The role of the hypothalamic dorsomedial nucleus in the central regulation of food intake

Ph.D. thesis

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Budapest
2013
1. Introduction

The central role of the hypothalamus in the regulation of food intake and energy expenditure has long been established. The hypothalamus receives hormonal input such as insulin, leptin, and ghrelin from the periphery. The gate for the most important adiposity signals is the arcuate nucleus, which contains neurons expressing orexigenic and anorexigenic peptides, respectively. These neurons convey peripheral input to the paraventricular and ventromedial nuclei, and the lateral hypothalamic area, which all play critical roles in body weight regulations. The hypothalamic dorsomedial nucleus (DMH) has also been implicated in the regulation of body weight homeostasis along with other hypothalamic nuclei including the arcuate, ventromedial, and paraventricular nuclei as well as the lateral hypothalamus. Lesions of the DMH affected ingestive behavior. Electrophysiological data suggested that neurons in this nucleus integrate hormonal input and ascending brainstem information and, in turn, modulate food intake and energy balance. In response to refeeding of fasted rats, Fos-activated neurons were reported in the DMH.

Major projections relay vagus-mediated signals from the gastrointestinal tract, and humoral signals to the hypothalamus from the nucleus of the solitary tract (NTS), a viscerosensory cell group in the dorsomedial medulla. In contrast to tonically active adiposity signals providing ongoing messages, the production of satiating signals is connected to the consumption of meal. Satiating signals are mediated to the NTS via afferent peripheral nerves, particularly the vagus nerve, as well as by circulating satiating signals including cholecystokinin. Both arcuate and NTS neurons project to the dorsomedial nucleus of the hypothalamus (DMH). Based on lesion studies, the DMH may be involved in body weight homeostasis. Hypothalamic neurons driven by adiposity signals influence the threshold of satiating hormones in the brainstem. In contrast, the influence of brainstem food intake regulatory centers on hypothalamic systems is less firmly established. Solitary-hypothalamic projections may represent the anatomical link between the medullary viscerosensory center and the hypothalamic circuitry regulating energy homeostasis.

The NTS contains neurons expressing prolactin-releasing peptide (PrRP) and glucagon-like peptide-1 (GLP-1). These neuropeptides are implicated in metabolic and autonomic regulations. Their central application inhibits food intake, reduces blood
glucose levels, and increases blood pressure and heart rate. GLP-1 exerts its actions via established G-protein coupled GLP-1 receptors, which are widely distributed in the brain, including the hypothalamus with particular abundance in the DMH. PrRP and GLP-1 fibers and fiber terminals were found in a variety of hypothalamic sites with the highest densities in the dorsomedial and paraventricular nuclei. Retrograde tracer studies indicated that a substantial number of nerve terminals in the DMH are of brainstem origin, arising from neurons located in the caudal part of the NTS. Thus, PrRP and GLP-1 fibers of brainstem origin are candidates that may activate dorsomedial neurons after refeeding.
2. Objectives

To understand the activation and function of dorsomedial neurons during refeeding and in satiety, the following points were addressed:

- Comparison of the Fos activation in the DMH in response to limited and unlimited food consumptions following 2-day fasting
- To identify the DMH regions that are sensitive to satiety
- The localization and chemical characterization of solitary-hypothalamic fibers that innervate DMH neurons
- Identification of the consequences of the transection of the ascending solitary-hypothalamic pathway on the elimination of PrRP and GLP-1 fibers
- To investigate the effect of the transections of solitary-hypothalamic pathway on re-feeding-induced Fos activation in the DMH
- The role of the arcuate nucleus was examined in the activation of DMH neurons in rats with chemical lesioning of the arcuate nucleus
- Demonstration of GLP-1 receptor expression in Fos-activating neurons of the DMHv in satiety
3. Materials and methods

A total of 84 adult, male Wistar rats (300–400 g body weight; Charles Rivers Laboratories, Isaszeg, Hungary) were used in this study and 18 of them had been neonatally injected with monosodium-glutamate.

