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Leptin receptor expressing neurons express phosphodiesterase-3B (PDE3B) and leptin induces STAT3 activation in PDE3B neurons in the mouse hypothalamus

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Abstract

Leptin signaling in the hypothalamus is critical for normal food intake and body weight regulation. Cumulative evidence suggests that besides the signal transducer and activator of transcription-3 (STAT3) pathway, several non-STAT3 pathways including the phosphodiesterase-3B (PDE3B) pathway mediate leptin signaling in the hypothalamus. We have shown that PDE3B is localized in various hypothalamic sites implicated in the regulation of energy homeostasis and that the anorectic and body weight reducing effects of leptin are mediated by the activation of PDE3B. It is still unknown if PDE3B is expressed in the long form of the leptin-receptor (ObRb)-expressing neurons in the hypothalamus and whether leptin induces STAT3 activation in PDE3B-expressing neurons. In this study, we examined co-localization of PDE3B with ObRb neurons in various hypothalamic nuclei in ObRb-GFP mice that were treated with leptin (5mg/kg, ip) for 2 hr. Results showed that most of the ObRb neurons in the arcuate nucleus (ARC, 93%), ventromedial nucleus (VMN, 94%), dorsomedial nucleus (DMN, 95%), ventral premammillary nucleus (PMv, 97%) and lateral hypothalamus (LH, 97%) co-expressed PDE3B. We next examined co-localization of p-STAT3 and PDE3B in the hypothalamus in C57BL6 mice that were treated with leptin (5mg/kg, ip) for 1 hr. The results showed that almost all p-STAT3 positive neurons in different hypothalamic nuclei including ARC, VMN, DMN, LH and PMv areas expressed PDE3B. These results suggest the possibility for a direct role for the PDE3B pathway in mediating leptin action in the hypothalamus.

Keywords

phosphodiesterase-3B; ObRb; p-STAT3; hypothalamus; leptin

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Introduction

Leptin signals nutritional status to key regulatory centers in the hypothalamus and regulates energy homeostasis by acting on the neurons expressing the long-form of the leptin receptor (ObRb) [16-18,41,42]. The neuron-specific deletion of Lepr leads to an obese phenotype [8], and transgenic supplementation of the Lepr in neurons of Lepr$^{db/db}$ mice results in an amelioration of the obese phenotype [21]. In addition, central leptin resistance contributes to the development of diet-induced obesity (DIO) and related disorders [13,23,24,37,45]. Besides its major role in energy homeostasis, leptin also plays important role in many other physiological functions including reproduction, growth, bone formation, and cardiovascular and immuno regulation [32,46]. Importantly, most, if not all, of these functions of leptin are mediated at the level of the hypothalamus. Thus, understanding the mechanisms of leptin signaling in the hypothalamus is very important. Cumulative evidence suggest that besides the classical Janus-kinase 2 (JAK2)-signal transducer and activator of transcription-3 (STAT3) pathway [4,17,18,433,44], leptin signaling in the hypothalamus is mediated through various non-STAT3 pathways including phosphatidylinositol 3-kinase (PI3K) [28,48], forkhead protein (FOXO1) [5,20], AMP-activated protein kinase (AMPK) [26], mammalian target of rapamycin (mTOR) [10], and SHP2-GRB2-Ras-Raf-MAPK (mitogen-activated protein kinase) [3,6,7,47]. Previously, we demonstrated that leptin action is also mediated through an insulin-like signaling pathway involving stimulation of PI3K and phosphodiesterase 3B (PDE3B) activities and reduction in cAMP levels in the hypothalamus [48]. Furthermore, PDE3 inhibitor, cilostamide, reverses the anorectic and body weight reducing effects of leptin [48] as well as the leptin-induced increase in proopiomelanocortin (POMC) and neurotensin (NT) gene expression in the hypothalamus [34]. Although these results suggest a role for the PDE3B pathway in mediating leptin action in the hypothalamus, it is unknown whether leptin directly or indirectly activates PDE3B in leptin-sensitive neurons.

In this regard, we recently demonstrated that PDE3B is expressed in the hypothalamic POMC and NPY neurons [37] that are the major targets of leptin action [2,9,14,30,32,41]. However, it is still unknown whether leptin directly activates PDE3B in other leptin-sensitive neurons, a possibility that could be demonstrated if PDE3B is expressed in ObRb neurons in various hypothalamic areas. Thus, we tested the hypothesis that PDE3B is expressed in hypothalamic ObRb neurons. To this end, we utilized ObRb-Cre in combination with Cre-inducible enhanced green fluorescent protein (EGFP) mouse reporter strains to visualize ObRb neurons and performed dual-label immunohistochemistry (IHC) with a specific PDE3B antibody and GFP (green fluorescence protein) antibody to examine PDE3B co-localization in hypothalamic ObRb neurons. In addition, although our previous demonstration of central cilostamide reversing the leptin–induced STAT3 activation in the hypothalamus suggested a cross talk between the PDE3B and STAT3 pathways [48], it is still unknown whether this phenomenon occurs within the same neurons. Thus, we also tested if leptin induces STAT3 activation in PDE3B neurons.

