Journal of Molecular Neuroscience

0895-8696/06/28:239–246/$30.00
JMN (Online)ISSN 1559-1166
DOI 10.1385/JMN/28:03:239

ORIGINAL ARTICLE

Emotional Behavior in Aged Neuropeptide Y (NPY) Y_2 Knockout Mice

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Received July 1, 2005; Accepted October 14, 2005

Abstract

Neuropeptide Y (NPY) was shown to modulate anxiety- and depression-related behaviors in various animal models. Previous studies demonstrated that NPY Y_2 receptor knockout (KO) mice display an anxiolytic- and antidepressant-like phenotype compared with control animals. However, the long-term effect of the deletion of this receptor in aged animals is unknown. Thus, anxiety- and depression-related behaviors were investigated in 2-yr-old NPY Y_2 KO mice. Aged NPY Y_2 KO mice display an anxiolytic-like profile as assessed in the elevated plus-maze and open field, providing further support for a role for Y_2 receptors in anxiety-related behaviors. Furthermore, aged NPY Y_2 KO mice have significantly lower immobility scores in the forced swim test; supporting the role for this receptor in antidepressant-like behaviors. These data provide further evidence that modulators of the NPY Y_2 receptor subtype are drug targets for the treatment of anxiety and mood disorders in human subjects.

DOI 10.1385/JMN/28:03:239

Index Entries: Neuropeptide Y; Y_2 receptor; knockout; aging; anxiety; depression.

Introduction

Neuropeptide Y (NPY) was isolated from porcine brain more than two decades ago (Tatemoto et al., 1982). This 36-amino-acid residue is one of the most abundant peptides found in the central nervous system (CNS) of all mammals, including humans (Chan-Palay et al., 1985, 1986; Chronwall et al., 1985). It is one of the most conserved peptides in evolution (Larhammar, 1996; Larhammar and Salaneck, 2004), suggesting an important role in the regulation of basic physiological functions (Larhammar et al., 1993). At present, five NPY receptor subtypes have been cloned and designated—Y_1, Y_2, Y_4, Y_5, and y_6 (Dumont et al., 1993; Gehlert, 1994; Michel et al., 1998)—all of which couple to G_1 proteins and inhibit the production of cyclic AMP (Palmiter et al., 1998). NPY has important modulatory functions in the immune and cardiovascular systems (Song et al., 1996; Michalkiewicz et al., 2001), circadian rhythms (Antonijevic et al., 2000), food intake (Jolicoeur et al., 1995), and seizure (Husum et al., 1998; Colmers and El Bahh, 2003). NPY is involved in anxiety-related behaviors (Thorsell and Heilig, 2002), and there is increasing support for the role of NPY in mood disorders such as depression (Redrobe et al., 2002a).

NPY is consistently implicated in the pathogenesis of anxiety disorders, given the significant number

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of findings that show NPY-induced anxiolytic activity in behavioral tests widely used for the screening of anxiolytic compounds. NPY is reported to elicit anxiolytic-like effects in exploratory behavior-based tests such as the open field, elevated plus-maze, and light/dark compartment test (Heilig et al., 1989; Pich et al., 1993); social interaction (Kask et al., 1998); punished responding tests (Britton et al., 1997); and fear-potentiated startle (Broqua et al., 1995). There is also evidence that NPY has antidepressant-like properties in animal models. For example, intracerebroventricular (icv) NPY displayed dose-dependent antidepressant-like activity in the rat (Stogner and Holmes, 2000) and mouse forced swim test (Redrobe et al., 2002b).

Further evidence for NPY in emotional behavior is supported by recent studies involving Y_2 receptor subtype knockout (KO) mice. Mice deficient in the Y_2 receptor subtype displayed an anxiolytic-like phenotype in the elevated plus-maze (Redrobe et al., 2003). NPY Y_2 KO mice also displayed increased preference for the central area of the open field when compared with wild-type (WT) control animals, without changes in locomotor activity (Redrobe et al., 2003). These findings have been confirmed by an independent laboratory using the open field, elevated plus-maze, and light-dark compartment test (Tschenett et al., 2003). It was also shown that Y_2 KO mice displayed approximately three times less immobility in the forced swim test than WT controls; indicating a stronger ability to cope with stress (Tschenett et al., 2003).

