Neuropeptides xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/ynpep

Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding

Yue Qi *, Melissa Fu, Herbert Herzog

Neuroscience Division, Garvan Institute of Medical Research, St Vincent's Hospital, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia

ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form 25 September 2015
Accepted 25 September 2015
Available online xxxx

Keywords:
Neuropeptide Y
Y2 receptors
Energy homeostasis
Bone formation
Food intake
Neuropeptides

ABSTRACT

Y2 receptors have been implicated in the development of obesity and are a potential target for obesity treatment due to their known role of inhibiting neuropeptide Y (NPY) induced feeding responses. However, the precise neuronal population on which Y2 receptors act to fulfil this role is less clear. Here we utilise a novel inducible, postnatal onset NPY neurons specific deletion model to investigate the functional consequences of loss of Y2 signalling in this population of neurons on feeding and energy homeostasis regulation. While the consequences of lack of Y2 signalling in NPY neurons are confirmed in terms of the uncoupling of suppression/increasing of NPY and pro-opiomelanocortin (POMC) mRNA expression in the arcuate nuclei (Arc), respectively, this lack of Y2 signalling surprisingly does not have any significant effect on spontaneous food intake. Fasting induced food intake, however, is strongly increased but only in the first 1 h after re-feeding. Consequently no significant changes in body weight are being observed although body weight gain is increased in male mice after postnatal onset Y2 deletion. Importantly, another known function of central Y2 receptor signalling, the suppression of bone formation is conserved in this conditional model with whole body bone mineral content being decreased. Taken together this model confirms the critical role of Y2 signalling to control NPY and associated POMC expression in the Arc, but also highlights the possibility that others, non-NPY neuronal Y2 receptors, are also involved in controlling feeding and energy homeostasis regulation.

1. Introduction

Neuropeptide Y (NPY) has been recognised as one of the most potent orexigenic neurotransmitters in the regulation of energy metabolism and pathological obesity (Loh et al., 2015). It is widely expressed in the central and peripheral nervous systems including the cerebral cortex, hypothalamus, brain stem and other brain regions, with the highest expression in the arcuate nuclei of the hypothalamus (Arc) (Baraban, 1998). Particularly these Arc NPY neurons, which also express agouti-related protein (AgRP) and gamma-aminobutyric acid (GABA) (Acuna-Goycolea et al., 2005), are known as the major population of orexigenic neurons in the hypothalamus. In addition to their role of promoting feeding by inhibiting the anorexic acting pro-opiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART) neurons, which are also located within the Arc, NPY neurons have also be shown to be critical for mediating the reduction in energy expenditure via modulating sympathetic outflow and controlling other peripheral organs like liver, pancreas and adipose tissue in order to conserve energy (reviewed in (Loh et al., 2015)). Importantly, the coordination of body weight and bone mass centrally is also mediated by hypothalamic NPY neurons (Baldock et al., 2009).

The NPY system exert their functions via G-protein-coupled receptors including Y1, Y2, Y4, Y5 and Y6 (Lin et al., 2004), each with varying distribution in central and peripheral organs and various biological responses. Like Y1 receptors, Y2 receptors are one of the most abundant receptors in the central nervous system and widely expressed in hypothalamus, hippocampus, brainstem, amygdala and lateral septum. Functionally, Y2 receptors are implicated with inhibition of food intake, regulation of bone formation and other functions such as anxiety, seizures, epilepsy and drug abuse, etc. (Parker and Balasubramaniam, 2008). Anatomically, Y2 receptors are characterised by being mostly expressed as auto-receptors on NPY neurons thereby modulating NPY neuronal function in an autocrine fashion. For example, administration of Y2 agonists, have been reported to restrict the presynaptic release of NPY in hypothalamic slices (King et al., 2000) and PC-12 cells (Chen et al., 1997). Within the hypothalamus these actions of Y2 receptors may lead to the inhibition of the release of GABA (Chen and van den Pol, 1996; Silva et al., 2007), as well as NPY itself. While there is not a lot of direct evidence one can assume that Y2 receptors are also exist on non-NPY neurons. For example, total hypothalamic deletion of Y2 receptors indicated that the lack of those Y2 receptors might play a role to increase body weight gain (Shi et al., 2010) and increase the formation of cortical and trabecular bone (Baldock et al., 2002).

In addition to NPY, the closely related peptides, peptide YY (PYY) and pancreatic polypeptide (PP) are also critical in controlling energy

* Corresponding author.
E-mail address: y.qi@garvan.org.au (Y. Qi).

