

Chronic High-Fat Diet Drives Postnatal Epigenetic Regulation of μ -Opioid Receptor in the Brain

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Opioid system dysregulation has been observed in both genetic and high-fat diet (HFD)-induced models of obesity. An understanding of the molecular mechanisms of MOR transcriptional regulation, particularly within an *in vivo* context, is lacking. Using a diet-induced model of obesity (DIO), mice were fed a high-fat diet (60% calories from fat) from weaning to > 18 weeks of age. Compared with mice fed the control diet, DIO mice had a decreased preference for sucrose. MOR mRNA expression was decreased in reward-related circuitry (ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC)) but not the hypothalamus, important in the homeostatic regulation of feeding. DNA methylation is an epigenetic modification that links environmental exposures to altered gene expression. We found a significant increase in DNA methylation in the MOR promoter region within the reward-related brain regions. Methyl CpG-binding protein 2 (MeCP2) can bind methylated DNA and repress transcription, and DIO mice showed increased binding of MeCP2 to the MOR promoter in reward-related regions of the brain. Finally, using ChIP assays we examined H3K9 methylation (inactive chromatin) and H3 acetylation (active chromatin) within the MOR promoter region and found increased H3K9 methylation and decreased H3 acetylation. These data are the first to identify DNA methylation, MeCP2 recruitment, and chromatin remodeling as mechanisms leading to transcriptional repression of MOR in the brains of mice fed a high-fat diet.

Neuropsychopharmacology (2011) **36**, 1199–1206; doi:10.1038/npp.2011.4; published online 16 February 2011

Keywords: μ -opioid receptor; preproenkephalin; DNA methylation; MeCP2; obesity; reward

INTRODUCTION

The μ -opioid receptor (MOR) is expressed broadly throughout the central nervous system (CNS) and participates in a wide array of physiological functions. Notably, MOR has a central role in coding the rewarding properties of natural stimuli such as palatable foods, as well as opiate drugs (eg, morphine, and heroin (Klein *et al*, 2009)) and nicotine (Berrendero *et al*, 2010) (also, see excellent, extensive recent review (Merrer *et al*, 2009)).

Little is known about MOR expression regulation in the brain. MOR expression can be altered by dopaminergic drugs, such as cocaine (Leri *et al*, 2006), whereas chronic exposure to opioid agonists or antagonists does not alter mRNA expression (physiological responses, such as tolerance and dependence are thought to involve post-transcriptional modifications that affect MOR function (Ammon-Treiber and Höllt, 2005)). Importantly, changes

in diet and/or metabolic state have been shown to influence expression of MOR. Hypothalamic MOR mRNA expression was increased following a 48-h fast (Barnes *et al*, 2008), whereas chronic exposure to a high-fat diet (HFD) can decrease MOR mRNA in the ventral tegmental area (VTA) (Blendy *et al*, 2005). Additionally, increased MOR binding has been shown in certain brain regions in the rat in response to diet-induced obesity (DIO) (Smith *et al*, 2002).

Epigenetic mechanisms of gene regulation, such as DNA methylation and histone modifications (eg, methylation, acetylation, ubiquitination) can regulate gene expression in response to environmental stimuli, such as drugs of abuse or diet. Recent reports have identified epigenetic modifications in the CNS in response to altered diet, particularly in the prenatal or early postnatal time period, when brain development is particularly vulnerable to perturbations (Vucetic *et al*, 2010a,b). Epigenetic regulation of gene expression outside of this early vulnerable time period has been documented as well, in response to cocaine (Schroeder *et al*, 2008), stress (Renthal *et al*, 2007), and learning and memory (Miller *et al*, 2010). Given that MOR expression can be affected by HF diet, the goal of these studies was to determine whether epigenetic modifications of the MOR occur during the postnatal period in response to chronic intake of HFD.

