IJAE
Vol. 118, n. 1 (Suppl.): 37-41, 2013
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Consequences of relaxin-3 null mutation in mice on food-entrainable arousal

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Summary

Relaxin-3/RXFP3 networks have been hypothesised to influence behavioural state based on their anatomical distribution and recent experimental findings in rat and mouse. Two arousal-related behaviours altered by changes in relaxin-3/RXFP3 signalling are feeding and voluntary running wheel activity. In particular, relaxin-3 null mutation (knockout) mice display a 'dark-phase hypoactivity' phenotype, reflected by reduced voluntary running wheel activity and increased sleeping behaviour, with no other major changes in basal behavioural profile. The present study compared the ability of relaxin-3 deficient (null mutation) and C57BL/6J wildtype littermate mice to entrain daily running wheel activity to timed food availability. Both genotypes adjusted to a restricted feeding paradigm of 3 hours access from ZT6 to ZT9 for 14 days and displayed increased running wheel activity in the 3 hour period prior to scheduled feeding, a phenomena termed food anticipatory activity. No significant difference in running wheel activity was observed between the genotypes, indicating that a whole-of-life relaxin-3 deficiency does not prevent entrainment to a restricted-feeding schedule. Further studies of the precise interaction between relaxin-3/RXFP3 signalling and the other major arousal networks are ongoing, using currently available and new strains of transgenic mice in combination with pharmacological and viral-based methods.

Key words

Relaxin-3, RXFP3, arousal, running wheel, food anticipatory activity, knockout mouse.

Introduction

Relaxin-3 is the most recently discovered member of the insulin-like peptide superfamily and acts as a neuropeptide in mammalian brain, signalling via its $G_{i/o}$-protein-coupled receptor, relaxin family peptide receptor 3 (RXFP3) (Ma et al., 2007; Smith et al., 2010, 2011). The function of relaxin-3/RXFP3 systems has been predominantly investigated in rats, but more recently in wildtype and relaxin-3 knockout (KO) mice (Smith et al., 2012; Watanabe et al., 2012). In rats, central injection of relaxin-3 and other RXFP3 agonists increases food intake (McGowan et al., 2005; Ganella et al., 2012; Shabanpoor et al., 2012), and deletion of the relaxin-3 gene in mice produces a prominent circadian hypoactivity phenotype, reflected by reduced voluntary running wheel and increased 'sleeping behaviour' during the dark/active phase (Smith et al., 2012). Voluntary wheel running is largely a nocturnal activity in mice occurring during their active, 'awake' phase. Sleep-wake cycles are regulated by

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the *central oscillator* located in the hypothalamic suprachiasmatic nucleus (Welsh et al., 2010). In nature, optimal feeding times dictate an alternative circadian clock termed the *food entrainable oscillator* (FEO). It has been demonstrated that if feeding is restricted to limited times of the day, in the hours prior to the provision of food there is an increase in body temperature, plasma corticosterone, and motor activity, which is termed food anticipatory activity (FAA) (see Mistlberger et al., 1994; Stephan, 2002); although the key brain regions underpinning this behaviour are unclear. This study investigated arousal related to food entrainment in relaxin-3 KO mice, to further explore the role of relaxin-3 in arousal-related behaviours.

Methods

Littermate female relaxin-3 KO and wildtype mice (backcrossed C57BL/6J genetic background; original breeder mice kindly supplied by Johnson & Johnson PR&D LLC, San Diego, CA, USA) were singly housed with access to a 37 cm circumference running wheel connected to a reed switch and activity wheel counters (Lafayette Instruments, Lafayette, IN, USA). Mice were maintained on a 12:12 h light:dark cycle and provided with water *ad libitum*. Baseline running wheel activity was established over a 2-3 week period with *ad libitum* access to food. Access to food was then gradually tapered over 4 nights and thereafter was provided only during the light, 'inactive/sleep' phase between Zeitgeber time (ZT) 6 and 9 for 14 days (n = 12 relaxin-3 KO; n = 11 WT littermates). The number of wheel revolutions during the 3 hours before the provision of food was measured as a reflection of FAA. Separate groups of wildtype and relaxin-3 KO mice (n = 3/group) were maintained with *ad libitum* access to food throughout the experiment to serve as additional controls. Food was weighed and intake measured daily. Data were analysed using one-way ANOVA and t- test. Data is presented as mean ± standard error of the mean (SEM).

Results

During the baseline period with *ad libitum* access to food, both genotypes displayed wheel running activity almost exclusively during the dark 'active' phase of the light/dark cycle (ZT12 to ZT0; see Fig. 1). As previously reported, relaxin-3 KO mice had significantly less average nocturnal running wheel revolutions over the baseline period than WT mice (WT = 12748 ± 1541, relaxin-3 KO = 6804 ± 844; P = 0.003). Upon food restriction however, the average number of wheel revolutions during the dark phase exhibited by relaxin-3 KO mice was not different to that of WT mice (WT = 12106 ± 1406, relaxin-3 KO = 13551 ± 1696; P = 0.52). Both relaxin-3 KO and WT mice displayed stable FAA by day 4 of food restriction, as reflected by increased running wheel activity during the 3 hours before provision of food at ZT6. This 3 h/day FAA was maintained throughout the duration of the experiment (14 days), whilst no activity was observed during this same 3 h time window in the *ad libitum* fed WT and relaxin-3 KO (control) mice (data not shown). Running wheel activity during the FAA period when normalised to the total daily activity revealed a trend for relaxin-3 KO mice to display a higher proportion of their 24 h wheel run-