http://dx.doi.org/10.1016/j.npep.2015.09.009
0143-4179/

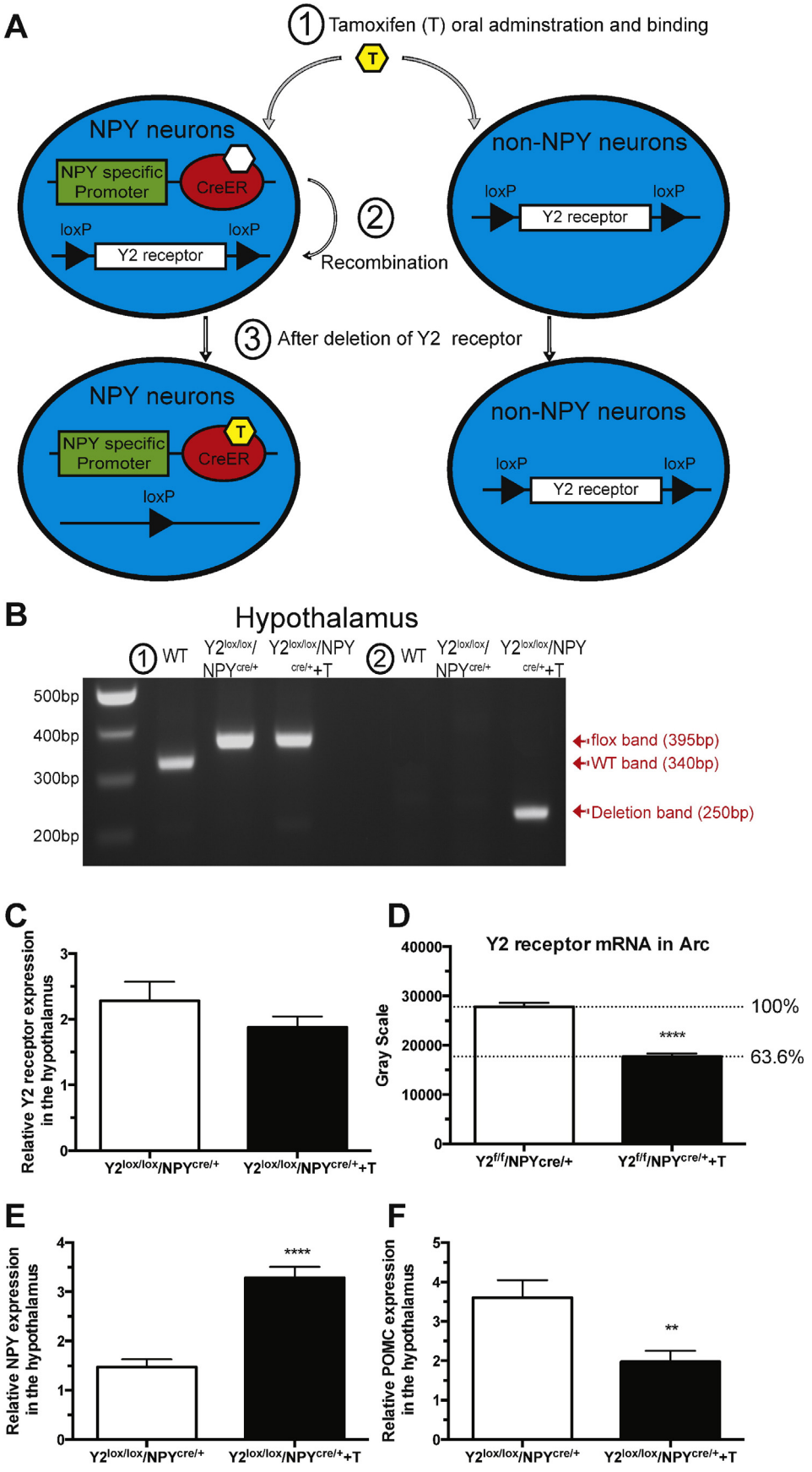

Fig. 1. Generation of $Y2^{lox/lox}/NPY^{cre/+}$ mice and confirmation of tamoxifen induced Y2 deletion in neuropeptide Y (NPY) neurons. A) Schematic representation showing the genomic organisation of the $Y2^{lox/lox}/NPY^{cre/+}$ mice and the selective deletion induced by tamoxifen (T). B) PCR genotyping results of $Y2^{lox/lox}/NPY^{cre/+}$ mice with/without tamoxifen and the wild type. C) The relative Y2 receptor mRNA expression in the hypothalamus detected by RT-PCR. D) The mRNA expression of Y2 receptors in the arcuate nuclei (Arc) detected by *in situ* hybridisation. E) The relative NPY mRNA expression in the hypothalamus detected by RT-PCR. F) The relative pro-opiomelanocortin (POMC) mRNA expression in the hypothalamus detected by RT-PCR. (**P < 0.01, and ****P < 0.0001).

Please cite this article as: Qi, Y., et al., Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding, Neuropeptides (2015), http://dx.doi.org/10.1016/j.npep.2015.09.009

homeostasis. However, while NPY centrally stimulates feeding and reduces energy expenditure, the peripherally released hormones PYY and PP mediate satiety (Loh et al., 2015). PYY is synthesised in endocrine L cells of the gut and is released in response to food intake. Two forms, PYY_{1-36} and PYY_{3-36} (Renshaw and Batterham, 2005; Unniappan et al., 2006) are found in the circulation. The latter one is being produced by the action of DPPIV leading to a truncated version that has lost its high affinity for all Y-receptors, specifically that for Y1 receptors, but retains affinity for the Y2 receptors (Blomqvist and Herzog, 1997; Keire et al., 2002; Nygaard et al., 2006). This property of PYY_{3-36} is now being pursed as a potential appetite-suppressing agent.

Germline deletion of Y2 receptors in mice has led to the discovery of various important functions previously unknown to require Y2 signalling including the control of bone formation as well as its role in anxiety and other behavioural aspects (reviewed in (Parker and Balasubramaniam, 2008)). But there were also discrepancies between different models particularly in the phenotypes related to feeding and body weight control. While mice generated by Naveilhan et al. (1999) produced a phenotype

