IJAE
Vol. 118, n. 1 (Suppl.): 52-55, 2013
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Pharmacological activation of RXFP3 is not orexigenic in C57BL/6J mice

Craig M. Smith[1,2,3], Ihaia T Hosken[1,3], Natalie L. Downer[1], Berenice E. Chua[1], Mohammad Akhter Hossain[1,2,4], John D. Wade[1,2,4], Andrew L. Gundlach[1,2,3,*]

[1]The Florey Institute of Neuroscience and Mental Health, [2]Florey Department of Neuroscience and Mental Health, [3]Department of Anatomy and Neuroscience and [4]School of Chemistry, The University of Melbourne, Victoria 3010, Australia

Summary

The neuropeptide relaxin-3 and its cognate G-protein-coupled receptor, RXFP3, have been implicated in the control of feeding behaviour in rats. For example, relaxin-3-positive projections and RXFP3 are present within hypothalamic feeding circuits, and icv injection of human relaxin-3 (~0.2 to 1.0 nmol) robustly increases feeding behaviour in satiated rats. To explore whether this action is conserved in other experimental species, the present study examined feeding behaviour in C57BL/6J mice following RXFP3 modulation, as mice display near identical regional distribution patterns of relaxin-3/RXFP3, and relaxin-3/RXFP3 signalling has been shown to modulate behavioural arousal in both species. Central injection of the RXFP3 agonists *R3/I5* or *H3 relaxin* (0.5 nmol, icv) did not alter chow consumption in satiated mice relative to vehicle controls, during the 60 min after treatment. Furthermore, relaxin-3 knockout mice displayed similar basal 24-h chow consumption and 1-h palatable food consumption to wildtype littermate controls; although further studies involving acute pharmacological antagonism of RXFP3 in WT mice are required to eliminate the likelihood of compensation in these life-long relaxin-3 deficient mice. Taken together, these findings are in contrast to the potent orexigenic effects of RXFP3 activation observed in rats, and may reflect differential RXFP3 expression within hypothalamic neuron populations in the rat and mouse, or differences in signalling upstream or downstream of relaxin-3/RXFP3 networks in these two species.

Key words

Relaxin-3, RXFP3, feeding, arousal, motivation, knockout

Introduction

The neuropeptide relaxin-3 is produced by neurons in the *nucleus incertus*, which send relaxin-3-positive projections to a broad range of brain regions that express the cognate receptor for relaxin-3, RXFP3 (Ma et al., 2007; Smith et al., 2010, 2011). The discovery of a high density of RXFP3 within hypothalamic regions associated with the control of feeding prompted several studies in the rat, which demonstrated that intracerebroventricular (icv) infusion of relaxin-3 or specific RXFP3 agonists robustly increase feeding behaviour at doses ranging from 180-1000 pmol (McGowan et al., 2005; Ganella et al., 2012). However, as it has not yet been reported whether or not these orexigenic effects are conserved in other experimental species, the present study assessed feeding behaviour in C57BL/6J mice following modulation of RXFP3.

* Corresponding Author: The Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Victoria, 3010, Australia; Email: andrew.gundlach@florey.edu.au; Ph: +61 3 9035 6507; Fax: +61 3 9035 0321.

http://www.fupress.com/ijae

Methods

Icv infusion studies: Adult male C57BL/6J mice were anaesthetised and surgically implanted with an indwelling guide cannula targeting the lateral cerebral ventricle. After recovery, the location of the guide cannula was verified by testing for a drinking response after injection of angiotensin II (25 ng). Satiated mice with *ad libitum* access to food were injected during the light phase with either artificial cerebrospinal fluid (aCSF) vehicle (1 μl), or 500 pmol of the RXFP3 agonists *R3/I5* or *H3 relaxin* (Liu et al., 2005). The amount of food consumed in their home-cage over the subsequent 60 min was then assessed. *Relaxin-3 KO mouse studies:* The amount of regular chow consumed by backcrossed adult male C57BL/6J relaxin-3 knockout (KO) and wild-type (WT) littermate mice (Smith et al., 2012) was measured daily over 4 weeks and averaged. To assess palatable food consumption, satiated adult, male relaxin-3 KO and WT littermate mice were provided with a pellet of palatable food (consisting of approximately equal parts fat, protein and sugar) in their home cage for 1 h during the light phase. Due to the variable and generally low amounts consumed during the first exposure, the experiment was repeated on 3 consecutive weeks, and data from the 3rd week was assessed. *Statistical analysis:* Feeding data were analysed by one-way RM ANOVA or unpaired t-tests, as appropriate.