3.1. The fasting-refeeding protocol

Rats in fasting-re-feeding experiments were divided into four major groups: 1) Controls were fed continuously ad libitum. This group contained 9 intact, 6 MSG treated, 6 brainstem transected, and 3 caudal hypothalamic transected rats. 2) Fasted rats were deprived of food for 48 h starting at 10.00 AM and then sacrificed. This group contained 6 intact, 3 MSG treated, 6 brainstem transected, and 3 caudal hypothalamic transected rats. 3) Refed rats were initially fasted for 48 h starting at 10.00 AM, then refed for 2h before they had been sacrificed. After fasting, rats started to eat immediately and they used to stop, drink, and eat again. Thus, these animals were sacrificed about 80-85 min after they stopped the continuous consumption of food. This group contained 12 intact, 9 MSG treated, 9 brainstem transected, and 3 caudal hypothalamic transected rats. Additional 3 animals were refed for only 1h and paired with 3 fasted rats for in situ hybridization. Another 2 rats was refed for 2h following 24h fasting to demonstrate a qualitatively similar response after shorter fasting period. 4) “Sham refed” rats (3 intact animals) were fasted for 48 h followed by presenting out-of-reach food for 2 hours before perfusion. The presentation of out-of-reach food was performed by placing 3 normal metal grid cage cover on top of each other. In this arrangement, the rats were not able to reach the pellet food placed in the upper metal grid cage cover.

Rats in fasting-refeeding experiments were divided into 3 major groups: 1) “Food showed” rats (n=6) were fasted for 48 hours followed by presenting out-of-reach food for 2 hours before perfusion. 2) “Limited refeeding” rats (n=4) were fasted for 48 hours followed by the consumption of 1.5 g food. This amount of food available for this group of rats consisted of less than one quarter that rats typically consume after fasting. 3) “Refed to satiety” rats were fasted for 48 hours followed by providing them with freely available food. Rats started to eat immediately as food became available and consumed
7.3±0.7 g within 30-35 min that was followed by a period of time without any food intake. Therefore, we considered this stage as satiation. Animals were sacrificed 2h after the starting time of eating. This group contained 14 intact and 6 transected rats. Additional 3 rats were perfused 6 hours after the onset of refeeding.

3.2. Transections of neuronal pathways

Line-shaped holes about 1x3 mm were drilled at a coronal plane, one side on the skull, according to the following coordinates: 1) for brainstem transections: antero-posterior: -9.0 mm, lateral: 1.3 mm, ventral: 7.5 mm; and 2) for caudal hypothalamic transections: antero-posterior: -4.0 mm, lateral: 1.6 mm, dorso-ventral: 9.0 mm (Paxinos & Watson, 2005). Unilateral transections were performed with 2.0-2.5 mm wide “glass knife” (Palkovits et al., 1982). After cutting the dura, the knife was vertically penetrated through the target area, as down as the basal surface of the brainstem at the pontomedullary junction, or down into the hypothalamus at the premamillary level. The animals were sacrificed on the 8th or 9th postoperative days. Twenty one brainstem and 9 hypothalamic transections have been performed.

3.3. Histological analyses

For immunocytochemistry, rats were deeply anesthetized and perfused transcardially with 150 ml saline followed by 300 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH=7.4 (PB). Brains were removed and postfixed in the same fixative solution for 24 hours and transferred to PB containing 20% sucrose for 2 days. Serial coronal sections were cut at 40 μm between -1.0 and +7.0 mm from the level of the bregma. For in situ hybridization, refeed rats were anesthetized and decapitated. The brains were dissected and frozen immediately on dry ice. Serial coronal sections (12 μm) were cut using a cryostat, dried, and stored at -80°C until use. The sections were examined using the following histological techniques:
3.3.1. Cresyl-violet staining
3.3.2. Luxol fast blue cresyl violet staining
3.3.3. Fos immunohistochemistry
3.3.4. Fluorescent Nissl staining combined with Fos immunolabeling
3.3.5. PrRP and GLP-1 immunohistochemistry
3.3.6. Double PrRP/GLP-1 and Fos immunocytochemistry
3.3.7. Double PrRP and TH immunohistochemistry
3.3.8. In situ hybridization histochemistry
3.3.9. Combination of in situ hybridization histochemistry for GLP-1R and immunolabeling for Fos