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Received 13 December 2010; revised 4 January 2011; accepted 5 January 2011

MATERIALS AND METHODS

Animals and Experimental Model

C57BL/6J females were bred to DBA/2J males. During pregnancy and lactation, females were fed a control-formulated diet (Test Diet, Richmond, IN) (#5755) that contained 18.5% protein, 12% fat, and 69.5% carbohydrate. At weaning, half the pups were placed on a HFD (#58G9; 18.5% protein, 60% fat, and 20.5% carbohydrate) and half continued on the control diet. Litter size was six to eight pups (one to two pups were culled when necessary). One animal per litter from 10–15 different dams was used in individual experiments, to control for any litter effect. Body weights were recorded weekly, and male mice ($n = 5\text{--}8/\text{group}$) aged 18–24 weeks of age were used in all experiments. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania.

Sucrose Preference

For the duration of the test, offspring were caged individually and fed *ad libitum*. Mice were maintained on their respective diets (control or high fat) during testing, as removal of a palatable diet is a stressor (Pankevich *et al*, 2010). Mice were given simultaneous access to two bottles, one with water and one with 4% sucrose solution for 48 h. Bottle order was random and was switched after 24 h. Intake of consumed water, sucrose solution, and animal weight were measured every 24 h. Water, sucrose, and the total fluid intake were normalized to body weight. Sucrose preference was calculated as a percentage of sucrose solution consumed in relation to total fluid intake.

Genomic DNA and Total RNA Isolation from Brain

Animals were euthanized with an overdose of carbon dioxide, followed by cervical dislocation, a method consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. After animals were killed, brains were rapidly removed and placed in RNA later (Ambion, Austin, TX) for 4 h before dissections. Brain dissections were performed as previously described (Cleck *et al*, 2008; Reyes *et al*, 2003; Vucetic *et al*, 2010a) and as detailed in Supplementary Methods. Genomic DNA (gDNA) and total RNA were isolated simultaneously using AllPrep DNA/RNA Mini Kit (Qiagen).

Gene Expression Analysis by Quantitative Real-Time PCR

For each individual sample, 500 ng of total RNA was used in reverse transcription using High Capacity Reverse Transcription Kit (ABI, Foster City, CA). Expression of target genes was determined by quantitative RT-PCR using gene-specific TaqMan probes (ABI) with TaqMan Gene Expression Master Mix (ABI) on the ABI7900HT Real-Time PCR Cycler. Probes used for RT-PCR are listed in Supplementary Material (Table S1). The relative amount of each transcript was determined using delta Ct values as previously described (Pfaffl, 2001). Changes in gene expression were calculated against endogenous, unchanged β -actin standard.

Methylated DNA Immunoprecipitation (MeDIP) Assay

Methylated DNA Immunoprecipitation (MeDIP) assay was performed as described (Weber *et al*, 2005). Methylated DNA was immunoprecipitated using 10 μg of mouse monoclonal 5-methylcytidine antibody (Eurogentec) or mouse pre-immune serum. Enrichment in the MeDIP fraction was determined by quantitative RT-PCR using chromatin immunoprecipitation (ChIP)-qPCR Assay Master Mix (SuperArray) on the ABI7900HT Real-Time PCR Cycler. For all genes examined, primers were obtained from Superarray (ChIP-qPCR Assays (–01) kb tile, SuperArray) for the amplification of genomic regions spanning the CpG sites located ~300–500-bp upstream of the transcription start sites (see Supplementary Material for primer sequences, Table S2). MeDIP results were expressed as fold enrichment of immunoprecipitated DNA for each specific site. To calculate differential occupancy fold change (% enrichment), the MeDIP DNA fractions' Ct values were normalized to the Input DNA fraction Ct value (see Supplementary Material). Finally, the normalized level of DNA methylation at a particular site was expressed as relative to control group set to 1.

ChIP Assays

Chromatin was prepared from dissected brain regions as previously described (Chahrour *et al*, 2008). ChIP-IT express (Active Motif) kit with enzymatic shearing protocol was used for ChIP assays according to manufacturer's instructions. Immunoprecipitations were performed using 5 mg of the following antibodies: normal serum IgG (Millipore), anti-di-methyl histone H3-K9 (Millipore), anti-acetyl histone H3 (Millipore), and anti-methyl CpG-binding protein 2 (MeCP2) (Millipore). Enrichment of genomic DNA was assessed using qPCR as described for MeDIP.