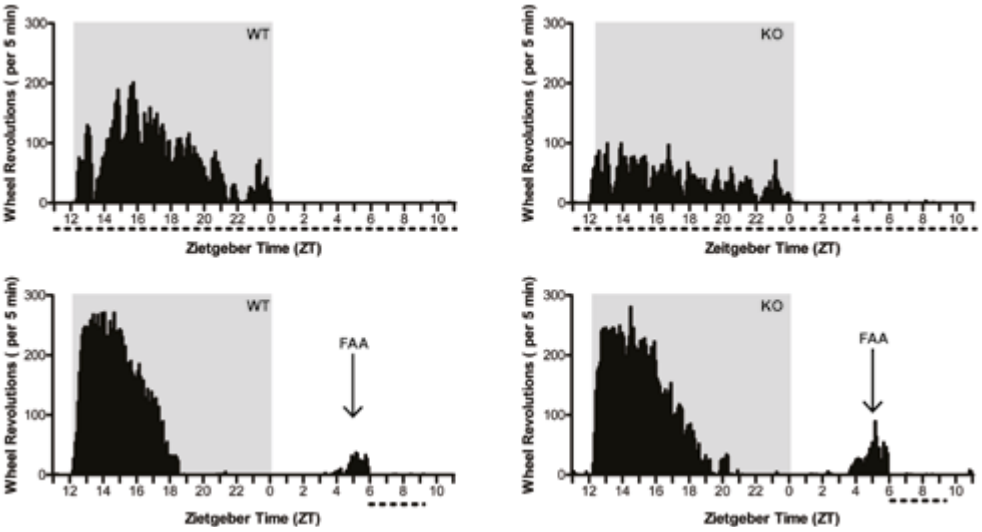

Figure 1. Average running wheel activity on representative days during *ad libitum* access to food (upper panels) and during food restriction (lower panels) of relaxin-3 KO (n = 12) and WT littermate mice (n = 11) plotted in 5 min bins over a 24 h period (mean data shown). Grey shading indicate dark phase, dashed lines indicate food availability, and arrows indicate food anticipatory activity (FAA).

ning activity during the FAA period compared to WT mice (WT 2.51 ± 1.57%, relaxin-3 KO 6.26 ± 1.37%; P = 0.085). There was no difference in the average daily food intake during the food restriction period (WT 3.49 ± 0.08 g and relaxin-3 KO 3.52 ± 0.05 g; P = 0.54), and there was no difference in body weight gain between genotypes before and after the 14 day food restriction paradigm (WT 3.17 ± 0.32 g; relaxin-3 KO 2.97 ± 0.22 g; P = 0.59).

Discussion

This experiment examined the ability of relaxin-3 deficient mice to entrain to a circadian schedule determined by food availability. Running wheel FAA in the hours prior to food availability was displayed in both relaxin-3 KO and WT littermate mice, and additionally no apparent differences between genotypes were detected in total 24 hour running wheel activity, food intake, or body weight change. Interestingly, relaxin-3 KO mice displayed a trend for increased normalised running wheel activity during the FAA period compared to controls. It is unknown whether the WT mice were engaged in other arousal-related activities during this time, as other parameters were not recorded in the current study, but it will be of interest to determine any preference for behaviours such as climbing and approaches to the food area during FAA.

The nocturnal hypoactive running wheel activity observed in relaxin-3 KO mice during the baseline period was in line with previous reports on these mice and appears robust (Smith et al., 2012), but the observed cessation of this dark-phase

hypoactivity during food restriction was unexpected. Complex neurochemical and physiological changes occur during restricted feeding, however, and some have been associated with changes in wheel running profiles, e.g. the suprachiasmatic nucleus-independent expression of the clock genes mPer1 and mPer2 shift from a nocturnal to a daytime peak in expression during food restriction (Wakamatsu et al., 2001). As both relaxin-3 KO and WT mice shifted to a similar running wheel activity profile, the physiological changes associated with the food restriction paradigm in this study appear to be independent of relaxin-3 signalling.

FAA is characterised by increased behavioural arousal that drives food seeking behaviour during a period of time prior to predicted food availability. This adaptive response maximises likely reward for food seeking in varied environments, rather than any strict adherence to a rigid genetically programmed nocturnal or diurnal activity pattern. Our findings indicate that mice with a whole-of-life relaxin-3 deficiency retain an intact ability to display FAA, but because FAA involves multiple homeostatic systems with many contributing elements, compensatory mechanisms may be present in these mice. Future analysis using conditional relaxin-3 or RXFP3 KO mice, or pharmacological studies of central infusion of RXFP3 antagonists, should yield a better understanding of the role of relaxin-3 in arousal behaviour related to feeding.

Acknowledgments

This research was supported by a National Health and Medical Research Council of Australia Dora Lush postgraduate scholarship (ITH) and project grant 509246 (ALG); by the Pratt and Besen Family Foundations; and by the Victorian Government Operational Infrastructure Support Program.

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