significantly increased their frequency in citing Cancer Statistics articles while showing a decreasing trend in citations by American researchers. Data for this correspondence were retrieved from the SCI-EXPANDED database of the *Web of Science* (*WoS*) of Thomson Reuters.

Figure 1 shows the annual number of citations of Cancer Statistics articles published from 2001 to 2011. During this time, these articles in general showed a citation peak in the second year of publication, as well as a higher citation peak with each successive year, indicating an increasing trend in their influence and readership. Table 1 shows the global influence of Cancer Statistics articles. As of the end of 2012, 'Cancer Statistics, 2001' by Greenlee *et al.*[1] has been cited in 2237 research papers by 10,401 authors from 2109 institutions in 56 countries, and was published in 685 journals belonging to 99 *WoS* categories in science. 'Cancer Statistics, 2008' by Jemal *et al.*[2] has the highest number of citations as of TC2012 (total number of times cited since the paper was published to 2012). It has been cited in 5544 research papers by 25,811 authors from 4530 institutions in 83 countries. In the last decade, Cancer Statistics articles have significantly increased their global influence. The more recent articles of 2009, 2010, and 2011 did not have as many citations as 'Cancer Statistics, 2008' (ref. 2), since the recent articles had a shorter life and time to accumulate citations. However, length of time may not be the only factor. Further analysis revealed that a reduction in the US papers citing Cancer Statistics articles has contributed to their lower citations after 2008.

Table 2 compares the number of citations, as well as the percentage of total citations that Cancer Statistics articles received from the top 10 leading countries. Since Cancer Statistics articles showed a citation peak in the second year of publication, we limit the time-frame to the first two years of article life. Citations at the end of their year can be used as a proxy of their visibility in cancer research. Furthermore, it does not vary with time, unlike the total number of citations, which can be affected by the article life, and thus can be used to provide a fair comparison among articles. Cancer Statistics articles published after 2011 were not included, as they may not have reached their citation peak.