Statistical Analyses

Data, presented as means \pm SEM, were analyzed using Prism 4 (GraphPad) and Excel tools for statistical analysis. Student's *t*-test was used to analyze differences in sucrose preference and gene expression between DIO and control animals, with Bonferroni's correction for multiple *t*-tests (multiple genes within a brain region) applied as warranted. A *p*-value of 0.05 or lower was considered significant.

RESULTS

Mice had continuous access to control diet (CD) or 60% HFD diet for 15–17 weeks (from weaning at 3 weeks until 18–20 weeks of age). At 20 weeks of age, HFD-induced obese (DIO) animals showed on average 27% greater body weight compared with controls (Figure 1a, *t*-test $p < 0.05$). Two-bottle sucrose preference test (4% sucrose) was performed to assess the response of control and DIO mice to a naturally rewarding stimulus. Although both groups of animals showed a preference for sucrose over water, DIO mice displayed a significantly reduced preference for the sucrose solution compared with control animals (Figure 1b, *t*-test, $p < 0.05$), suggesting a state of reward hypofunction in the DIO animals.

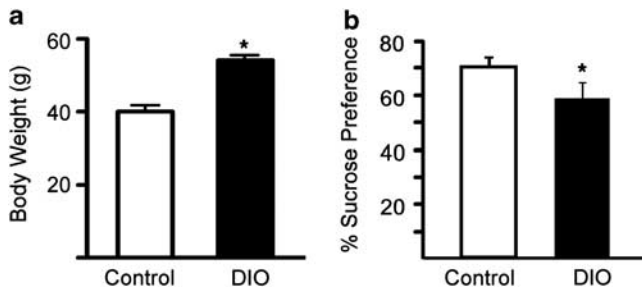


Figure 1 Long-term exposure to high-fat diet (HFD) induces obesity in mice, and decreases preference for sucrose. Mice had continuous access to control or 60% HFD from weaning at 3 weeks until 18–20 weeks of age. (a) Dietary-induced obese mice (DIO) were 27% heavier than controls at 20 weeks. (b) Preference for 4% sucrose solution was evaluated using 48-h two-bottle preference test in control mice (white bar) and DIO mice (black bar). DIO mice showed a decreased preference for sucrose compared with control-diet fed animals. Values represent mean \pm SEM. * $p < 0.05$, $n = 6-8$ /group.

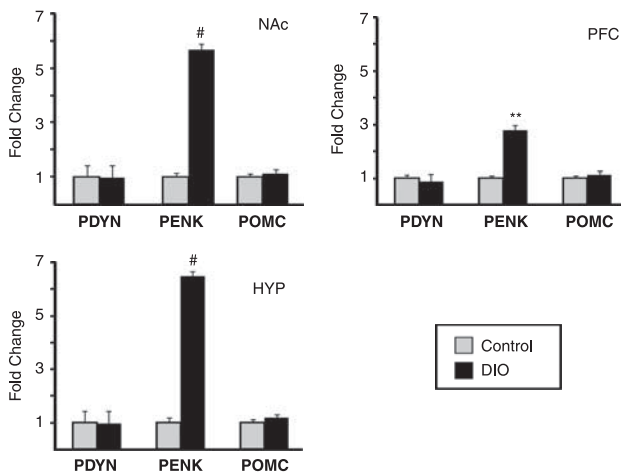


Figure 2 Expression of endogenous opioid ligand precursors in control and obese mice. RT-qPCR was used to assay mRNA levels of prodynorphin (PDYN), preproenkephalin (PENK) and pro-opiomelanocortin (POMC) mRNA in nucleus accumbens (NAc), prefrontal cortex (PFC), and HYP of control (gray bars) and obese mice (Dietary-induced obese mice (DIO), black bars). PENK levels were significantly increased in DIO mice in all CNS regions tested. Mean \pm SEM. ** $p < 0.01$, # $p < 0.005$.