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Materials and methods

LepRb^{EGFP} (Lepr^{cre}–Gt(ROSA)26Sor^{tm2Sho}) mice were generated by breeding Lepr^{cre} (ObRb^{cre}) mice [22] with Gt(ROSA)26Sor^{tm2Sho} mice in the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan, as previously described [22]. C57BL/6 mice were obtained from the Jackson Laboratory and maintained at the Animal Care Facility of the Magee Womens Research Institute of the University of Pittsburgh School of Medicine. All mice were housed in a 12 h light/12 h dark cycle and had *ad libitum* access to food and water. All care and procedures were in accordance with the guidelines and with the approval of the University of Michigan Committee on the Use and Care of Animals and the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Twenty week old, female and eighteen-wk old, male LepRb^{EGFP} (ObRb-GFP) mice were injected with recombinant murine leptin (5 mg/kg body weight, i.p; A.F. Parlow, NHPP, Torrance, CA) in the laboratory of Dr. Martin G. Myers (University of Michigan, Ann Arbor, Michigan, USA). Two hour later, animals were anesthetized with a lethal dose of intraperitoneal (ip) pentobarbital and immediately perfused with 0.1 M PBS followed by 10% formalin. Brains were removed, postfixed and cryoprotected as previously described [27]. Cryoprotected brains were shipped to the University of Pittsburgh, where brains were frozen on dry ice and kept at −80 °C until sectioning. Five series of coronal 25 μm free-floating sections were cut through the mediobasal hypothalamus on a freezing microtome (Leica Sliding Microtome), and stored in cryoprotectant at −20 °C until use.

For dual-label IHC for PDE3B and GFP (for ObRb), ICC for PDE3B was performed first followed by GFP staining as described previously [39]. Free-floating tissue sections were pretreated with 1% NaOH and 1% H_2O_2 in H_2O for 20 min. Sections were then blocked for 1 h and incubated with goat anti-PDE3B (1:500, FabGennix Inc., Frisco, TX) at 4 °C for 48 h, followed by washing and incubation with Cy3-conjugated donkey anti-goat secondary antibody (1:800, 90 min RT). Sections were washed and then incubated with chicken anti-GFP (1:1200, Abcam, Cambridge, MA) at 4 °C for overnight, followed by washing and incubation with DyLight 488-conjugated donkey anti-chicken secondary antibody (1:500, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 90 min at RT. Finally, sections were stained with DRAQ5 (fluorescence DNA dye, 1:2000), mounted on Superfrost slides (Fisher Scientific, Pittsburgh, PA) using Fluoromount-G (Southern Biotech), and visualized with an Olympus FluoView Confocal Microscope for green GFP (ObRb), red PDE3B expressing neurons and blue nuclear stain. The specificity of the PDE3B antibody was validated as previously described [39]. First, pre-absorption of primary antibody with the PDE3B peptide used as immunogen blocked all staining in dual-label IHC procedures (Fig. 1). Second, substitution of isotypic serum for primary antibody eliminated all staining (data not shown).

To demonstrate p-STAT3 and PDE3B co-localization in various hypothalamic nuclei following leptin administration, adult male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) were injected with recombinant murine leptin (5 mg/kg body weight, i.p) followed 60 minutes later by transcardial perfusion with saline and 4% paraformaldehyde. Brains were processed for sectioning as described above. For dual-label ICC for PDE3B and

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p-STAT3, ICC for p-STAT3 was performed first as previously described [25], followed by PDE3B staining as described above. Briefly, free-floating tissue sections were pretreated with 1% NaOH and 1% H_2O_2 in H_2O for 20 min, 0.3% glycine for 10 min, and 0.03% sodium dodecyl sulfate for 10 min. Sections were then blocked for 1 h with blocking solution (5% normal goat serum in PBS, 1%BSA, 0.4% Triton X-100), followed by incubation with p-STAT3^{Tyr705} antibody (1:1500 in blocking solution, Cell Signaling Technology, Inc., Danvers, MA) for overnight at 4 °C. On the next day, the sections were washed and incubated with goat anti-rabbit Alexa-488 secondary antibody (1:500, 90 min RT). Sections were washed and then incubated with goat anti-PDE3B at 4 °C for 48 h, followed by washing and incubation with Cy3-conjugated donkey anti-goat secondary antibody (1:800, 90 min RT). Finally, sections were mounted on Superfrost slides using Fluoromount-G, and visualized with an Olympus FluoView Confocal Microscope for green (p-STAT3) and red PDE3B-expressing neurons.