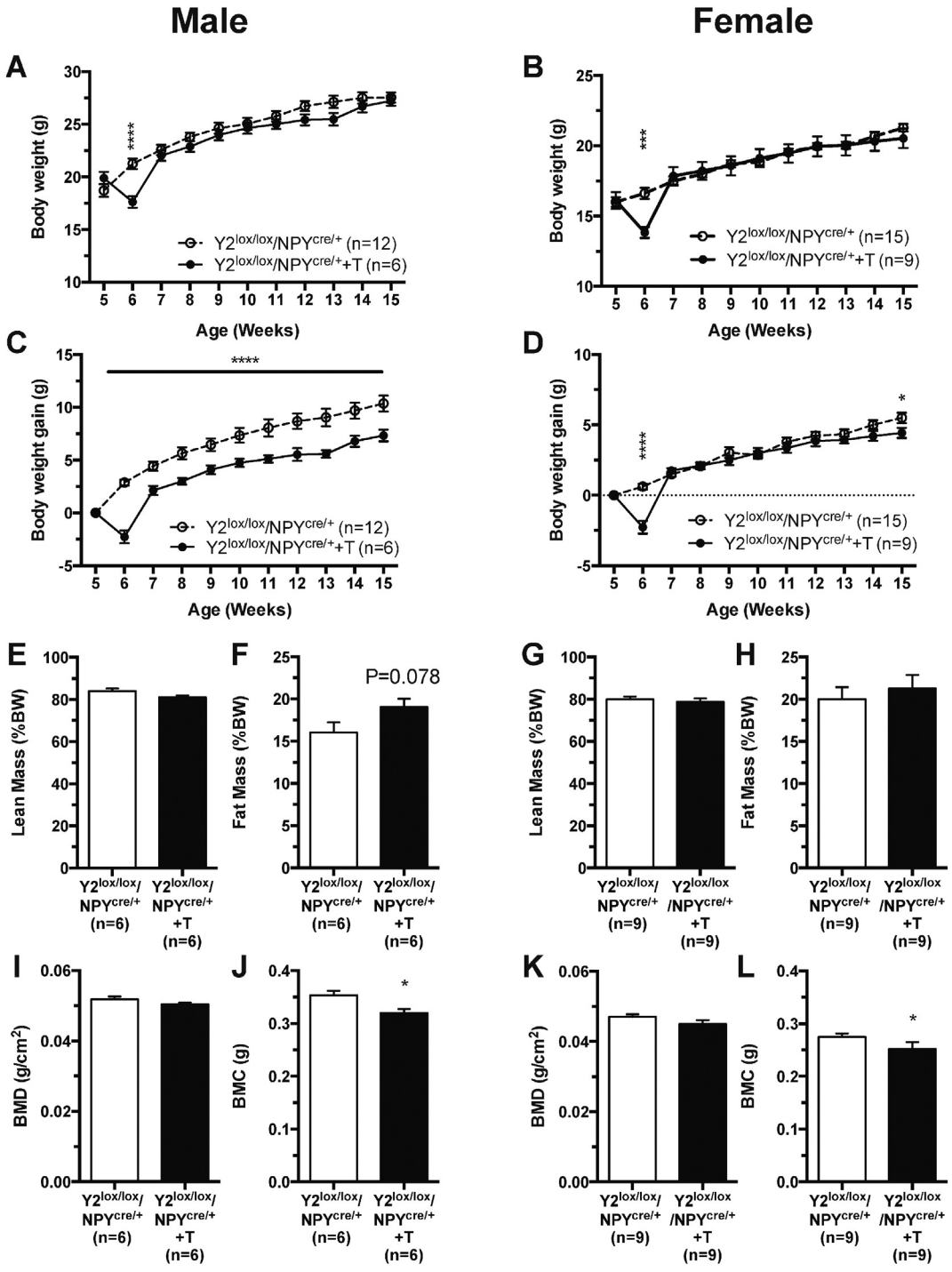

Fig. 2. Body weight and body composition in male (left column) and female (right column) of $Y2^{lox/lox}/NPY^{cre/+}$ mice with tamoxifen (+T) (—●—; black bars) vs. the ones without tamoxifen (– – –O– – –; white bars). A and B) Absolute weekly body weight from 5 to 15 weeks of age of male and female mice, respectively; C and D) Body weight gain normalised to their body weight (BW) at 5 weeks of age in male and female mice respectively; E) Lean mass as a percentage of BW (%BW) in male mice; F) Fat mass (%BW) in male mice; G) Lean mass (%BW) in female mice; H) Fat mass (%BW) in female mice; I) Bone mineral density (BMD) of male mice; J) Bone mineral content (BMC) of male mice; K) BMD of female mice; L) BMC of female mice. (*$P < 0.05$, ***$P < 0.001$, and ****$P < 0.0001$).

Please cite this article as: Qi, Y., et al., Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding, Neuropeptides (2015), http://dx.doi.org/10.1016/j.npep.2015.09.009

with increased body weight and fat mass, germline mice produced by our group revealed lack of Y2 signalling is associated with significantly lower body weight and adiposity and different pattern of food intake between genders (reduced in male and increased in female) and results in increased NPY and reduced POMC mRNA expression (Sainsbury et al., 2002a, 2002b). Some of the discrepancies between these models may be explained by the different targeting strategy as well as different background strains used to generate the mice. To refine the analysis of Y2 receptor signalling function in the hypothalamus a previous study of ours utilised viral vectors to delete Y2 receptors in the adult mice only in the hypothalamus. Importantly, this study confirmed that hypothalamic Y2 receptors are critical for the control of feeding and lack of it results in an increase in food intake and subsequent body weight gain. In an attempt to even more precisely investigate the function of Y2 receptor signalling specifically on NPY neurons we generated a conditional model that allows for the adult onset deletion of Y2 receptors in NPY neurons, which was triggered by the treatment with doxycycline. Interestingly, these mice did not show a difference in food intake and body weight, however, reproduced the phenotype with increased NPY and reduced POMC mRNA expression (Shi et al., 2010). This result was somewhat surprising as it raised the question whether Y2 receptors located on NPY neurons are really the critical ones for controlling feeding. The slight problem with the doxycycline-induced model is that leakage can occur and deletion may already occur during development and that this may not be a true inducible knockout. Therefore, we generated a new and more reliable inducible NPY specific Cre line and repeated the experiments to comprehensively evaluate the consequences of postnatal onset NPY neuron specific Y2 deletion on feeding and whole body energy homeostasis, results of which are described below.