To examine whether chronic HFD affected opioid expression within structures of the reward circuitry (VTA, nucleus accumbens (NAc), prefrontal cortex (PFC)) or hypothalamus, RT-qPCR was used to measure mRNA levels of the endogenous ligand precursors, prodynorphin (PDYN), preproenkephalin (PENK), and pro-opiomelanocortin (POMC). Expression of PENK mRNA was robustly increased (three to six fold) in the NAc, PFC and HYP of DIO animals ($p < 0.005$, $p < 0.01$ and $p < 0.005$, respectively), whereas levels of preprodynorphin and pro-opiomelanocortin remained unchanged (Figure 2). In addition to the opioid ligands, mRNA for the receptors was also examined. Expression of MOR mRNA was significantly decreased in areas of the brain related to reward processing (NAc, PFC, VTA) in DIO animals ($p < 0.01$, $p < 0.005$ and $p < 0.005$, respectively), whereas remaining unchanged in the hypothalamus (Figure 3). Further, in the VTA, expression of

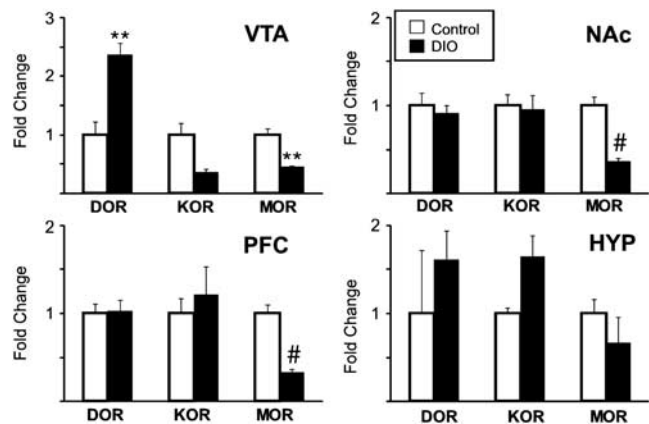


Figure 3 Expression of opioid receptors in control and obese mice. RT-qPCR was used to assay mRNA levels of delta (DOR), kappa (KOR) and mu-opioid (MOR) receptors in ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC) and HYP of control (Control; gray bars) and obese mice (Dietary-induced obese mice (DIO), black bars). DOR was significantly increased in VTA, whereas MOR levels were significantly reduced in VTA, PFC and NAc of DIO mice. KOR levels displayed a trend toward decreased levels in the VTA ($p < 0.05$, Bonferroni corrected p -value = 0.0165). Values are mean \pm SEM. ** $p < 0.01$, # $p < 0.005$.

kappa opioid receptor was decreased ($p < 0.05$, although not significant at Bonferroni corrected $p = 0.05/3 = .016$), whereas expression of mRNA for the delta subtype of the opioid receptor was significantly increased ($p < 0.01$).

Given the consistent downregulation of the MOR mRNA expression within the reward circuitry and the importance of MOR for coding reward, further experiments were designed to examine *in vivo* epigenetic modifications related to these expression differences. The promoter region of the MOR was found to be significantly hypermethylated in the VTA, NAc, and PFC of DIO animals, brain regions in which mRNA expression was decreased (Figure 4). As expected, methylation of the MOR promoter did not differ in samples taken from the hypothalamus. DNA methylation within the promoter region of PENK was also examined and did not differ between groups in any brain region (data not shown).

MeCP2 can function as a transcriptional repressor and has been reported to interact with the MOR receptor (Hwang *et al*, 2007, 2009), so ChIP assays were completed to determine whether MeCP2 was differentially bound to the MOR promoter in the CNS samples from DIO animals. Binding of MeCP2 to a region of the MOR promoter 0.5-kb upstream of the transcriptional start site was significantly increased in the NAc and PFC of DIO animals, and significantly decreased in the hypothalamus (Figure 5). Importantly, MeCP2 binding in regions of the MOR not known to be involved in transcriptional control, either 2-kb upstream or 0.5-kb downstream of the start site, were not differentially bound by MeCP2, nor was there differential binding to GAPDH. Next, ChIP was used to examine the association of additional epigenetic marks, associated with either inactive chromatin (H3-K9 dimethylation, (Snowden *et al*, 2002)) or active chromatin (H3 acetylation, Lee *et al*, 1993), with the MOR promoter region. There was an increase in H3-K9 methylation associated with the MOR