NPY Y_2 receptors are considered to be autoreceptors that provide negative feedback to NPY-ergic nerve terminals to modulate NPY release (Wahlestedt et al., 1986). In the rodent brain, NPY Y_2 receptor mRNA and protein are abundantly expressed in the hippocampus and brain stem, whereas moderate levels of Y_2 receptors are detected in the hypothalamus (Dumont et al., 1993; Parker and Herzog, 1999; Dumont et al., 2000). Consequently, Y_2 KO mice are predicted to have increased endogenous peptide expression that might contribute to the underlying mechanism responsible for anxiolytic-like behaviors regulated by NPY (Redrobe et al., 2003).

Although the behavioral phenotype of NPY Y_2 receptor KO mice and WT controls is known, the long-term effect of the deletion of this receptor in aged animals is unknown. Therefore, anxiety- and depression-related behaviors were investigated in 2-yr-old NPY Y_2 receptor KO mice and WT controls using the elevated plus-maze, open field, and forced swim test.

Materials and Methods

Animals

NPY Y_2 KO and NPY Y_2 WT mice (C57/B16-129SvJ background) were developed using cre/loxP technology, as described previously (Baldock et al., 2002; Smith-White et al., 2002), and were received from Dr. Herbert Herzog at the Garvan Institute of Medical Research (Sydney, Australia). These mice were tested at approx 24 mo of age. All animals were housed under standard laboratory conditions (12/12 h light/dark cycle, lights on at 07:00, food and water *ad libitum*). Animal care was provided according to protocols and guidelines approved by McGill University and the Canadian Council of Animal Care.

Elevated Plus-Maze

The test was performed as described previously (Pellow et al., 1985; Handley and McBlane, 1993; Kask et al., 2002; Redrobe et al., 2003). The experimental apparatus consists of a plus-formed maze elevated 50 cm above the ground. The four arms are 37.5 cm long and 5 cm wide. Two opposing arms are surrounded by black Plexiglas walls, 15 cm high (closed walls); the other arms are devoid of walls (open arms). The animal is placed in the center of the maze facing an open arm, after which the cumulative time spent in each arm and the number of entries into the open or closed arms are recorded during a 5-min test session. An individual entry into the arm is defined as the animal placing all four paws in that arm. Total number of entries onto any arm is presented as a measure of general locomotor activity on the maze so as to rule out any nonspecific effects that might have interfered with the interpretation of the data.

Open Field Test

The open field consists of a square base (70 × 70 cm) surrounded by a 75-cm-high wall. Illumination is provided by a 40 W bulb, positioned 90 cm above the floor of the apparatus. The animals are placed into the center of the apparatus, and the number of crossings, as well as the time spent in the central area of the open field, is recorded. Total crossings are presented as a measure of general locomotor activity in the arena. Testing is conducted over a 10-min period and recorded by a video tracking system connected to a computer equipped with the commercially available HVS image system (HVS, UK) for the analysis of the open field activity.