Sections were scanned at 1024 × 1024 pixels, 40X objective, 0.3μm pixel size, using two or three color image collection (488 nm laser, 543 nm, 633 nm) together with appropriate dichroics and barrier filters. Image planes throughout the depth of the specimen were collected and the neurons expressing both GFP (green) and PDE3B (red) (yellow color in merged images) for ObRb and PDE3B co-localization or both p-STAT3 (green) and PDE3B (red) for p-STAT3 and PDE3B co-localization were counted on at least ten different sections through the entire rostro-caudal extent of the ARC of each brain using the MetaMorph software (Molecular Devices, Sunnyvale, CA). Co-expression values were calculated as percentages of the total number of ObRb or p-STAT3-positive cells expressing PDE3B. All values were expressed as means ± standard error (SE).

To examine the total number of PDE3B expressing neurons and PDE3B-positive neurons that express ObRb in various hypothalamic nuclei, sections stained with PDE3B (red), GFP (ObRb) and DRAQ5 or DAP1 (blue, nuclear stain; Sigma, St Louis, MO, USA) were scanned at 1024 × 1024 pixels, 20X objective, 0.3μm pixel size, using three color image collection together with appropriate dichorics and barrier filters. Total number of cells (blue nuclear stain), the number of PDE3B-positive (red) cells and the number of ObRb-positive (GFP, green) cells expressing PDE3B in each hypothalamic nuclei were counted from at least three matched sections through the rostro-caudal extent of the ARC. To demonstrate whether PDE3B is also expressed in amygdala region that does not express ObRb, we counted PDE3B-positive neurons in this area of the brain. Total number of PDE3B expressing cells was expressed as percentage of all cells counted in specific nuclei. The number of PDE3B + ObRb cells was presented as percentage of PDE3B cells expressing ObRb.

To demonstrate whether PDE3B is expressed in neuronal and/or glial cells, few hypothalamic sections were processed for dual–label IHC for PDE3B + NeuN (NEUronal Nuclei marker) or PDE3B + GFAP (glial fibrillary acidic protein, glial cell marker). IHC was done as described above using PDE3B, NeuN (mouse anti-NeuN monoclonal, cat# MAB377; 1:1000; Millipore, Bedford, MA) and GFAP (Chicken polyclonal, 1:4000, #ab4674, Abcam) antibodies as appropriate. The sections for PDE3B +GFAP localization were also stained with DAP1 for blue nuclear stain. Sections stained with PDE3B+GFAP or

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PDE3B+ NeuN were scanned at 1024 × 1024 pixels, 40X objective, 0.3μm pixel size, using three-color image collection together with appropriate dichorics and barrier filters.

Finally, because a recent study by Kelly et al. suggested that the hypothalamus does not express PDE3B [19], although we and others have consistently measured PDE3B activity in the hypothalamus [29, 36, 38, 40, 48] and we have shown PDE3B expression in the hypothalamus by IHC [39], we re-examined PDE3B expression by RT-PCR to demonstrate and confirm that the mouse hypothalamus expresses PDE3B. Thus, we examined PDE3B expression in the medial basal hypothalamus (MBH). Because PDE3B is highly expressed in the liver and adipose tissue [11], and Kelly et al. reported cerebellum to express PDE3B [19], we also included these tissues as positive controls for PDE3B expression. Two adult male mice (C57BL/6J), available in our mouse colony, were killed and the MBH, cerebellum, liver and WAT were harvested, which was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. The tissues were processed for total RNA extraction by Trizol reagent (Life Technologies, Grand Island, NY, USA) and assessed for purity and concentration using spectrophotometric analysis. 15ug of total RNA was subjected to DNase treatment using RNase-Free DNase (Promega, Madison, WI) according to the manufacturer's protocol and re-extracted with Trizol. cDNA was made from 1 μg of re-extracted total RNA using the high-capacity RT kit as described by the manufacturer (Life Technologies) and 50 ng of cDNA was used to examine the presence of PDE3B by PCR using the following primers (Forward: 5'-agtaccgcggaggaaaaagt-3', reverse: 5'-aagtcccagtcccagaggat-3'). The PCR machine was run at 94 °C for 3 min, and then the cycle of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 1 min for 28 or 35 cycles followed by 10 min at 72 C. The PCR product (164 bp) was visualized on 1% agarose gel containing ethidium bromide under UV light.

Statistical analysis

All values are expressed as means ± SEM. Statistical significance in the values in different brain nuclei were analyzed by one-way ANOVA followed by Fisher's least significant difference (LSD) multiple-range tests. All statistical analyses were done using GB-Stat software for the Macintosh (Dynamic Microsystems, Silver Spring, MD). $P < 0.05$ were considered to be significant.

