TIME-DEPENDENT EXPRESSION OF AHR AND ARNT MRNAS IN MULTIPLE MOUSE TISSUES

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Introduction As a member of the basic-helix-loop-helix(bHLH)-PAS family of transcription factors, the AhR mediates many of the biological responses to exogenous ligands such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds[1]. The Arnt protein, also a member of the bHLH-PAS family, seems to have numerous functions but appears to be a necessary component of the AhR signal transduction pathway[1,2,3]. It is known that bHLH-PAS proteins play a role in biological rhythms[4] (Crews, 1998) and studies from this laboratory have shown a daily cycle of AhR and Arnt protein expression in multiple tissues of Sprague-Dawley rats[5]. Since, the AhR shares a common PAS domain with a number of genes that exhibit a pronounced circadian rhythm, the purpose of this study was to examine the daily cycle of AhR and Arnt mRNA expression in multiple untreated tissues of male C57BL/6N mice.

Methods

Animals, and treatment Male C57BL/6N mice (12 weeks old; ~30 g), purchased from Charles River Laboratories (Raleigh, NC), were allowed free access to food and water. Mice were maintained in a diurnal cycle of 12 h of light/dark (6 AM/6 PM). The mice were euthanized at 4 AM, 7 AM, 11 AM, 4 PM, 7 PM, and 11 PM by CO_2 asphyxiation (n= 4 animals/time point). All animals were euthanized within a 15 minute period. The liver, lung, spleen, and thymus were removed, weighed and immediately placed in liquid nitrogen. All samples were stored at -80°C until use.

RNA isolation, relative real-time RT-PCR RNA was isolated using the Chomczynski and Sacchi method[6]. Real-time RT-PCR was performed using the ABI Prism 7700.

Results and Discussion

AhR and Arnt mRNA expression in liver Figure 1 shows an almost identical daily pattern in the mRNA expression of AhR and Arnt. Though not statistically significant, AhR and Arnt expression decreases after 4 AM and then recovers at the 4 PM time point.

AhR and Arnt mRNA expression in lung Figure 2 demonstrates an increase in relative AhR mRNA expression that was observed between the hours of 4 and 7 AM which was followed by a decrease between the hours of 7 and 11 AM. AhR mRNA expression increased between 11 AM and 4 PM, decreased between 4 and 7 PM, increased between 7 and 11 PM, and then decreased between 11 and 4 AM (day 2). Arnt exhibited a similar oscillation pattern except mRNA expression decreases between 7 and 11 AM, but does not start to increase again until between 4 and 7 PM.

AhR and Arnt mRNA expression in spleen Figure 3 shows a slight oscillation between 4 and 11 PM in AhR mRNA expression. No cycling pattern in Arnt mRNA was seen, demonstrating a lack

of coordinating regulation between AhR and Arnt expression. Previous studies have shown that TCDD-treatment results in a rapid depletion of AhR protein in multiple tissues with little change in Arnt protein, also suggesting a lack of coordinate regulation[7].

AhR and Arnt mRNA expression in thymus Figure 4 exhibits a similar oscillation pattern between AhR and Arnt mRNA expression. Between the hours of 4 and 7 PM there is a slight decrease and then an increase between 7 and 11 PM in mRNA expression.

Summary These data show that the mRNA expression of the bHLH/PAS proteins AhR and Arnt, exhibit apparent tissue specific daily oscillations within multiple tissues of male C57BL/6N mice. With protein expression still to be examined from this study, this data gives a good indication that AhR and Arnt protein expression may resemble the daily cycle of that found in Sprague-Dawley rats[5].

