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Behavioral analysis of relaxin-3 deficient mice

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Summary

Relaxin-3 is a neuropeptide belonging to the relaxin/insulin superfamily. Studies using rodents have revealed that relaxin-3 is predominantly expressed in neurons in the nucleus incertus of the pons, projecting axons to forebrain regions including the hypothalamus. There is evidence that relaxin-3 is involved in several functions, including food intake and stress responses. We generated relaxin-3 gene knockout (KO) mice and examined them using a battery of behavioral tests of sensory/motor functions and emotion-related behaviors. Relaxin-3 KO mice exhibited normal growth and appearance. There was no difference in bodyweight among genotypes in both normal and high fat diet feeding. In addition, there were no significant differences between wild-type and KO mice in social interaction, depression-like behavior, and short memory test. However, in the elevated plus maze test, KO mice exhibited a robust increase in the tendency to enter open arms, although they exhibited normal performance in a light/dark transition test and showed no difference from wild-type mice in the open field test. Taken together, these results indicate that relaxin-3 KO mice exhibit mild anxiolytic characteristics relative to wild-type mice, suggesting that this peptide is involved in anxiety-related behavior.

Key words

Relaxin-3; knockout; battery of behavioral tests; elevated plus maze test; high fat diet; mice

Relaxin-3/insulin-like peptide7 is a new member of the insulin/relaxin family. We first reported the detail distribution of relaxin-3 in the rat brain using immunohistochemistry and in situ hybridization (Tanaka et al., 2005). Relaxin-3 is mainly expressed in neurons of the nucleus incertus (NI) of the pons, but minor population of expressing cells are found in other regions of brainstem such as periaqueductal gray and dorsal area to the substantia nigra. The fibers of relaxin-3 neurons ascend to widely forebrain regions, particularly dense in the areas which belong to the limbic system and in electron microscopy, this peptide is localized in the dense-cored vesicles and presumably released into the synaptic cleft. Developmentally relaxin-3 is detected along the fourth ventricle from embryonic day 18 at mRNA level and from postnatal day 0 at peptide level (Miyamoto et al., 2008). Several functions of relaxin-3 have been suggested by studies using rats and mice, including a role in food intake and stress responses, neuroendocrine function (McGowan et al, 2005; Banerjee et al, 2010). We reported that relaxin-3 gene expression in the NI was increased in response to restraint stress and intracerebroventricular (icv) administration of relaxin-3 induced c-Fos expression in the paravenricular nuleus and enhanced CRF

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mRNA expression. Moreover plasma ACTH was increased after relaxin-3 icv injection (Watanabe et al., 2011a).

In the present study, we generated mice carrying a null mutation in the relaxin-3 gene in order to investigate the function of this peptide in the whole body. We disrupted exon 1 and exon 2 of the relaxin-3 gene by replacing with a PGK-neomycin resistance cassette. A targeting vector was transfected into ES cells derived from 129/SvJ mouse strain by electroporation. After eight generations of backcrossing with C57BL/6N, relaxin-3 null mice were generated by mating heterozygous mice and confirmed that the mRNA and peptides of relaxin-3 were never exhibited (Watanabe et al., 2011b). These mice grow normally, and are fertile. There were no evident abnormalities in the physical characteristics of relaxin-3 KO mice, and no significant differences in body temperature, grip strength or wire-hanging time between relax-

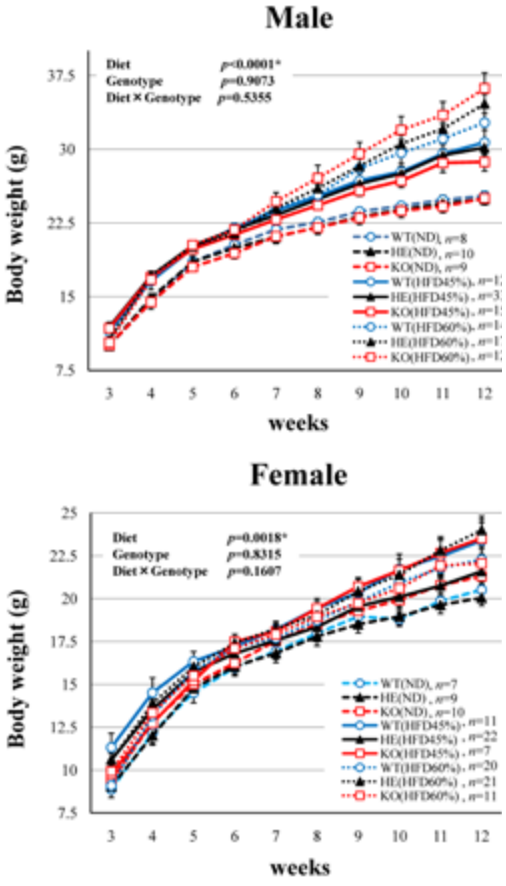

Figure 1. Body weight gain of relaxin-3 KO mice under a high fat diet. Body weight gain for *relaxin*-3$^{-/-}$ (KO), *relaxin*-3$^{+/-}$ (HE), and wild-type littermates (WT) was measured weekly. Male (upper) or female (lower) mice were fed a normal diet (ND), a 45% high fat diet (HFD), or a 60% HFD from 3 weeks of age. Data were presented as mean ± SEM and analyzed with repeated measures ANOVA. Statistical results were shown in graphs.

Table 1. Results of comprehensive behavioral test of relaxin-3 KO mice.

Tests	Results
1. General health / Neurological Screening	n.s.
2. Light / Dark Transition Test	n.s.
3. Open Field Test	n.s.
4. Elevated Plus Maze	decreased anxiety
5. Hot Plate Test	n.s.
6. Social Interaction Test	n.s.
7. Rotarod Test	n.s.
8. Social Interaction Test (Crawley version)	n.s.
9. Porsolt Swim Test	n.s.
10. Y-Maze Test	n.s.
11. Fear Condition Test (Contextual & Cued)	n.s.
12. Tail Suspension Test	n.s.
13. Body Weight	n.s.

in-3 KO and wild-type littermates. A previous study reported that relaxin-3 KO mice of mixed 129S5:B6-background showed leaner and smaller than control animals when they were fed with high fat diet. We examined feeding normal (13%) diet, and 45% and 60% calorie of high fat diet to wild, heterozygote, and KO mice of both genders. Animals with high fat gained body weight faster than mice with normal diet. However, we did not observe any significant change in body weights between genotypes until 12 weeks (Fig. 1).

Using male wild and KO mice of litter mate at 10 weeks, we performed a battery of behavioral tests to examine such as anxiety, pain sensation, social interaction, short memory and depression (Table1). Among tests for measuring anxiety-like behaviors, KO mice showed a significant change compared with the wild phenotype only in the elevated plus maze test. They performed a significantly higher percentage of entries into the open arms, and spent a higher percentage of their time in the open arms. However, there were no significant differences in distance traveled, or the number of entries into the arms. There were also no significant differences in light/dark transition test and open filed test. In other behavioral tests, we did not detect the significant differences between genotypes (Table 1). In conclusion from these behavioral screenings, relaxin-3 deficient mice have mild anxiolytic characteristics relative to wild-type mice, suggesting that this peptide is involved in anxiety-related behavior.

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