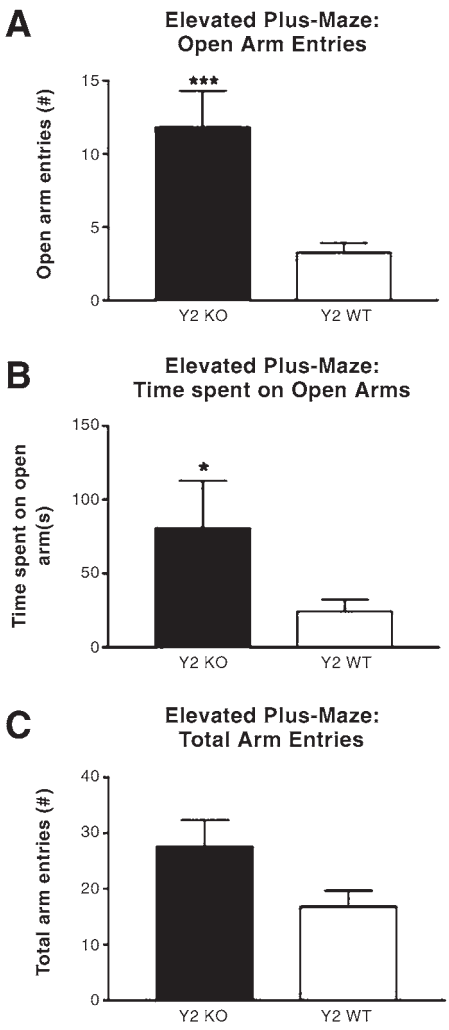

Fig. 1. Behavioral profile of aged neuropeptide Y (NPY) $Y_2^{-/-}$ [Y2 knockout] and $Y_2^{+/+}$ [Y2 wild-type] mice in the elevated plus-maze. Results are expressed as mean ± S.E.M. of open arm entries **(A)**, time spent on open arms **(B),** and total arm entries **(C)**. $p < 0.05$ (*) and $p < 0.0001$ (***) by two-tailed unpaired *t*-test.

Forced Swim Test

The Porsolt forced swim test is a reliable tool for screening potential antidepressant drugs (Porsolt et al., 1977). In the mouse forced swim test, mice are individually placed in a 40-cm-diameter cylinder filled with 24–25°C water to a depth of 30 cm for 6 min. Immobility time is recorded during the last 4 min. The immobility score is associated with a positive antidepressant-like effect.

Statistical Analysis

Behavioral data were analyzed with unpaired Student's *t*-tests (two-tailed) using GraphPad Prism software (San Diego, CA). All results are reported as mean ± S.E.M. For all statistical analyses, $p < 0.05$ is considered statistically significant.

Results

Behavioral Profile of Aged NPY $Y_2^{-/-}$ and $Y_2^{+/+}$ Mice in the Elevated Plus-Maze

NPY Y_2 KO mice ($n = 6$) made more entries (Fig. 1A, Open Arm Entries; mean ± S.E.M.: 11.83 ± 2.469 [KO] vs 3.250 ± 0.6643 [WT]; $t = 4.422$; df = 16; $p = 0.0004$) and spent significantly more time (Fig. 1B, Time Spent on Open Arms; mean ± S.E.M.: 80.50 ± 32.30 [KO] vs 24.33 ± 8.059 [WT]; $t = 2.250$; df = 16; $p = 0.0389$) on the open arms of the elevated plus-maze compared with WT control mice ($n = 12$). This effect was not attributable to nonspecific changes in locomotor activity, as the number of closed arm entries did not differ significantly between groups (Fig. 1C, Total Crossings; mean ± S.E.M.: 27.67 ± 4.709 [KO] vs 16.83 ± 2.878 [WT]; $t = 2.066$; df = 16; $p = 0.0554$).

Behavioral Profile of Aged NPY $Y_2^{-/-}$ and $Y_2^{+/+}$ Mice in the Open Field

NPY Y_2 KO mice ($n = 5$) displayed increased preference for the central area of the open field (Fig. 2A, Entries into Central Area; mean ± S.E.M.: 31.60 ± 8.016 [KO] vs 7.083 ± 1.041 [WT]; $t = 4.720$; df = 15; $p = 0.0003$) and spent more time (Fig. 2B, Time Spent in Central Area; mean ± S.E.M.: 68.20 ± 17.04 [KO] vs 17.25 ± 2.346 [WT]; $t = 4.587$; df = 15; $p = 0.0004$), when compared with WT animals ($n = 12$). There was also a significant change in locomotor activity in Y_2 KO mice (Fig. 2C, Total Crossings; mean ± S.E.M.: 310.4 ± 50.59 [KO] vs 117.4 ± 13.83 [WT]; $t = 5.079$; df = 15; $p = 0.0001$) when compared with WT controls.

Behavioral Profile of Aged NPY $Y_2^{-/-}$ and $Y_2^{+/+}$ Mice in the Forced Swim Test

An unpaired *t*-test (two-tailed) revealed a significant difference in mean immobility scores between NPY Y_2 KO mice ($n = 5$) and WT controls ($n = 12$) (Fig. 3, Immobility; mean ± S.E.M.: 4.400 ± 2.619 [KO] vs 24.83 ± 3.593 [WT]; $t = 3.465$; df = 15; $p = 0.0035$) in the forced swim test.