Results

GFP-positive cells identifying the ObRb neurons in ObRb-GFP mice were distributed in various hypothalamic nuclei including the arcuate nucleus (ARC), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), ventral premammillary nucleus (PMv) and lateral hypothalamic areas (LH) as previously described [15,22]. Systematic examination of sections through the rostro-caudal axis of the hypothalamus showed expression of PDE3B (red) in ObRb neurons (green) as shown by the development of yellow color in the merged figures in various nuclei (Fig. 1). Since there was no difference in PDE3B and ObRb colocalization in male and females, the data from one male and three female mice were combined for the analysis. Analysis of various sections throughout the hypothalamus in four ObRb-GFP mice showed that PDE3B was expressed in almost all ObRb neurons in the ARC

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(92.74%), VMN (94.27%), DMN (95.6%), LH (96.73%) and PMv (97.22%) (Fig.2). As previously described [15], there were very few ObRb-expressing neurons in the PVN, however all ObRb neurons in this area co-expressed PDE3B (data not shown).

We also counted the total number of PDE3B expressing neurons in the hypothalamic nuclei and the amygdala. We observed that within the hypothalamus, the number of PDE3B expressing cells was highest in the ARC (58.90%) followed by PMv (57.03%) VMN (50.81%), PVN (49.71%), DMN (49.00%) and LH (44.41%). The percentage of PDE3B expressing neurons in the ARC and PMv was significantly higher ($P < 0.05$) than that in the LH. On the other hand, PDE3B expression in the amygdala region, which does not express ObRb, was only in 25.73% of the cells counted and it was significantly ($P < 0.01$) lower than that was observed in any of the hypothalamic nuclei (Fig. 3A). Also, there were no ObRb-GFP-positive cells in the amygdala, as expected. When we counted the number of PDE3B expressing cells (red) that also expressed ObRb (GFP) in the hypothalamic nuclei, we observed that most of the PDE3B neurons in the ARC (64.15%) and PMv (59.14%) expressed ObRb, whereas only 28.57%, 27.89% and 27.73% of PDE3B neurons expressed ObRb in the VMN, DMN and LH, respectively (Fig. 3B). The percentage of PDE3B neurons that express ObRb was significantly higher ($P < 0.01$) in the ARC and PMv as compared to that in the VMN, DMN or LH.

As reported previously [15,22], leptin-induced p-STAT3-positive cells were localized in various nuclei in the mouse hypothalamus including the ARC, VMN, DMN, PMv and LH (data not shown). As expected, p-STAT3 was localized in the nucleus. We counted all p-STAT3-positive (green) and p-STAT3+PDE3B-positive (green + red) cells in the hypothalamic areas and expressed as percentage of p-STAT3-positive cells colocalized with PDE3B. We observed that PDE3B (red) was expressed in almost all p-STAT3-positive (green) cells in the hypothalamic nuclei (ARC: 98.78%, VMN: 99.25%, DMN: 98.10%, PMv: 99.82%, LH: 100%) (Figs. 4 and 5). In the PVN, p-STAT3 positive cells were negligible (data not shown).

Dual-label IHC demonstrates that PDE3B is expressed in the neuronal cells but not in the glial cells, because PDE3B and NeuN (neuronal marker) were co-localized but not PDE3B and GFAP (glial marker) (Fig 6). RT-PCR with total RNA showed that PDE3B is expressed in the liver, WAT and cerebellum as expected [19, 11]. Most importantly, RT-PCR clearly shows that PDE3B is also expressed in the MBH (Fig. 7).

Discussion

In this investigation, we studied co-localization of ObRb with PDE3B, and p-STAT3 with PDE3B in various hypothalamic nuclei by immunohistochemical double labeling. We observed that PDE3B is expressed in almost all ObRb and p-STAT3-positive neurons in various hypothalamic nuclei that have been implicated in mediating leptin action. These results further support the possibility of a direct role for PDE3B in transducing leptin action in the hypothalamus.

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ObRb-expressing neurons utilize various intracellular signaling pathways to transduce leptin action in the hypothalamus. Discovery of the leptin receptor as a member of the class-1 cytokine receptor family promptly established the JAK2-STAT3 pathway as the major signaling mechanism for the action of leptin in the hypothalamus [43, 44]. However, subsequently several non-STAT3 pathways have been identified as an integral part of the signaling network in the hypothalamus that regulates energy homeostasis [32, 34]. Using pharmacological approach, we have identified the PDE3B pathway as one of the non-STAT3 pathways of leptin signaling in the hypothalamus [48]. Thus, PDE3 inhibition by cilostamide reverses the anorectic and body weight reducing effects of leptin [48]. In addition, reversal of the leptin-induced STAT3 activation in the hypothalamus by PDE3 inhibition demonstrates a cross talk between the PDE3B and STAT3 pathways of leptin signaling [48]. Recently, we have demonstrated that PDE3 inhibition reverses the leptin-induced increase in POMC and NT gene expression [34], and PDE3B is expressed in the POMC and NPY neurons in the hypothalamus [39], suggesting a potential role for the PDE3B pathway in transducing leptin signaling in these neurons. Along this line, the PI3K-PDE3B-cAMP pathway but not the STAT3 pathway of leptin signaling in the hypothalamus is impaired during the development of leptin resistance in POMC and NPY neurons following chronic central leptin infusion [31, 33, 36]. Recently, we have demonstrated PI3K as an upstream regulator of the PDE3B pathway of leptin signaling in the rat hypothalamus [38]. In addition, most recently, we have shown that the PDE3B-cAMP pathway of leptin signaling in the hypothalamus is impaired during the development of diet-induced obesity in mice [39].