2. Materials and methods

2.1. Animals

Research procedures and animal care for this study were approved by Garvan Institute/St. Vincent's Hospital Animal Ethics Committee and were in agreement with the Australian Code of Practise for the Care and Use of Animals for Scientific Purpose. Mice were housed under conditions of controlled temperature (22 ± 1 °C as room temperature) and illumination (12-h light–dark cycle, with lights on at

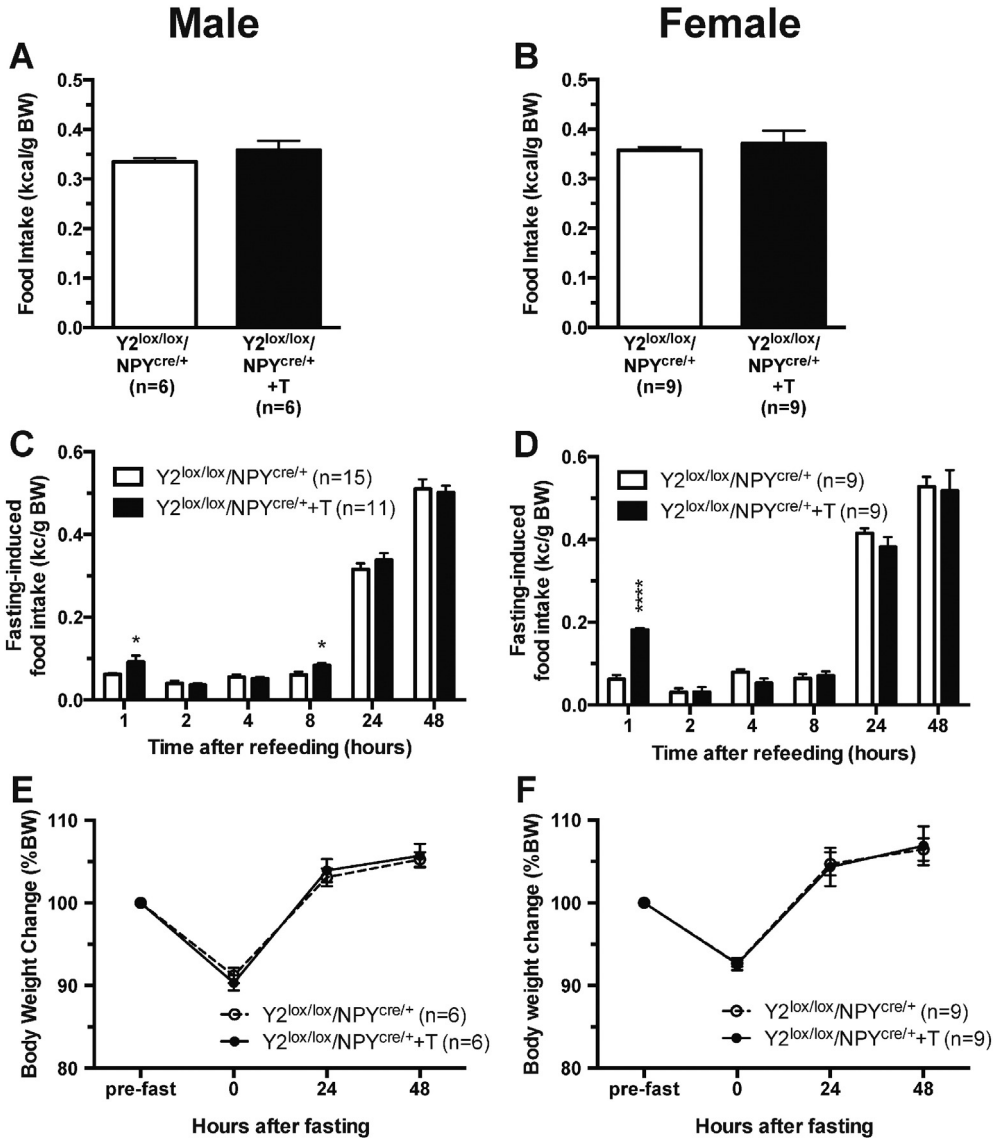

Fig. 3. Unaltered energy intake and transient increased fasting induced food intake in $Y2^{lox/lox}/NPY^{cre/+}$ mice with tamoxifen (+T) (—●—; black bars) vs. control mice (– – –O– – –; white bars). A and B) spontaneous 24-hour calorie intake as a percentage of body weight (%BW) in male (A) and female (B) mice, expressed as the average of triplicate readings over three consecutive days; C and D) fasting induced calorie intake (%BW) in male and female mice; E and F) Body weight change during the test of fasting induce food intake for male (E) and female (F) mice. (*P < 0.05 and ****P < 0.0001).

Please cite this article as: Qi, Y., et al., Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding, Neuropeptides (2015), http://dx.doi.org/10.1016/j.npep.2015.09.009

07:00 am and off at 07:00 pm). Animals had ad libitum access to a normal chow diet (8% calories from fat, 21% calories from protein, 71% calories from carbohydrate, and 2.6 kcal/g; Gordon's Specialty Stock Feeds, Yanderra, NSW, Australia). Water was unrestrictedly available. For the treatment group, tamoxifen (T) (T5648, Sigma-Aldrich, Castle Hill, NSW, Australia) mixed with chow diet was provided at the age of week 5 and last for one week. Body weight was measured weekly from the beginning of tamoxifen treatment.