3.4. Quantitative analysis of Fos expression

By using a projection microscope under 10X magnification, the number of Fos-positive neurons was counted in the ventral subdivision of the DMH on sections 3.2, 3.4, and 3.6 mm caudal to the bregma, on both sides of the brain. The counts were summarized and the individual mean values from 5 animals per group were analyzed except for the sham refed group, which consisted of only 3 animals. Statistical analysis was performed using one-way ANOVA followed by Bonferroni’s Multiple Comparison Tests.
4. Results

5.1. The effect of satiety on the activity of the neurons of the DMH

5.1.1. Body weight and eating time

Body weight and eating time were measured during our experimental protocol in order to demonstrate food consumption and the presence of a normal behavioral sequence of satiety following fasting for 48 h. It should be noted that the body weight we measured was biased by the altered amount of ingested food and water during fasting and re-feeding. Therefore, the data we present mean only apparent body weight and not long-term physiological alterations of body weight. One day (9.00 AM) before fasting, the body weight of rats (n=10) was 364±7.5 g. Within 24h, the rats gained 5.8±1.5 g. In the first day of fasting, the loss of body weight was 21.6±1.4 g, which was followed by a 11.0±1.4 g on the second day. Thus, instead of gaining about a calculated 11.6 g in 2 days, the animals lost 31.6±1.1 g, as a result of fasting. During re-feeding, the body weight of the rats increased by 10.3±1.8 g within 2h. The total weight of the consumed dry food in this period was 7.8±0.8 g, or somewhat less because we did not measure spillage. After presentation of food, the rats started to eat within 1-3 min. They typically ate for about 15 min when they stopped eating to drink. After that, rats continued to eat for about another 15 min, drank, and soon went back to sleep. The time of cessation of eating from the start of re-feeding was 36.8±3.1 min.

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5.1.2. Fos expression following re-feeding of fasted rats

There was a very low density of Fos-containing cells in DMH and surrounding hypothalamic areas in *ad libitum* fed control rats, as well as in fasted rats. Following re-feeding, the number of Fos expressing cells increased throughout the DMH, however, one well circumscribed region in the nucleus, contained a particularly high density of activated cells throughout the rostro-caudal extent of the nucleus in 48h fasted as well as in 24h fasted animals. This region corresponds to the ventral subdivision of the DMH in Nissl-stained sections. The position of the activating cells was further confirmed in sections double labeled with Fos immunoreactivity and a fluorescent Nissl dye. The average numbers of Fos-ir neurons in control, fasted, and ‘sham refed’ groups were 8.0±2.4, 7.4±1.2, and 9.8±0.9, respectively, which increased highly significantly (p < 0.001) to 96.7±7.7 Fos-ir neurons after re-feeding.

5.1.3. The topographical localization of Fos-ir neurons in the DMH

Except the ventral subdivision of the DMH, there was no other group of cells in the hypothalamus that exhibited such an intense Fos signal in response to re-feeding. In a group of the experimental animals, when food was presented, but rats were not able to reach and consume it, the density of Fos-ir neurons showed a moderate increase evenly in all parts of the DMH, without any specific intense labeling in the ventral subdivision of the DMH.

5.1.4. The appearance of Fos mRNA in response to refeeding

The specificity of the Fos-immunoreactive (Fos-ir) signal in the ventral subdivision of the DMH was verified by *in situ* hybridization histochemistry for *c-fos* mRNA. It was clearly demonstrated that *c-fos* mRNA was expressed exclusively in the ventral subdivision of the DMH, exactly where the Fos protein immunoreactivity appeared in this nucleus.

5.1.5. Fos activation in the DMv following satiation

Fos-containing cells were seen in very low density throughout the DMH and the surrounding hypothalamic areas in fasted rats. In animals, which were presented with
food in a way that they were not able to reach and consume it, the density of Fos-immunoreactive (Fos-ir) neurons showed a moderate increase evenly in all parts of the DMH, without any specific intense labeling in the ventral subdivision of the nucleus. Here, the average number of Fos-ir neurons was 16.1±3.6 (mean±SEM) (per section, one side). In the group where rats were allowed to eat only a limited amount of food (1.5 g), we did not observe any further increase in the density of Fos-ir neurons in any subdivisions (including the ventral one) of the DMH. In these animals, the number of Fos-ir neurons was 17.3±1.9 in the DMHv. In rats refed to satiety the density of Fos-ir neurons in the compact and the dorsal subdivisions of the DMH did not show any changes, meanwhile a particularly high density of activated cells appeared throughout the rostro-caudal extent of the ventral subdivision of the DMH (Fig. 1C). The average numbers of Fos-ir neurons in the ventral subdivision of the DMH of rats refed to satiety was elevated as high as 75.9±3.8. This value (over 4fold) represents a highly significant increase (p < 0.001) in the number of Fos-ir neurons in response to satiation. The number of Fos-ir neurons and the intensity of Fos signal were markedly reduced 6 hours after the onset of refeeding.