Results

Icv infusion of RXFP3 agonists did not increase feeding in mice: In contrast to observations in rats under similar conditions, icv infusion of 500 pmol *R3/I5* or *H3 relaxin* in satiated mice during the light phase did not alter food consumption, compared to vehicle controls (Fig. 1A). This lack of effect was not due to a lack of biological activity of the *R3/I5* or *H3 relaxin* samples, as these batches of peptide were observed to increase feeding in rats at the same bolus dose in studies in our laboratory (Shabanpoor et al., 2012), despite the relatively larger brain size and cerebrospinal fluid volume in this species.

Life-long relaxin-3 deficiency did not alter feeding behaviour in mice: In line with previous observations that backcrossed C57BL/6J relaxin-3 KO mice display normal bodyweights (Smith et al., 2012), no differences between relaxin-3 KO and WT littermates were observed in measures of average 24-h food consumption (Fig. 1B), or in the 1-h consumption of palatable food (Fig. 1C).

Discussion and Conclusion

The absence of an orexigenic effect following icv infusion of RXFP3 agonists in mice suggests this RXFP3 action may not be strongly conserved across species. This apparent discrepancy was not predicted, as both species display an essentially identical regional distribution pattern of RXFP3 mRNA and protein (binding sites) (Ma et al., 2007; Smith et al., 2010). However, the precise cellular distribution of RXFP3 within the hypothalamus and the relative downstream targets and signalling effects are currently unknown; and hence it is possible that RXFP3 activation directly or

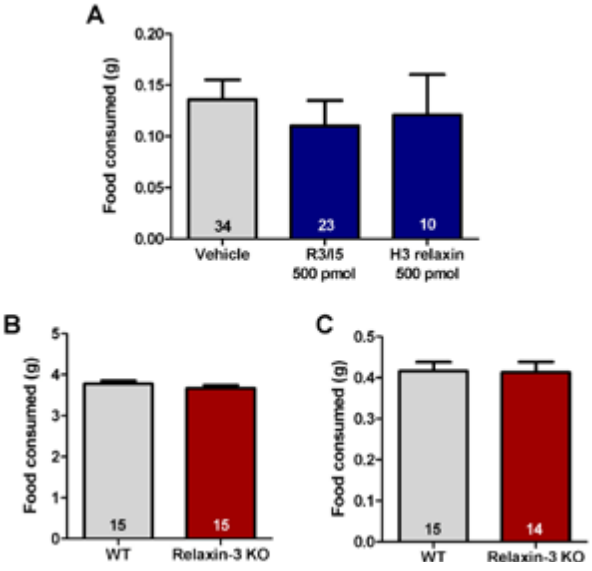

Figure 1. A: Equivalent amounts of regular chow consumed by satiated C57BL/6J mice in 1 h during the light phase following icv infusion of aCSF vehicle or 500 pmol of the RXFP3 agonists R3/I5 or H3 relaxin. B: Equivalent amounts of regular chow consumed by relaxin-3 knockout and wildtype littermate mice during an average 24 h period. C: Equivalent amounts of palatable food consumed by relaxin-3 knockout and wildtype littermates during a 1-h access period. Group sizes (n) are indicated within columns. Data are expressed as mean ± SEM.

indirectly influences feeding-related circuits within the hypothalamus in rats, but not mice. This conclusion is in line with observations that C57BL/6J relaxin-3 KO mice do not display any overt differences in feeding behaviour and body weight compared to WT controls, although due to the potential for compensation in these life-long knockout mice, examining the effects of acute pharmacological antagonism of RXFP3 in WT mice remains an important future direction.