To better understand the role of PDE3B in mediating leptin signaling in the hypothalamus, in the present study we examined if PDE3B was localized in ObRb-expressing neurons. The finding of PDE3B expression in almost all ObRb-expressing neurons in the ARC, VMN, DMN, LH, pMV and PVN neurons is in favor of the possibility of a direct role of the PDE3B pathway in mediating leptin action in these hypothalamic areas. Leptin-signaling in the above-mentioned hypothalamic sites plays a critical role in many physiological functions including the regulation of energy homeostasis and reproduction [12]. Thus, our finding that almost all ObRb neurons in these areas express PDE3B further suggests a physiological role of this pathway in leptin action. In this study, we also determined the percentage of neurons that expressed PDE3B and the percentage of PDE3B neurons expressing ObRb in the hypothalamic nuclei under investigation. We included the amygdala region, which does not express ObRb, to determine if PDE3B expression in this area was comparable to that in the hypothalamic nuclei. Our finding that the percentage of PDE3B-positive neurons was very low in the amygdala region and it was significantly lower than any hypothalamic nuclei examined suggests the enrichment of PDE3B in the hypothalamic areas expressing ObRb neurons. Interestingly, within the hypothalamus, majority of neurons (above 50%) in the ARC and PMv expressed PDE3B, and approximately 60% of the PDE3B neurons in these two hypothalamic nuclei also expressed ObRb. These results show enrichment of PDE3B in the ARC and PMv and further suggest an important role of PDE3B in mediating leptin signaling in these two hypothalamic areas. Along this line, we have recently demonstrated a decrease in cAMP levels, a result of increased PDE3B activity, only in the ARC following thirty-minute exposure of peripheral leptin in mice [40]. Notably, in that particular study

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PMv was not examined. Nevertheless, although the specific role of the PDE3B pathway of leptin signaling in various hypothalamic nuclei is yet to be documented, the role of this pathway in mediating leptin signaling in NPY, POMC and NT neurons is becoming apparent [34, 39]. Whereas co-localization of PDE3B and ObRb in various hypothalamic nuclei suggests the possibility of a direct role of PDE3B in mediating leptin action in these nuclei, whether leptin action is mediated also by PDE3B expressing non-ObRb neurons in the hypothalamus remains to be determined, and if there is any role, it would be an indirect one.

To further assess the physiological role of the PDE3B pathway of leptin signaling, we examined whether leptin treatment would induce STAT3 activation in PDE3B expressing neurons. Because STAT3 activation leads to p-STAT3 localization in the nucleus, we were able to identify the neurons that express both p-STAT3 (nuclear) and PDE3B (mostly membrane localization) [11]. Our revelation that all p-STAT3 positive neurons expressed PDE3B further suggests leptin utilization of both STAT3 and PDE3B pathways in the same neuron. This finding along with our previous demonstration of the reversal of STAT3 activation in the hypothalamus by PDE3B inhibition [48] further suggest a direct cross talk between these two pathways in hypothalamic neurons; and provide additional evidence in favor of a physiological role of the PDE3B pathway of leptin signaling in the hypothalamus.

It is worthy to mention that recently, using *in situ* hybridization (ISH) technique, Kelly et al. reported that PDE3B expression in the mouse brain is only restricted to the cerebellum [19], which would suggest that PDE3B is not expressed in the hypothalamus. However, in our studies with rats and mice, we have consistently demonstrated leptin to increase PDE3B activity in the hypothalamus [36,38,40,48]. Most importantly, a recent study from the Laboratory of Dr. Emilio Hirsch has measured PDE3B activity in the mouse hypothalamus and GT1.7 hypothalamic neuronal cells [29]. It is also reasonable to assume that without enough expression of PDE3B in the hypothalamus, it is impossible to measure PDE3B activity in a biochemical assay. Along this line, using ICC approach, we have demonstrated PDE3B expression in the rat [32] and mouse hypothalamus [39] and in hypothalamic POMC and NPY neurons [39]. Furthermore, we have demonstrated that cilostamide, a specific PDE3 inhibitor, reverses the anorectic and bodyweight reducing effects of central leptin [48]. Recently, by RT-PCR we have shown PDE3B to express in the hypothalamus and in mHypoE-46 hypothalamic neurons that express NPY/AgRP [1]. In the present study, we have shown by IHC that in the hypothalamus PDE3B is expressed in the neuronal cells but not in the glial cells and confirmed by RT-PCR the expression of PDE3B in the hypothalamus as well as in the cerebellum, liver and white adipose tissue. Notably, although PDE3B expression in the hypothalamus was lower than in the cerebellum, it reaffirms our previous finding that PDE3B is expressed in this part of the brain that regulates energy homeostasis [1, 39]. Thus, altogether these studies have clearly established the expression of PDE3B in the mouse and rat hypothalamus. However, the underlying factor(s) behind Kelly *et al.* not observing PDE3B expression in the mouse hypothalamus using ISH method is currently unknown, although these authors recorded very low level of PDE3B expression in the hypothalamus by qPCR [19].