Discussion

It has been shown previously that mice deficient in the Y_2 receptor subtype have an anxiolytic-like phenotype in the elevated plus-maze and open field

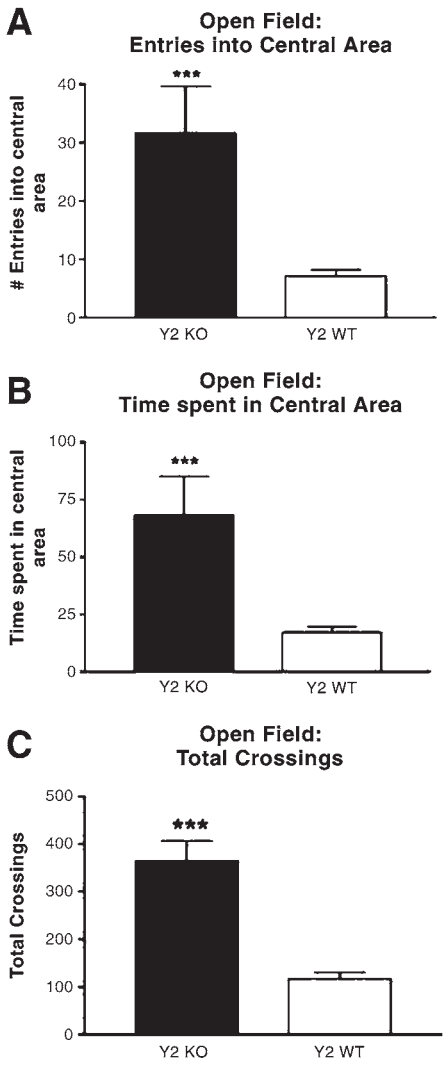

Fig. 2. Behavioral profile of aged neuropeptide Y (NPY) $Y_2^{-/-}$ [Y2 knockout] and $Y_2^{+/+}$ [Y2 wild-type] mice in the open field. Results are expressed as mean ± S.E.M. of entries into central area **(A)**, time spent in central area **(B),** and total crossings **(C)**. $p < 0.0001$ (***) by two-tailed unpaired *t*-test.

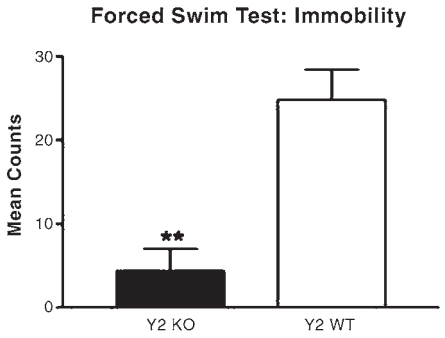

Fig. 3. Behavioral profile of aged neuropeptide Y (NPY) $Y_2^{-/-}$ [Y2 knockout] and $Y_2^{+/+}$ [Y2 wild-type] mice in the forced swim test. Results are expressed as mean ± S.E.M. $p < 0.02$ (**) by two-tailed unpaired *t*-test.

test without changes in locomotor activity (Redrobe et al., 2003). These findings were confirmed independently using the open field, elevated plus-maze, and light-dark compartment test (Tschenett et al., 2003). An increase in locomotor activity was observed in the open field when mice were tested with light but not when tested in the dark (Tschenett et al., 2003). The anxiolytic-like profile of the Y_2-deficient mouse was confirmed in 2-yr-old Y_2 KO mice using the elevated plus-maze and open field test. In agreement with the second study, an increase in locomotor activity in the open field was observed in NPY Y_2 KO mice compared with WT controls. This is most likely because NPY Y_2 KO and WT control mice were tested in the open field with light, rather than in the dark.

It was also shown previously that Y_2 KO mice displayed approximately three times less immobility in the forced swim test compared with WT controls, indicating a stronger ability to cope with stress (Tschenett et al., 2003). The results of this study also reveal that 2-yr-old NPY Y_2 receptor subtype KO mice had lower immobility scores in the forced swim test compared with WT control mice. Together, these studies support the antidepressant-like properties of NPY and the Y_2 receptor subtype.