Neuronal circuits and transmitters that control behavioural arousal often also modulate feeding behaviour, as exemplified by orexin (Sakurai, 2007); an effect that is not surprising given that acquiring food is a major goal to ensure survival. Although at first the present data appear to suggest profound differences between rat and mouse relaxin-3 systems, anatomical and functional evidence suggests relaxin-3/RXFP3 signalling primarily modulates behavioural arousal in *both* species. For example, relaxin-3 appears to be associated with an ascending arousal pathway in both rats (Ma et al., 2007) and mice (Smith et al., 2010, 2011). Furthermore, local infusion of an RXFP3 agonist or antagonist into the septum of rats increases or decreases hippocampal theta rhythm respectively, which is a process closely associated with and required for behavioural arousal (Ma et al., 2009); while in mice with voluntary access to running wheels, deletion of the relaxin-3 gene results in circadian hypoactivity (Smith et al., 2012). Therefore, it seems likely that relaxin-3/RXFP3 signalling plays a similar broad role in promoting arousal in both species, although differences exist in how this manifests as specific behaviours.

Acknowledgments

This research was supported by National Health and Medical Research Council of Australia project grants 509246 (ALG) and 508995 (JDW), and by the Pratt and Besen Family Foundations (ALG) and the Victorian Government Operational Infrastructure Support Program.

References

Ganella D.E., Ryan P.J., Bathgate R.A.D., Gundlach A.L. (2012) Increased feeding and body weight gain in rats after acute and chronic activation of RXFP3 by relaxin-3 and receptor-selective peptides: functional and therapeutic implications. Behav Pharmacol. 23: 516-525.

Liu C., Chen J., Kuei C., Sutton S., Nepomuceno D., Bonaventure P., Lovenberg T.W. (2005) Relaxin-3/insulin-like peptide 5 chimeric peptide, a selective ligand for G protein-coupled receptor (GPCR)135 and GPCR142 over leucine-rich repeat-containing G protein-coupled receptor 7. Mol Pharmacol. 67: 231-240.

Ma S., Bonaventure P., Ferraro T., Shen P.J., Burazin T.C.D., Bathgate R.A.D., Liu C., Tregear G.W., Sutton S.W., Gundlach A.L. (2007) Relaxin-3 in GABA projection neurons of nucleus incertus suggests widespread influence on forebrain circuits via G-protein-coupled receptor-135 in the rat. Neuroscience. 144: 165-190.

Ma S., Olucha-Bordonau F.E., Hossain M.A., Lin F., Kuei C., Liu C., Wade J.D., Sutton S.W., Nunez A., Gundlach A.L. (2009) Modulation of hippocampal theta oscillations and spatial memory by relaxin-3 neurons of the nucleus incertus. Learn Mem. 16: 730-742.

McGowan B.M., Stanley S.A., Smith K.L., White N.E., Connolly M.M., Thompson E.L., Gardiner J.V., Murphy K.G., Ghatei M.A., Bloom S.R. (2005) Central relaxin-3 administration causes hyperphagia in male Wistar rats. Endocrinology. 146: 3295-3300.

Sakurai T. (2007) The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nat Rev Neurosci. 8: 171-181.

Shabanpoor F., Hossain M.A., Ryan P.J., Belgi A., Layfield S., Kocan M., Zhang S., Samuel C.S., Gundlach A.L., Bathgate R.A.D., Separovic F., Wade J.D. (2012) Minimization of human relaxin-3 leading to high-affinity analogues with increased selectivity for relaxin-family peptide 3 receptor (RXFP3) over RXFP1. J Med Chem. 55: 1671-1681.

Smith C.M., Hosken I.T., Sutton S.W., Lawrence A.J., Gundlach A.L. (2012) Relaxin-3 null mutation mice display a circadian hypoactivity phenotype. Genes Brain Behav. 11: 94-104.

Smith C.M., Ryan P.J., Hosken I.T., Ma S., Gundlach A.L. (2011) Relaxin-3 systems in the brain - The first 10 years. J Chem Neuroanat. 42: 262-275.

Smith C.M., Shen P.J., Banerjee A., Bonaventure P., Ma S., Bathgate R.A.D., Sutton S.W., Gundlach A.L. (2010) Distribution of relaxin-3 and RXFP3 within arousal, stress, affective, and cognitive circuits of mouse brain. J Comp Neurol. 518: 4016-4045.