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In summary, we have demonstrated expression of PDE3B in ObRb neurons and leptin induction of p-STAT3 in PDE3B neurons in various hypothalamic nuclei that have been implicated in energy homeostasis. These findings suggest an important role of the PDE3B pathway in mediating action of leptin in different hypothalamic areas and further support the possibility of a direct cross talk between the STAT3 and PDE3B pathways in the leptin-sensitive hypothalamic neurons.

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Highlights

- PDE3B is expressed in various hypothalamic areas that play a key role in energy homeostasis.
- Almost all ObRb-expressing neurons in the hypothalamic nuclei express PDE3B.
- Almost all p-STAT3 positive neurons in different hypothalamic nuclei express PDE3B.
- This study suggests a possible direct role for PDE3B in mediating leptin action in the hypothalamus.

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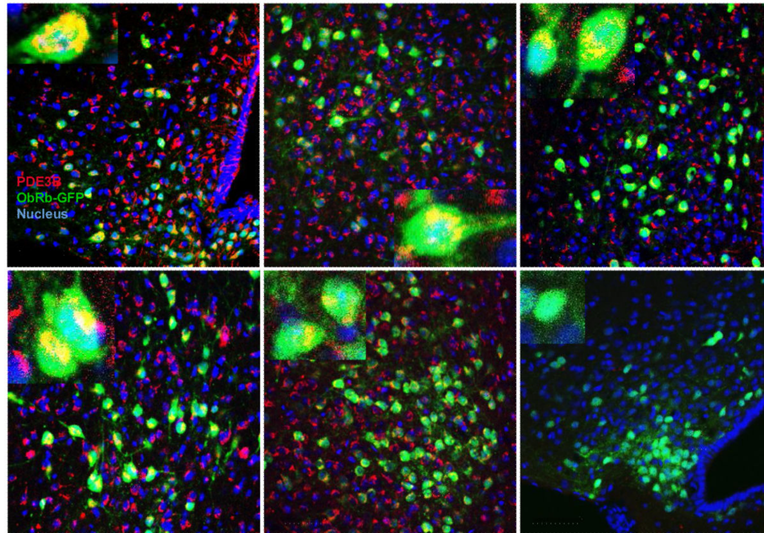

Fig. 1.

Confocal microscopy of double labeled IHC for PDE3B (red) and GFP-ObRb in various hypothalamic nuclei of ObRb-GFP mice (A-E). Blue = nuclear stain. Note that almost all ObRb-GFP neurons in the arcuate nucleus (ARC, A), ventromedial nucleus (VMN, B), dorsomedial nucleus (DMN, C), lateral hypothalamus (LH, D) and ventral premammiliary nucleus (PMv, E) also express PDE3B as shown by yellow color (for details see inset). 3v = third ventricle. Note that preabsorption of PDE3B antibody with PDE3B peptide used as immunogen blocked all PDE3B staining in a section through the ARC (F). Scale bar = 50 μm

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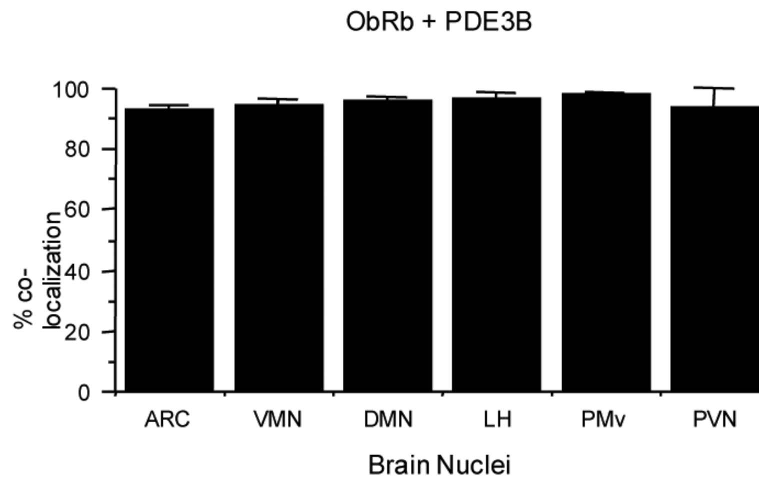

Fig. 2.