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Figure 1

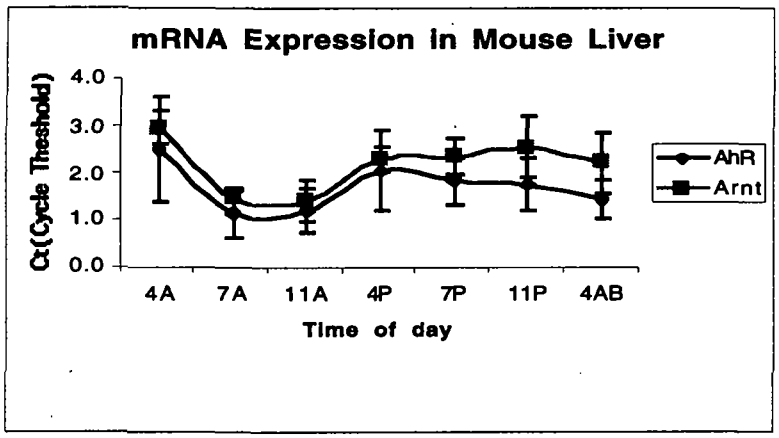

Figure 2

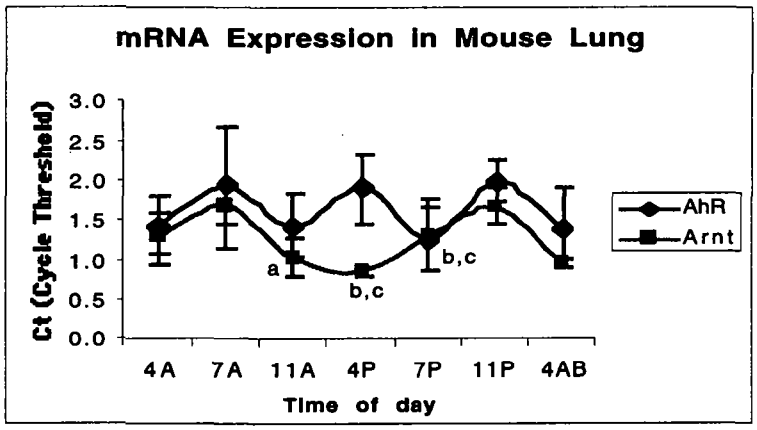

Figure 1 and 2. Daily cycle of AhR and Arnt mRNA expression in male C57BL/6N mice. All data are represented as the means ± standard deviation (n=4). Data are normalized to β-Actin. The statistical comparisons were determined using a one-way analysis of variance (ANOVA) followed by Fisher's test with $P< 0.05$. [a]statistically different than the 4 AM time-point. [b]statistically different than the 7 AM time-point. [c]statistically different than the 11 PM time-point.

Figure 3

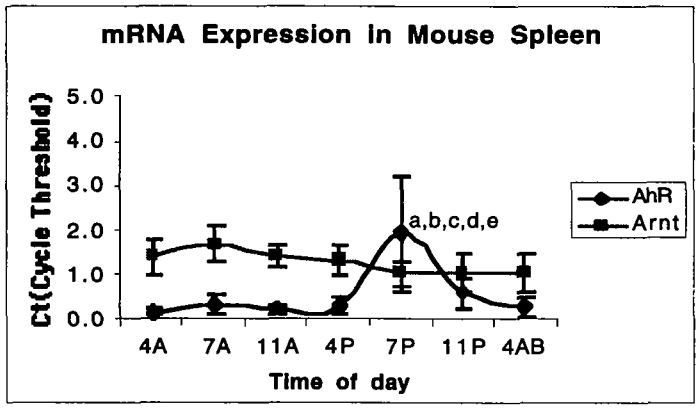

Figure 4

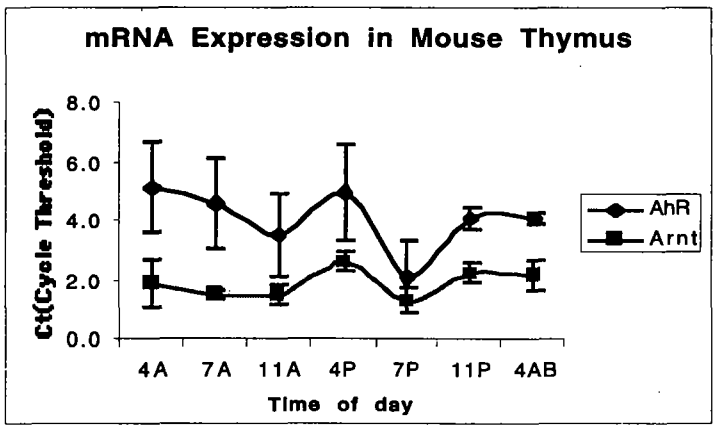

Figure 3 and 4. Daily cycle of AhR and Arnt mRNA expression in male C57BL/6N mice. All data are represented as the means ± standard deviation (n=4). Data are normalized to β-Actin. The statistical comparisons were determined using a one-way analysis of variance (ANOVA) followed by Fisher's test with $P< 0.05$. [a]statistically different than the 4 AM time-point. [b]statistically different than the 7 AM time-point. [c]statistically different than the 11 AM time-point. [d]statistically different than the 4 PM time-point. [e]statistically different than the 11 PM time-point.