2.2. Feeding and energy homeostasis studies

To investigate energy intake, both 24-h spontaneous food intake and fasting induced food intake were measured. Mice were individually housed and fed powder diet for 3 days in preparation for feeding studies. They were then transferred into Nalgene metabolic cages (Medtex, Notting Hill, VIC), which are specially designed for monitoring food intake, water consumption, faeces and urine production, and acclimatised for 24 h. Spontaneous 24-h food intake was monitored over three consecutive days and the average of triplicate readings was taken as basal food intake. For fasting induced food intake, food consumption was monitored at the time points of 1, 2, 4, 8, 24 and 48 h after an 18-hour fast. Body weight was recorded daily during the measurement of spontaneous food intake and at the time points of 0, 24 and 48 h for fasting induced food intake. Food intake was calculated as calorie intake and normalised to body weight of individual mice.

Energy expenditure was assessed using an eight-chamber open-circuit Oxymax system (Oxymax Series; Columbus Instruments, Columbus, OH, USA). Mice were acclimatised in the chambers for 24 h, followed by a 24-h continuous measurement of oxygen consumption and carbon dioxide production. Respiratory exchange ratio (RER) and heat production were calculated from these parameters. Energy expenditure was determined by correcting heat production for total lean and fat mass. Physical activity was continuously recorded using a passive infrared detector (OPTO-M3 sensor). Physical activity was expressed as the hourly sum of total X and Y plane beam breaks over 24 consecutive hours; calorimetry data were expressed as hourly means over 24 consecutive hours.

2.3. Dual energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) was used to determine body composition. Mice anaesthetised with isoflurane were scanned using a Lunar PIXImus2 mouse densitometer (GE healthcare, Waukesha, WI, USA). Whole body lean mass, fat mass, bone mineral density (BMD) and bone mineral content (BMC) were determined for individual mice.

2.4. Tissue collection

Mice were culled by cervical dislocation followed by decapitation. Trunk blood was collected, from which serum was obtained after

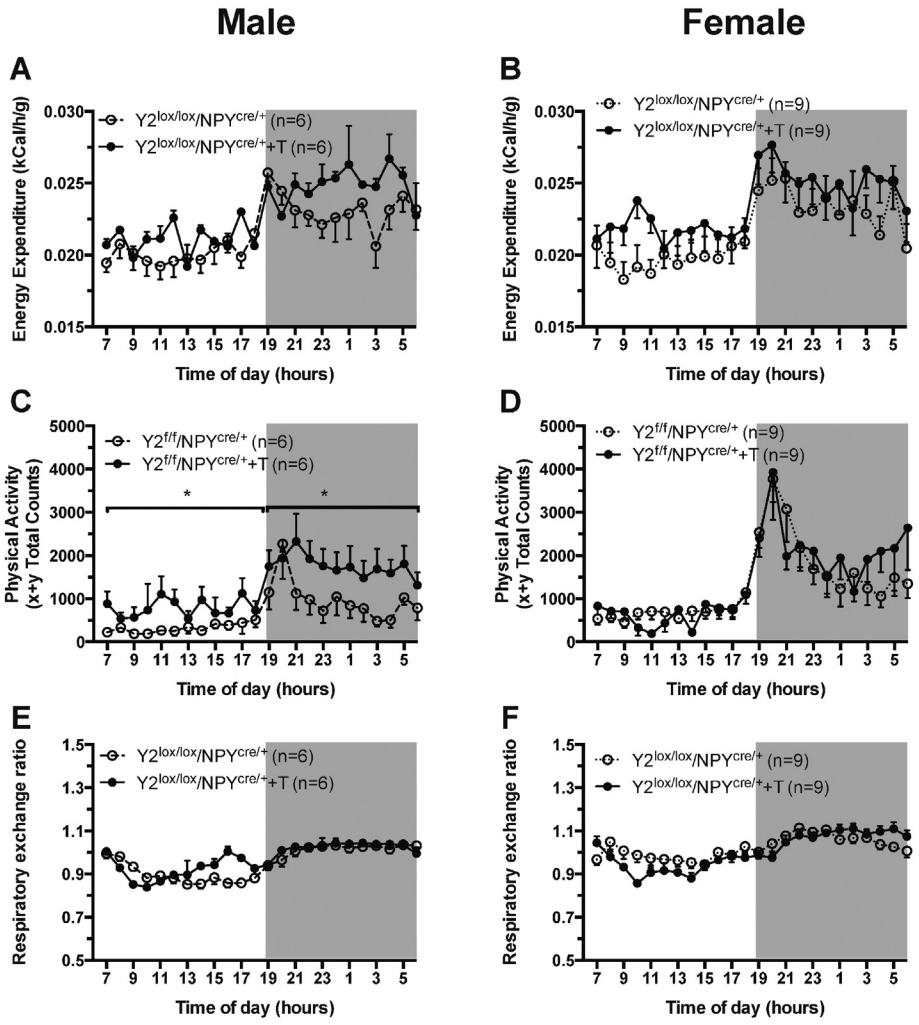

Fig. 4. Unaltered energy expenditure in both male and female $Y2^{lox/lox}/NPY^{cre/+}$ mice with tamoxifen (+T) (—●—) compared to their controls (– – – O– – –) and increased physical activity in male conditional knockout mice. A and B) 24-h energy expenditure for male and female mice, respectively; C and D) 24-h physical activity of male and female mice; E and F) 24-h respiratory exchange ratio of male and female mice. (*$P < 0.05$).

Please cite this article as: Qi, Y., et al., Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding, Neuropeptides (2015), http://dx.doi.org/10.1016/j.npep.2015.09.009

centrifugation at 13 000 rpm for 4 min and stored at −20 °C for further analysis. Fresh brains were collected and frozen flat on dry ice, then stored at −80 °C. The interscapular brown adipose tissue (BAT) and white adipose tissue (WAT) from the inguinal, epididymal, retroperitoneal and mesenteric deposits were excised and weighed. The organs, including gonads, spleen, pancreas, kidney, liver and heart, were also excised and weighed. Tissue and organ weight was expressed as a percentage of body weight.