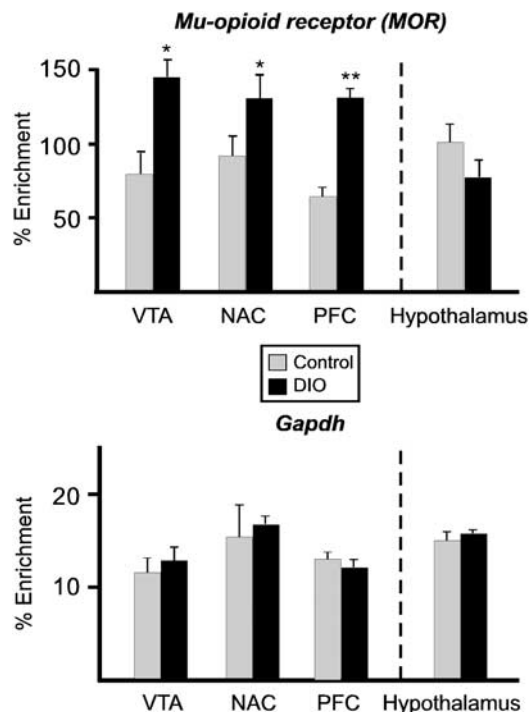


Figure 4 DNA methylation status of μ -opioid receptor (MOR) promoter in dietary-induced obese mice (DIO) mice. Genomic DNA was isolated from dissected ventral tegmental area (VTA), nucleus accumbens (NAC), prefrontal cortex (PFC), and HYP of control (gray bars) and obese (DIO) mice, sheared by sonication and immunoprecipitated with 5-methylcytosine antibody. The enrichment of DNA methylation relative to input genomic DNA at the proximal promoter region of MOR was quantified by qPCR. DIO mice displayed hypermethylation of MOR. GAPDH methylation was not altered in DIO mice. Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, $n = 6$ /group, Two-tailed t -test.