Percent of ObRb neurons co-localized with PDE3B in the hypothalamus of ObRb-GFP mice. Values represent the mean ± SEM for 4 animals.

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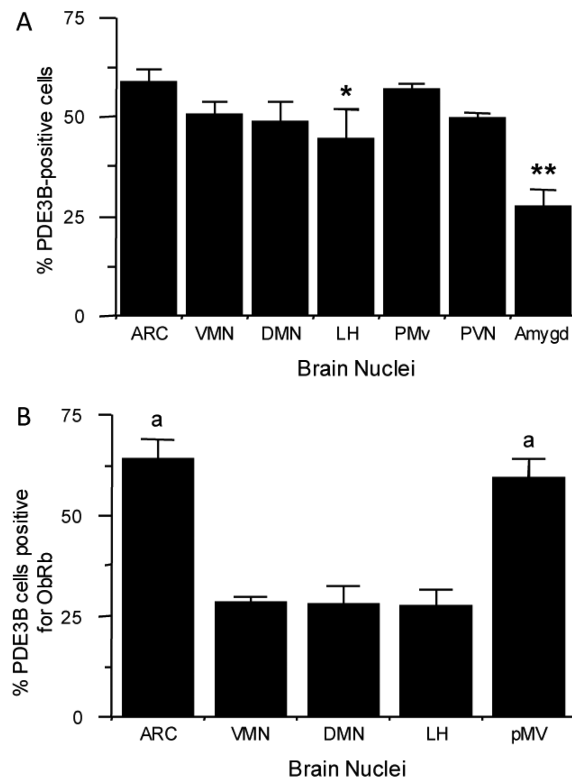

Fig. 3.

Changes in percent of PDE3B-positive cells (A) and percent of PDE3B cells expressing ObRb (B) in various brain nuclei. ARC = arcuate nucleus, VMN = ventromedial nucleus, DMN = dorsomedial nucleus, LH = lateral hypothalamus, PMv = ventral premammiliary nucleus, PVN = paraventricular nucleus, Amygd = amygdala. Values represent the mean ± SEM for 3 animals. * $P < 0.05$ vs ARC and PMv, ** $P < 0.01$ vs all other nuclei, ^{a} $P < 0.01$ vs VMN, DMN and LH.

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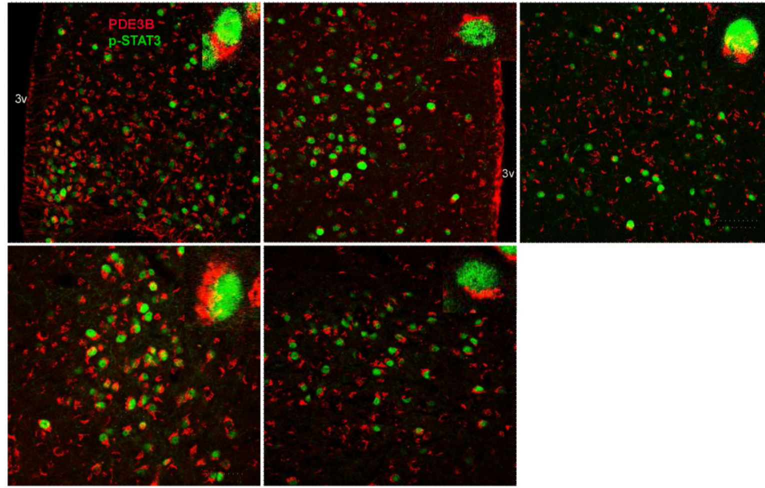

Fig. 4.

Confocal microscopy of double labeled IHC for PDE3B (red) and p-STAT3 in various hypothalamic nuclei of C57BL6 mice (A-E). Note that almost all p-STAT3-positive neurons in the arcuate nucleus (ARC, A), ventromedial nucleus (VMN, B), dorsomedial nucleus (DMN, C), lateral hypothalamus (LH, D) and ventral premammiliary nucleus (PMv, E) also express PDE3B (see inset for details). 3v = third ventricle. Scale bar = 50 μm.