2.5. In situ hybridisation and densitometry

Fresh frozen brains were sectioned at 30 μm thickness and thaw-mounted on Superfrost Plus® glass microscope slides (Lomb Scientific Pty Ltd., NSW 2229, Australia). *In situ* hybridisation was performed, as previously described (Sainsbury et al., 2002a). Briefly, matching hypothalamic sections of deletion and control mice were hybridised with oligonucleotide of mouse Y2 receptors mRNA (5′-TTTGTGCCTTCGCTGATGGTAATGGTCACTTGCAGCTCCAG GACT-3′), which were labelled with [35S] thio-dATP (Amersham Pharmacia Biotech, Buckinghamshire, UK) using terminal deoxynucleotidyltransferase (Roche, Mannheim, Germany). Silver grain densities of labelled mRNAs were analysed and compared using ImageJ software (US National Institutes of Health).

2.6. Quantitative real-time PCR

To test the mRNA expression of Y2 receptors, NPY and POMC in the hypothalamus, total RNA from the hypothalamic region was isolated using TRIzol® Reagent (Sigma, St. Louis, MO, USA). Quantitative real-time PCR was carried out following the protocol as previously described (Shi et al., 2010). Briefly, the total RNA was reverse transcribed into cDNA by the Superscript III First-strand Synthesis System (Invitrogen, Mount Waverley, VIC, Australia). Expression of Y2-receptor, NPY and POMC genes as well as their housekeeping gene-ribosomal protein L13A (RPL-13A), was quantified on a LightCycler® (LightCycler® 480, Roche Applied Science, Germany) using SensiMix™ Probe (Bioline Australia Pty Ltd., Alexandria, NSW, Australia). The latter was used to normalise the relative expression level of the tested mRNAs.

2.7. Statistical analyses

All data are presented as means ± SEM. T-test was used to compare the data difference obtained from the real-time PCR, *in situ* hybridisation, DXA, and spontaneous food intake between the conditional knockout group and the control. Difference of body weight, fasting induced food intake, RER and physical activity was analysed by two-way ANOVA with Bonferroni post-tests to compare each time point to the others. ANCOVA was used to analyse energy expenditure data. Statistical analyses were performed with SPSS for Mac OS X version 16.0.1 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $P < 0.05$.

3. Results

In order to determine the metabolic phenotype caused by the specific, postnatal-onset deletion of Y2 receptors on NPY neurons we crossed our homozygous Y2 receptors floxed mice ($Y2^{lox/lox}$) (Sainsbury et al., 2002a) with a our newly generated tamoxifen inducible, NPY-specific Cre line ($NPY^{cre/+}$) (Shi et al., 2013), both of which are on a mixed C57BL/6-129SvJ background, to generate double heterozygous mice ($Y2^{lox/+}/NPY^{cre/+}$), which were then crossed again with $Y2^{lox/lox}$ mice to finally generate ($Y2^{lox/lox}/NPY^{cre/+}$) mice. To induce the NPY neuron specific gene deletion of the Y2 receptor, $Y2^{lox/lox}/NPY^{cre/+}$ mice were then fed for one week with tamoxifen containing food (+T) from the age of week 5 onwards. $Y2^{lox/lox}/NPY^{cre/+}$ mice fed normal chow diet for the whole period were used as controls (Fig. 1A).

The successful deletion of the Y2 receptor gene was confirmed by PCR analysis of genomic DNA isolated from hypothalamic tissue of mice with the various genotype and treatment. The results from the PCR analysis demonstrate that only $Y2^{lox/lox}/NPY^{cre/+}$ mice treated with tamoxifen produce an additional product of the correct size of 250 bp's corresponding to the deletion product, while the others only produce the floxed band of 395 bp (Fig. 1B). To assess the contribution NPY-specific Y2 receptors make to the total pool of hypothalamic neurons that expressed Y2 receptors, RT-PCR analysis was performed. Interestingly, although there is a slight trend to reduced levels, results from this experiment showed no significant difference in Y2 mRNA expression between the genotypes suggesting that the population of Y2 receptors expressed on NPY neurons is considerably small compared to all other Y2 expressing neurons in the hypothalamus (Fig. 1C). To more specifically look at the proportion of Y2 receptors on NPY neurons only in the Arc we also performed *in situ* hybridisation analysis. Consistent with the high concentration of NPY neurons in this nucleus this analysis did show a significant reduction in Y2 receptor expression further confirming the successful deletion of Y2 receptors in our new model (Fig. 1D).

The lack of inhibitory Y2 signalling in NPY neurons would predict that certain regulatory mechanism are no longer in place and that particularly NPY mRNA should be up-regulated. Employing *in situ* hybridisation confirmed this prediction (Fig. 1E). In addition, this altered expression profile of NPY neurons with increased NPY should also lead to a down-regulation of POMC mRNA expression in neighbouring POMC/CART neurons. Again *in situ* hybridisation analysis confirmed this theory (Fig. 1F) and together these results demonstrate the inhibitory role of Y2 receptors on the expression of NPY, as well as the inhibitory action of NPY neurons over POMC neurons.