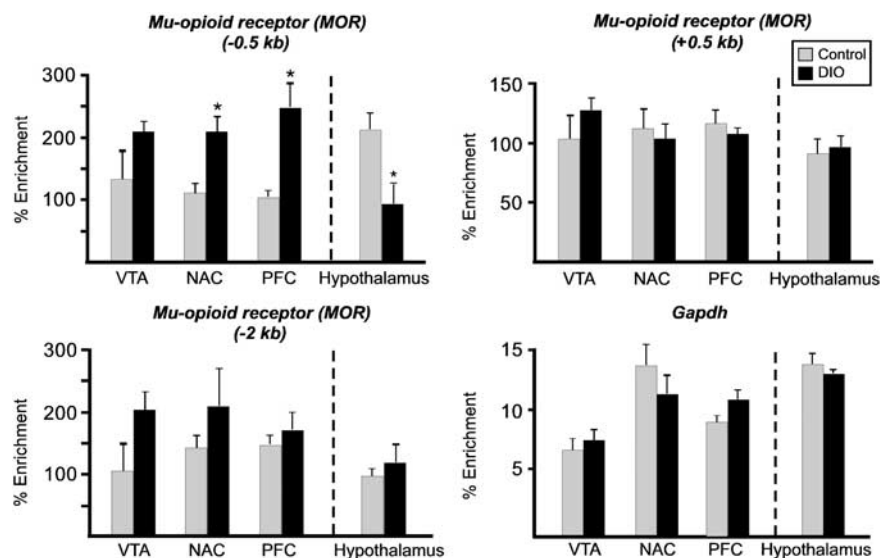


Figure 5 Association of methyl CpG-binding protein 2 (MeCP2) on μ -opioid receptor (MOR) promoter in central nervous system (CNS) of dietary-induced obese mice (DIO) mice. Chromatin was isolated from dissected ventral tegmental area (VTA), nucleus accumbens (NAC), prefrontal cortex (PFC), and HYP of control (gray bars) and DIO mice (black bars), enzymatically digested and immunoprecipitated with IgG and anti-MeCP2. The enrichment of genomic DNA associated with immunoprecipitated chromatin around proximal promoter (-0.5 and $+0.5$ kb) and 2 kb distal of MOR was quantified by qPCR. DIO mice displayed increased association of MeCP2 only in the -0.5 -kb region of the MOR promoter in NAC and PFC, and decreased MeCP2 association in the hypothalamus. There was no difference in MeCP2 binding either $+0.5$ or -2.0 kb from the MOR promoter and no differential binding to GAPDH. Values are mean \pm SEM. * $p < 0.05$, $n = 3$ /group, Two-tailed t -test.

receptor promoter in the VTA, NAC, and PFC of DIO animals, whereas there was a decrease in H3 acetylation associated with the MOR in the NAC and PFC from the DIO animals (Figure 6). Neither of the histone modifications was differentially associated with MOR in the hypothalamus.

DISCUSSION

These experiments provide important new data describing epigenetic alterations linking chronic consumption of HFD with MOR gene repression in the CNS. We found that a decrease in MOR gene expression within reward-related circuitry (NAC, PFC and VTA) was associated with epigenetic changes suggestive of a repressed transcriptional state at the histone and DNA level (DNA hypermethylation within the promoter region of MOR, increased binding of MeCP2 within the active promoter region of the MOR, an increase in H3-K9 methylation and a decrease in H3 acetylation). Observations within the VTA were consistent, but more variable, such that increased MeCP2 binding to the MOR promoter and decreased H3 acetylation were not significantly different. Decreased MeCP2 binding to the MOR promoter was observed in the hypothalamus; however, this did not lead to altered MOR expression.

Chronic consumption of HFD can result in a state of reward hypofunction ((Cottone *et al*, 2009; Johnson and Kenny, 2010; Stice *et al*, 2008) and supported here in the observed decrease in sucrose preference). It has been suggested that reduced function of the reward system can increase motivation to consume palatable foods or seek drugs of abuse in an effort to mitigate the 'state' of reduced reward (Johnson and Kenny, 2010; Koob and le Moal, 2008). Reward hypofunction in obesity has been linked to

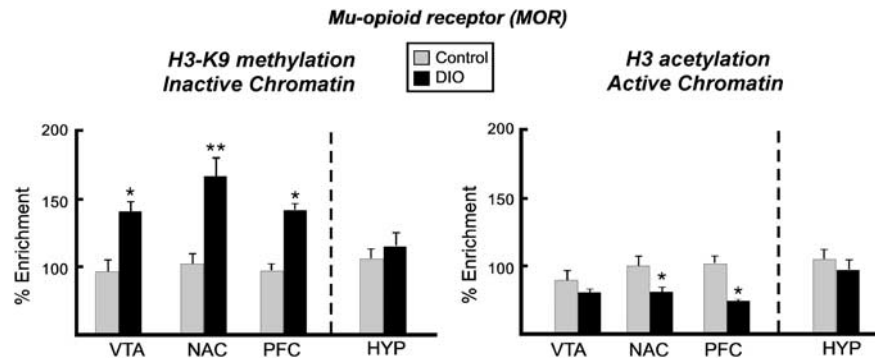


Figure 6 Histone modifications on μ -opioid receptor (MOR) promoter in central nervous system (CNS) of dietary-induced obese mice (DIO) mice. Chromatin was isolated from dissected ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), and HYP of control (gray bars) and DIO mice (black bars), enzymatically digested and immunoprecipitated with IgG, anti di-methyl-H3K9 (inactive chromatin) and anti-acetyl-H3 antibody (active chromatin). The enrichment of genomic DNA associated with immunoprecipitated chromatin at proximal promoter regions of the MOR was quantified by qPCR as described for MeDIP. DIO mice displayed increased amount of methylated histone on the MOR promoter, whereas the amount of acetyl-histone associated DNA was reduced in NAc and PFC of DIO mice. Changes in histone modifications were not observed on GAPDH promoter in DIO mice. This suggests an epigenetically repressed state of the MOR promoter in DIO mice. Values are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, $n = 6$ /group, Two-tailed t -test.