5.2. Fibers in the DMv and their brainstem origin

5.2.1. PrRP-containing nerve fibers and terminals in the DMH

PrRP immunolabeling was detected in different parts of the hypothalamus. PrRP-ir cell bodies appeared exclusively in the most caudal part of the DMH, while PrRP-ir fibers were present in the dorsal and ventral subdivisions of the DMH, as well as in the arcuate nucleus, the hypothalamic paraventricular nucleus, and randomly in the lateral hypothalamic area. The distribution of PrRP-containing fibers in the ventral subdivision of the DMH overlapped completely with the area that contained the Fos-immunoreactive neurons. It was shown that essentially all of these PrRP-ir fibers and terminals contained tyrosine-hydroxylase (TH), like PrRP cell bodies in the caudal part of the nucleus of the solitary tract, as well. In contrast, PrRP-ir cell bodies in the most caudal part of the DMH did not co-express TH suggesting that PrRP-TH double labeled fibers in the DMH are of medullary origin.
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After emanating from a portion of A2 noradrenaline cells situated in the caudal part of the commissural subdivision of the nucleus of the solitary tract, PrRP-ir fibers projected in rostral direction along the solitary tract in the medulla. They did not form a bundle but rather projected along larger fascicles. At the level of the pons, the fibers were along the superior cerebellar peduncle, then they joined the ventral noradrenergic bundle and entered the midbrain. Here, they turned ventrally and continued their way further rostrally between the medial lemniscus and the substantia nigra into the ventral tegmental area. In the lateral hypothalamus, the PrRP-immunoreactive fibers were situated among the fibers of the medial forebrain bundle. At the premamillary level of the hypothalamus, some of the fibers turned dorsomedially, entered the DMH, and terminated in the ventral subdivision of the nucleus. The other PrRP fibers in the medial forebrain bundle coursed further rostral towards the hypothalamic paraventricular nucleus, the bed nucleus of the stria terminalis, and the septal nuclei.

5.2.3. GLP-1-containing nerve fibers and terminals in the DMH

GLP-1 immunolabeling was detected in fibers and terminals in different parts of the hypothalamus including the dorsomedial, the arcuate, the paraventricular nuclei and some parts of the lateral hypothalamic area. The highest density of GLP-1-ir fibers was present in the DMH. However, the comparison of the sections with sections stained with Luxol fast blue cresyl violet indicated that GLP-1 fibers were distributed apparently and
almost exclusively in the ventral subdivision of the DMH. The topographical distribution of GLP-1-containing fibers in the ventral subdivision of the DMH clearly and entirely overlapped with the area that contained Fos-immunoreactive neurons in very high density in this nucleus.

5.3. The effect of pathway transection and lesion on the PrRP és GLP-1 fibers and Fos activation in the DMH

5.3.1. The effects of transection of PrRP fibers

Unilateral transections of the ascending solitary-hypothalamic pathway containing PrRP-containing fibers was performed at 2 different rostro-caudal levels using coronal knife cuts. Transections in the pons, as well as in the caudal part of the hypothalamus resulted in the accumulation of PrRP immunoreactivity immediately caudal to the knife cuts. Both transections elicited a marked decrease in the density of PrRP-containing fibers in the ventral subdivision of the DMH, as well as in other target areas of PrRP-containing fibers in the hypothalamus, ipsilateral to the knife cut. In contrast, we observed no change in the density of PrRP-ir fibers contralateral to the transections, as compared to intact refed animals. Parallel to the disappearance of PrRP-ir fibers, a dramatic decrease occurred in the density of Fos-expressing neurons following re-feeding ipsilateral to the transections. The number of Fos-ir neurons in response to re-feeding decreased significantly (p < 0.001) from 96.7±7.7 (intact rats) to 25.1±3.1 ipsilateral to the transection. This value is also markedly lower (p < 0.01) than the 79.2±4.7 in the contralateral side of the transected rats, which was only slightly reduced as compared to intact control rats.