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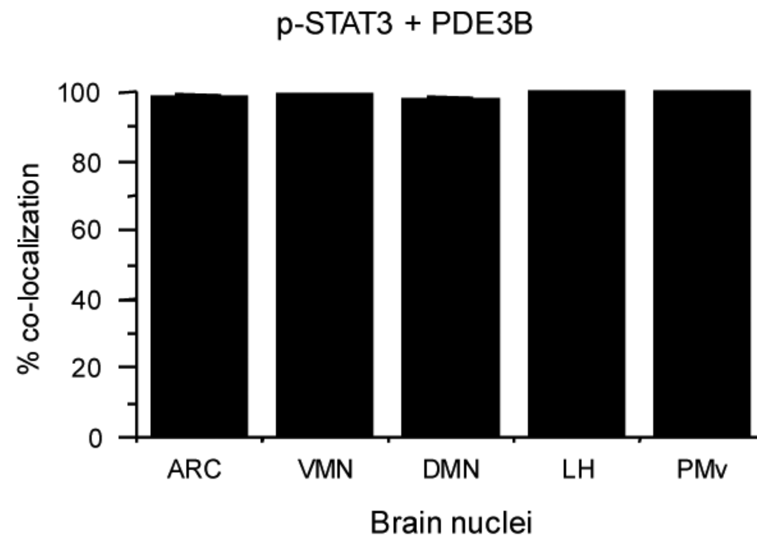

Fig. 5.
Percent of p-STAT3-positive neurons co-localized with PDE3B in the hypothalamus of C57BL6 mice treated with leptin (5mg/kg, ip) for one hour. Values represent the mean ± SEM for 4 animals.

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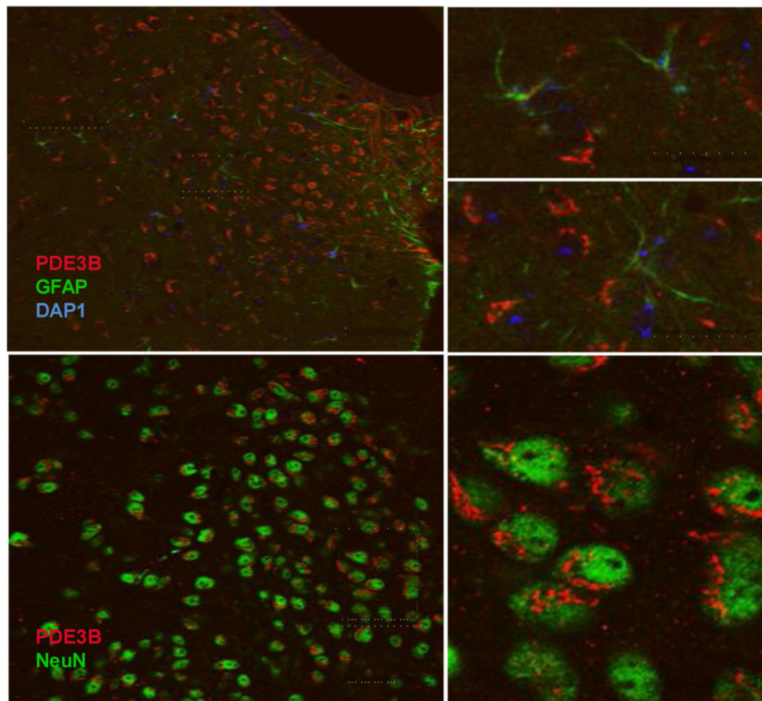

Fig. 6.

Confocal microscopy of double labeled IHC for PDE3B (red) and GFAP (green) (A-C), and for PDE3B (red) and NeuN (green) (D, E) in the section through the ARC-VMN area of the mouse hypothalamus. Blue = nuclear stain. Note that PDE3B is expressed only in the neuronal cells (NeuN-positive) but not in the glial cells (GFAP-positive). 3v = third ventricle. Scale bar = 50 μm in A and D, and 10 μm in B, C, E.

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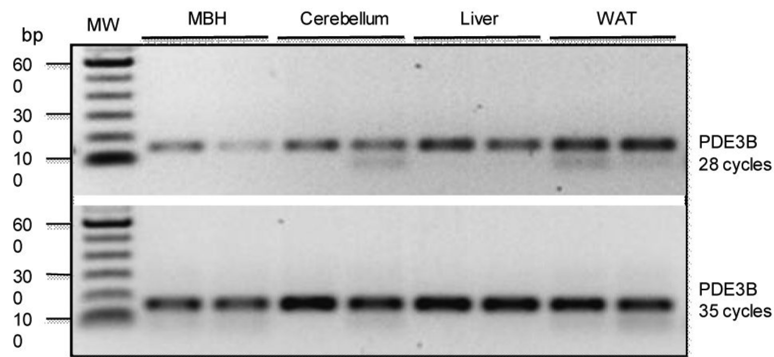

Fig. 7.

PDE3B expression in the medial basal hypothalamus (MBH), cerebellum, liver and epididymal white adipose tissue (WAT) of C57BL/6J mice (n = 2). RT-PCR products (164 bp) from RNA isolated from the indicated tissues were electrophorosed in a 1% agarose gel containing ethidium bromide and visualized under UV light. MW = 100 bp DNA ladder (Invitrogen). Upper and lower panels show RT-PCR products at 28 and 35 PCR cycles, respectively. Please note that only 4 μl of the total 22 μl reaction mixture (20 μl RT reaction and 2 μl dye) was loaded in the gel for each sample.