decreased dopamine D2 receptor expression and function in both animal models of obesity, as well as in humans (Johnson and Kenny, 2010; Stice *et al*, 2008; Wang *et al*, 2001). Further, obese rats were shown to have decreased basal levels of dopamine in the nucleus accumbens, as well as attenuated dopamine release in response to chow or amphetamine (Geiger *et al*, 2009). Additionally, reward hypofunction can result from repeated cycling between a highly palatable and a chow diet, and the CRF system has been implicated in this response (Cottone *et al*, 2009). These data identify downregulation of the MOR as an additional mechanism that may contribute to a diminished state of reward in obese animals. Similar findings, including decreased MOR expression in the VTA and diminished nicotine-related reward behavior, were reported in a different mouse strain (129SvEv/C57BL/6) (Blendy *et al*, 2005) and complement the present findings.

A limitation of the current experiments is the fact that concurrent consumption of calorically dense HFD in the DIO mice could contribute to the decreased sucrose preference in DIO mice. And in fact, consumption of a HFD in the absence of obesity has been shown to attenuate responding for sucrose in an operant task (Davis *et al*, 2008). However, in this same report, the authors also showed that obesity, independent of consumption of the HFD, also reduced responding for sucrose and eliminated the development of a conditioned place preference for amphetamine. In contrast, in a separate study that included a pair-fed cohort, deficits in dopamine function in obesity were linked specifically to consumption of a HFD, not weight gain, *per se* (Alsiö *et al*, 2010). Collectively, these data support the finding of reward hypofunction in obesity and also identify a complex interplay of diet, metabolic state and reward-related behaviors.

The increased expression of PENK was also notable, as increased PENK mRNA, particularly in the NAc (Ellgren *et al*, 2007), may be related to drug abuse vulnerability (Nikoshkov *et al*, 2008). Further, increased PENK expression may potentiate the obesity cycle, as injections of enkephalin peptides stimulate consumption of fat-rich

diets, particularly that of a HFD over a low-fat diet and a fat-rich diet over a carbohydrate-rich diet (Leibowitz, 2007). Additionally, increased expression of the delta opioid receptor (*DOR*) in the VTA was observed. Although the functional relevance of this increase is unclear at the present, inactivation of the *DOR* in the VTA increased the rewarding properties of cocaine (Ward and Roberts, 2007), suggesting that increased *DOR* in the VTA may also participate in establishing reward hypofunction. Given the focus of this manuscript on transcriptional regulation, the current studies examined gene expression at the mRNA level and did not examine protein expression. However, our results closely parallel findings from a similar study that determined the concentration of binding sites for the opiate receptor subtypes in lean and genetically obese mice (Khawaja *et al*, 1989), in which obese mice demonstrated an increase in *DOR* binding, a decrease in *MOR* binding and no change in *KOR* binding. Collectively, these data support the idea that chronic consumption of a HFD leads to opioid dysfunction within reward circuitry.

Altered DNA methylation in the brain in response to adverse conditions, either *in utero* or in the early postnatal timeperiod, has been documented across multiple challenge conditions (eg, altered diet or prenatal stress) (Coupé *et al*, 2010; Murgatroyd *et al*, 2009; Niculescu *et al*, 2006; Niculescu and Lupu, 2009; Plagemann *et al*, 2009; Vucetic *et al*, 2010a,b; Weaver *et al*, 2004). Given the relative decrease in CNS plasticity across developmental time, the question of whether altered DNA methylation occurs in the adult brain remains an important issue, and one that has been recently addressed. Differential methylation of *BDNF* during memory consolidation (Lubin *et al*, 2008), and altered DNA methylation of *Fkbp5* in response to chronic corticosterone exposure (Lee *et al*, 2010b) has been observed. Further, the importance of balanced DNA methylation for behavioral responses to chronic cocaine or chronic social defeat has recently been reported (LaPlant *et al*, 2010). To the best of our knowledge, only one other paper has reported differential DNA methylation in DIO mice (hypomethylation of melanocortin 4 receptor in whole

brain) (Widiker *et al*, 2010). The current manuscript significantly extends this work by examining DNA methylation of *MOR* and *PENK* across multiple brain regions.