The results of this study confirm that NPY has a significant role in emotional behavior throughout the life span of an animal model. NPY Y_2 KO mice had a similar emotional profile when tested at both a young and senescent age. Further support for NPY as a neuromodulator in the regulation of anxiety-related behaviors throughout life is provided by NPY transgenic rats. Both young and old NPY transgenic rats were resistant to acute physical restraint stress measured by the elevated plus-maze (Thorsell et al., 2000; Carvajal et al., 2004). Given the conflicting and limited results concerning emotional behavior in aged animals, these studies provide insight into the regulation of anxiety- and depression-like behavior by NPY during aging. For example, studies examining the anxiety profile of young vs aged animals using the elevated plus-maze have yielded contradictory results. No change in anxiety levels between young and old Long-Evans rats (Rowe et al., 1998) or between 5- and 25-mo-old C57BL/6NIA mice (Frick et al., 2000) has been found, although increased anxiety levels were observed in aged Wistar rats (Darwish et al., 2001; Boguszewski and Zagrodzka, 2002). In contrast to these studies, decreased anxiety

levels have also been seen in aged animals. For instance, aged Fisher rats had increased open arm activity compared with young control animals in the elevated plus-maze (Pisarska et al., 2000). A recent study found lower anxiety levels in aged (24 mo) Wistar rats compared with young (3 mo) rats that were not subjected to any prior behavioral testing (Torras-Garcia et al., 2005). However, the behavioral profile of the aged animals in this study might be attributable to the dietary restriction paradigm used by the investigators to promote healthy aging in the 24-mo-old rats (Torras-Garcia et al., 2005). Given the inconsistent findings of previous studies, additional research on emotional behavior in aged animals is essential.

Studies have shown that anxiety disorders are highly prevalent in elderly patients (Beekman et al., 1998). However, anxiety disorders in late life have received less attention than the same disorders in younger patients (Flint, 2005). Interestingly, depression with psychiatric comorbidity, particularly anxiety, has been found to be associated with greater severity of depression and anxiety, poorer or delayed response to antidepressants, functional impairment, and decreased responsiveness to treatment (for review, *see* Chisholm et al., 2003). In elderly patients, high levels of comorbidity are found among major depressive disorder and anxiety disorders (Lenze, 2003). Anxiety disorders such as generalized anxiety disorder (GAD) and comorbid major depressive disorders are associated with increased severity (such as suicidal thought and poorer functioning) and diminished well-being (de Beurs et al., 1999). Furthermore, the most common lifetime pattern of comorbidity was GAD preceding major depressive disorder (Lenze et al., 2005). Depression is traditionally viewed as the manifestation of an inability to cope with various lifetime stressors (Nestler et al., 2002a, 2002b) and is presumably determined by genetics. Consequently, NPY presents a novel approach in the search for effective antidepressant treatment strategies given its significant role in both anxiety and depression-related behaviors.

The interaction between corticotropin-releasing factor (CRF) and NPY has been proposed as a means by which emotional behavior is regulated (for review, *see* Sajdyk et al., 2004). In contrast to the anxiolytic-like effects of NPY (Kask et al., 2002), evidence exists that CRF has significant anxiogenic-like effects in animal models (Contarino and Gold, 2002; Lin et al., 2004). For example, icv administration of CRF increases anxiety-like behavior in the elevated plus-maze, and CRF antagonists can block the behavioral effects of a stress challenge (Menzaghi et al., 1994). The anxiogenic-like role of CRF has been confirmed in studies using CRF-overexpressing and CRF KO mice (Stenzel-Poore et al., 1994; Muglia et al., 1995). It has been shown that NPY can counteract the anxiogenic-like effect of CRF in the hippocampus (Kagamiishi et al., 2003), hypothalamus (Hastings et al., 2001), locus coeruleus (Charney, 2004), periaqueductal gray (Kask et al., 1998; Charney, 2004), and the septal nucleus (Kask et al., 2001). Moreover, deletion of Y_2 receptors causes a 60% reduction in CRF mRNA expression (Sainsbury et al., 2002), which might contribute to the anxiolytic-like behavior of NPY Y_2 receptor KO mice (Tschenett et al., 2003). Interestingly, a lower level of CRF mRNA expression in the amygdala of 12- and 24-mo-old rats compared with that in young 4-mo-old rats is associated with decreased anxiety-like behavior in the aged animals (Pisarska et al., 2000). Additional studies are required to fully explore the interaction between CRF and NPY in aged animals.

Overall, these data provide further evidence that the NPY system offers an attractive target for drug development. Small molecule antagonists of the NPY Y_2 receptor subtype are potential drug targets for the treatment of anxiety and mood disorders in both young and elderly human subjects.

Acknowledgments

This work was supported by the Canadian Institutes for Health Research (CIHR).

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