Having confirmed these fundamental control mechanisms of Y2 receptor signalling on NPY neurons we set out to investigate the metabolic effects of this alteration of NPY signalling in these mice. For this we monitored $Y2^{lox/lox}/NPY^{cre/+}$+T and control mice over a period of 11 weeks from the induction of gene deletion by tamoxifen at the age of 5 weeks till 16 weeks of age. As shown in (Fig. 2A and B), the treatment with tamoxifen food lead to an initial reduction in body weight compared to the non-treated mice, but mice recovered shortly thereafter and no obvious difference in absolute body weight was observed between the genotypes. However, there was a slight gender difference with female mice regaining quicker then males. This difference was more obvious when displayed as body weight gain where male mice were lacking significantly behind their controls whereas females only showed a minor difference (Fig. 2C and D). However, this reduced body weight gain had no significant influence on body composition in either gender with both showing similar fat and lean mass compared to their controls when determined by DXA analysis (Fig. 2E–H). Interestingly however, whole body BMC was reduced in both genders with NPY specific Y2 deletion (Fig. 2I–L). This is again consistent with the known effects of hypothalamic NPY on bone homeostasis with elevated NPY being suppressive of bone formation.

From the results of the analysis of the peptide mRNA expression in the Arc a major feature that would be expected is an increase in food intake and a reduction in energy expenditure that would lead to the development of obesity. However, the body weight data do not indicate that this is happening. Analysis of spontaneous food intake in these mice also does not show any significant alterations, thought there is a slight trend to increased food intake in both males and female mice with NPY neuron deleted Y2 receptors (Fig. 3A and B). Interestingly, however, 24 h fasting induced a significant increase in food intake in both genders, but only at the 1 h time point and this was then no longer observed at the later time points (Fig. 3C and D). However, this initial increase in food intake was not enough to lead to an overall increase in 24 h food intake after fasting nor did it influence body weight recovery after fasting (Fig. 3E and F).

Please cite this article as: Qi, Y., et al., Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding, Neuropeptides (2015), http://dx.doi.org/10.1016/j.npep.2015.09.009

To further test whether the other function of the NPY system, controlling energy expenditure was affected by the lack of Y2 signalling in NPY neurons we utilised indirect calorimetry. Not to surprisingly from the lack of any effect of feeding there was also no significant difference in energy expenditure in both male and female $Y2^{lox/lox}/NPY^{cre/+}+T$ mice compared to controls (Fig. 4A and B). However, increased physical activity was seen in male $Y2^{lox/lox}/NPY^{cre/+}+T$ mice but not in female mice (Fig. 4C and D). This increase in activity could be a contributing factor to the slight reduction in body weight gain that is observed in male mice missing Y2 signalling in NPY neurons. RER again showed no difference, indicating there is no difference in fuel usage (Fig. 4E and F). Taken together, this confirms the previous observed phenotype of lack of control of NPY Y2 receptors on normal feeding and energy homeostasis control as well as bone homeostasis.

4. Discussion

Results from this study confirm the role of NPY Y2 receptors to control the expression and release of NPY in these neurons. These results also confirm that as a consequence of this NPY dis-regulation the expression of POMC mRNA in the neighbouring neurons is decreased, which is consistent with the known fact that these neurons are controlled by NPY neurons in an inhibitory fashion. Interestingly however, similar to a previous observation in a related model, there was no significant influence on normal food intake. There was a short lasting increase in fasting induced food intake consistent with the predicted response to elevated NPY and reduced POMC levels under these conditions. On the other hand, this short elevated feeding response did not translate into altered body weight gain. The lack of Y2 signalling in NPY neurons however did replicate the bone phenotype that is expected from such a lack with decreased BMC in both male and female mice.

The transience of the observed increase in fasting induced food intake (first hour after re-feeding) demonstrates the inhibitory role of Y2 receptors on the release of NPY, however, also highlights that other factors play a role in this process and eventually overcome the initial stimulatory effect. Compared to the previous conditional knockout models, which induced the deletion when the experimental animals were 12 weeks, the present study induced the deletion in mice that were 5 weeks old, a period where they are still in a strong growth phase and the differences in feeding and growth in general might be more pronounced that could mask any significant differences between the genotypes. Importantly, the lost inhibition from Y2 receptor signalling leading to an increased expression of NPY and the consequent decrease in the expression of POMC provides evidence that the neuronal connectivity between these two neuronal populations is unaltered and functional.

The discrepancies observed between the results of this study and previous analysis of germline, pharmacological treated and viral induced conditional models indicates that Y2 receptors located on NPY neurons fulfil an important role in the local control of these neuronal population and neighbouring neurons, but also highlight the fact that other Y2 receptors, particularly within the hypothalamus are also critical involved in the control of feeding and energy homeostasis. Most intriguing here are the results from the viral induced Y2 deletion in the adult hypothalamus that fully replicate the feeding inhibitory role of the Y2 signalling by increasing food intake and body weight in their absence. That Y2 receptors expressed on NPY neuron only play a limited role in the control of feeding regulation is also consistent with the relative low impact this selective deletion has on the overall content of hypothalamic Y2 mRNA expression determined by RT-PCR. Furthermore this is consistent with the low level of co-localisation that can be seen by double immune histochemical analysis of NPY and Y-receptor in the mouse hypothalamus (Stanic et al., 2011).

Together, these are important novel findings and highlight the importance to more clearly define the regional expression of Y2 receptors in the hypothalamus specially the Arc as well as to identify the neuronal signature of the these neurons to better understand the integral role of NPY signalling particularly through Y2 receptors and how interfering with these processes could be utilised to control feeding and related aspects of whole body energy homeostasis.

References

Acuna-Goycolea, C., Tamamaki, N., Yanagawa, Y., Obata, K., van den Pol, A.N., 2005. Mechanisms of neuropeptide Y, peptide YY, and pancreatic polypeptide inhibition of identified green fluorescent protein-expressing GABA neurons in the hypothalamic neuroendocrine arcuate nucleus. J. Neurosci. 25, 7406–7419.