These findings have important implications for reward circuitry function in obesity, as gene expression changes driven by differential DNA methylation may prove relatively more stable. Although removal of the HFD and/or normalization of body weight was not examined in the present experiments, related data in the literature suggest the relative stability of reward-related changes in obesity. Decreased expression of the dopamine D1 receptor in the accumbens of obese rats persisted 18 days after the withdrawal of the HFD (Alsö *et al*, 2010) and increased brain reward thresholds in obesity persisted 2 weeks after the removal of a HFD, even as body weight was normalized (Johnson and Kenny, 2010). Additionally, it should be noted that in these studies high fat diet consumption began when animals were 3 weeks of age, approximately mid-adolescence, which is clearly a critical period in brain development (Ernst *et al*, 2009). Related experiments that have examined gene expression changes in the CNS in obesity have identified the length of diet exposure and the timing of the exposure as critical variables (eg, early and chronic exposure to HFD altered CNS gene expression (Lee *et al*, 2010a), whereas 4-week HFD exposure in adult animals did not (de Leeuw van Weenen *et al*, 2009)). The present experiments were designed to cover the broadest possible range of postnatal exposure, and future experiments will be directed at defining the critical period of exposure for epigenetic alterations.

It was striking to note that decreased MOR expression and DNA hypermethylation were not universally observed throughout the brain, as we observed these changes within the reward circuitry but not within the hypothalamus. This suggests the existence of a mechanism whereby regional specificity is encoded. Given that the promoter sequence across these different regions does not differ, differential/preferential activation of neurons within the reward circuitry during the chronic consumption of the HFD likely participates in driving some of the epigenetic modifications. This would be in line with similar observations linking acute neuronal activation to epigenetic modifications (Ma *et al*, 2009; Zhou *et al*, 2006). One possible contributor is the increase in MOR agonist stimulation (provided by increased *PENK*), as this has been shown to downregulate MOR mRNA expression *in vitro* (Gach *et al*, 2008). Additionally, there are other likely contributors to the initiation of the epigenetic changes that drive altered MOR expression. Increased adiposity and elevated glucose and leptin are well-characterized responses in the DIO paradigm (Matysková *et al*, 2008). Further, pro-inflammatory cytokines are elevated in obesity, and IL-4 has been shown to modify epigenetic regulation of MOR expression in T-cells (Kraus *et al*, 2010). The direct contribution of the HFD in combination with the physiological responses to the diet are likely to have synergistic roles in driving changes in MOR expression.

Epigenetic control of MOR expression during development involves altered MeCP2 binding and histone modifications, seen both *in vitro* (Hwang *et al*, 2007) and *in vivo* (Hwang *et al*, 2009). Histone modifications associated with MOR expression changes have been demonstrated in the

hippocampus in response to ischemia (Formisano *et al*, 2007), and an excellent recent review (Wei and Loh, 2011) describes the current state of the literature regarding transcriptional regulation of the MOR. This report is the first to show both altered DNA methylation and chromatin changes around the MOR promoter in response to chronic HFD consumption. Defining whether these changes are reversed upon removal of the diet and/or normalization of body weight will have significant implications for understanding the way the CNS is altered in obesity.

The prevalence of obesity, at epidemic proportions and continuing to rise (Sherry *et al*, 2010), is driven by the chronic intake of highly palatable (high fat, high sugar), energy-dense foods (coupled with reduced activity levels). Maintenance of weight loss after dieting is typically low (with 67 (Phelan *et al*, 2010) to 80% (Field *et al*, 2001) of patients regaining the lost weight). These data identify postnatal epigenetic regulation of MOR repression as a novel CNS response to the chronic intake of HFD; a response which may, among other things, contribute to the difficulty in establishing healthier eating patterns after chronic exposure to highly palatable foods.

ACKNOWLEDGEMENTS

We thank Kathy Totoki for technical assistance. The current work was supported by NIH DK064086 (Reyes) and MH091372 (Reyes).

DISCLOSURE

The authors declare no conflict of interest.

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