Baldock, P.A., Lee, N.J., Driessler, F., Lin, S., Allison, S., Stehrer, B., Lin, E.J., Zhang, L., Enriquez, R.F., Wong, I.P., McDonald, M.M., During, M., Pierroz, D.D., Slack, K., Shi, Y.C., Yulyaningsih, E., Aljanova, A., Little, D.G., Ferrari, S.L., Sainsbury, A., Eisman, J.A., Herzog, H., 2009. Neuropeptide Y knockout mice reveal a central role of NPY in the coordination of bone mass to body weight. PLoS One 4, e8415.

Baldock, P.A., Sainsbury, A., Couzens, M., Enriquez, R.F., Thomas, G.P., Gardiner, E.M., Herzog, H., 2002. Hypothalamic Y2 receptors regulate bone formation. J. Clin. Invest. 109, 915–921.

Baraban, S.C., 1998. Neuropeptide Y and limbic seizures. Rev. Neurosci. 9, 117–128.

Blomqvist, A.G., Herzog, H., 1997. Y-receptor subtypes–how many more? Trends Neurosci. 20, 294–298.

Chen, G., van den Pol, A.N., 1996. Multiple NPY receptors coexist in pre- and postsynaptic sites: inhibition of GABA release in isolated self-innervating SCN neurons. J. Neurosci. 16, 7711–7724.

Chen, X., DiMaggio, D.A., Han, S.P., Westfall, T.C., 1997. Autoreceptor-induced inhibition of neuropeptide Y release from PC-12 cells is mediated by Y2 receptors. Am. J. Physiol. 273, H1737–H1744.

Keire, D.A., Bowers, C.W., Solomon, T.E., Reeve Jr., J.R., 2002. Structure and receptor binding of PYY analogs. Peptides 23, 305–321.

King, P.J., Williams, G., Doods, H., Widdowson, P.S., 2000. Effect of a selective neuropeptide Y Y(2) receptor antagonist, BIIE0246 on neuropeptide Y release. Eur. J. Pharmacol. 396, R1–R3.

Lin, S., Boey, D., Herzog, H., 2004. NPY and Y receptors: lessons from transgenic and knockout models. Neuropeptides 38, 189–200.

Loh, K., Herzog, H., Shi, Y.C., 2015. Regulation of energy homeostasis by the NPY system. Trends Endocrinol. Metab. 26, 125–135.

Naveilhan, P., Hassani, H., Canals, J.M., Ekstrand, A.J., Larefalk, A., Chhajlani, V., Arenas, E., Gedda, K., Svensson, L., Thoren, P., Ernfors, P., 1999. Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. Nat. Med. 5, 1188–1193.

Nygaard, R., Nielbo, S., Schwartz, T.W., Poulsen, F.M., 2006. The PP-fold solution structure of human polypeptide YY and human PYY3-36 as determined by NMR. Biochemistry 45, 8350–8357.

Parker, S.L., Balasubramaniam, A., 2008. Neuropeptide Y Y2 receptor in health and disease. Br. J. Pharmacol. 153, 420–431.

Renshaw, D., Batterham, R.L., 2005. Peptide YY: a potential therapy for obesity. Curr. Drug Targets 6, 171–179.

Sainsbury, A., Schwarzer, C., Couzens, M., Fetissov, S., Furtinger, S., Jenkins, A., Cox, H.M., Sperk, G., Hokfelt, T., Herzog, H., 2002a. Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. Proc. Natl. Acad. Sci. U. S. A. 99, 8938–8943.

Sainsbury, A., Schwarzer, C., Couzens, M., Herzog, H., 2002b. Y2 receptor deletion attenuates the type 2 diabetic syndrome of ob/ob mice. Diabetes 51, 3420–3427.

Shi, Y.C., Lau, J., Lin, Z., Zhang, H., Zhai, L., Sperk, G., Heilbronn, R., Mietzsch, M., Weger, S., Huang, X.F., Enriquez, R.F., Baldock, P.A., Zhang, L., Sainsbury, A., Herzog, H., Lin, S., 2013. Arcuate NPY controls sympathetic output and BAT function via a relay of tyrosine hydroxylase neurons in the PVN. Cell Metab. 17, 236–248.

Shi, Y.C., Lin, S., Wong, I.P., Baldock, P.A., Aljanova, A., Enriquez, R.F., Castillo, L., Mitchell, N.F., Ye, J.M., Zhang, L., Macia, L., Yulyaningsih, E., Nguyen, A.D., Riepler, S.J., Herzog, H., Sainsbury, A., 2010. NPY neuron-specific Y2 receptors regulate adipose tissue and trabecular bone but not cortical bone homeostasis in mice. PLoS One 5, e11361.

Silva, A.P., Lourenco, J., Xapelli, S., Ferreira, R., Kristiansen, H., Woldbye, D.P., Oliveira, C.R., Malva, J.O., 2007. Protein kinase C activity blocks neuropeptide Y-mediated inhibition of glutamate release and contributes to excitability of the hippocampus in status epilepticus. FASEB J.

Stanic, D., Mulder, J., Watanabe, M., Hokfelt, T., 2011. Characterization of NPY Y2 receptor protein expression in the mouse brain. II. Coexistence with NPY, the Y1 receptor, and other neurotransmitter-related molecules. J. Comp. Neurol. 519, 1219–1257.

Unniappan, S., McIntosh, C.H., Demuth, H.U., Heiser, U., Wolf, R., Kieffer, T.J., 2006. Effects of dipeptidyl peptidase IV on the satiety actions of peptide YY. Diabetologia 49, 1915–1923.

Please cite this article as: Qi, Y., et al., Y2 receptor signalling in NPY neurons controls bone formation and fasting induced feeding but not spontaneous feeding, Neuropeptides (2015), http://dx.doi.org/10.1016/j.npep.2015.09.009