5.3.2. GLP-1-containing medullary-hypothalamic pathway and effect of its transections on Fos activity in DMH neurons

GLP-1 neurons are located mainly in the caudal part of the nucleus of the solitary tract. Unilateral transections of the ascending solitary-hypothalamic pathway in the caudal pons using coronal knife cuts resulted a marked decrease in the density of GLP-1-containing fibers and terminals rostral to the cut, and an almost complete disappearance in the ventral subdivision of the DMH, ipsilateral to the transection. Parallel to the disappearance of GLP-1-ir fibers, a dramatic decrease occurred in the
density of refeeding-elicited Fos-expressing neurons in the DMHv: the number of Fos-ir neurons decreased to 26.7±3.4 (mean±SEM) ipsilateral to the transection compared to the 75.9±3.8 average value counted in the intact rats (p < 0.05). In contrast, we did not observe any visible changes in the density of GLP-1-ir fibers in the DMHv contralateral to the transaction. The number of Fos-positive cells was significantly higher contralateral than ipsilateral to the transaction (57.4±5.1 vs. 26.7±3.4, p < 0.05) although the contralateral value was slightly reduced as compared to that in the intact, unoperated refed rats.

5.3.3. GLP-1 receptors (GLP-1R) in the DMH

GLP-1Rs are abundant in the hypothalamus. Particularly high density of GLP-1R-expressing neurons was found in the ventral subdivision of the DMH but also in the arcuate and periventricular nuclei, as well within the posterior hypothalamic area. For demonstration of the presence of Fos activity in GLP-1 receptor expressing neurons in the dorsomedial nucleus, in situ hybridization for GLP-1R mRNA and immunostaining for Fos were applied. Immunohistochemistry for Fos revealed that 83.2±1.4% of GLP-1R mRNA-containing neurons expressed Fos in response to refeeding to satiation. Vice versa, 76.8±1.3% of Fos activated cells were labeled for GLP-1R mRNA.

5.3.4. The effect of MSG treatment on the Fos activation in the DMH

The early postnatal monosodium glutamate treatment led to the lesion and essential complete disappearance of neurons of the arcuate nucleus throughout its rostrocaudal axis, as demonstrated on Nissl-stained sections. Other hypothalamic nuclei, including the adjacent ventromedial nucleus did not show visible cytoarchitectural changes. Re-feeding of fasted MSG-treated animals resulted in the appearance of Fos-ir neurons in the ventral subdivision of the DMH, and their density was comparable to that in intact animals. MSG treatment had no effect on the number of re-feeding induced Fos-ir neurons. The number of Fos-ir neurons in response to re-feeding in MSG-treated rats was 90.3±6.8 versus 96.7±7.7 (controls).
5. Conclusions

- We reported that a high number of neurons expressed c-fos mRNA and protein in response to unlimited food intake in fasted rats in the DMH. In contrast, limited food consumption failed to induce Fos expression in DMH neurons suggesting that satiation should be one of the important signals that activate these neurons.

- Based on double staining of Fos and fluorescent Nissl stains, the neurons activated by satiation are specifically located in the ventral subdivision of the DMH suggesting that this part of the nucleus plays a role in mediating satiety.

- The possible origin of fibers conducting satiation signals to the DMH could be in the lower brainstem, especially in PrRP- and GLP-1-containing neurons in the nucleus of the solitary tract (NTS) whose fiber terminals topographically overlap with activated Fos-positive neurons in the DMH in re-fed rats.

- Unilateral transections of ascending solitary-hypothalamic fibers from the NTS inside the pons in re-fed rats (unlimited food consumption) resulted in a dramatic decrease in the density of PrRP and GLP-1 fibers ipsilateral to the transection. Contralateral to the transection, however, neither the GLP-1 nor the PrRP fiber density changed significantly. Meanwhile, the density of PrRP and GLP-1 immunoreactivity was markedly accumulated in transected nerve fibers caudal to the knife cuts, as a consequence of the interruption of the ascending transport route.

- In correlation with the decrease in the DMH levels of PrRP and GLP-1 fibers ipsilateral to the transection, the number of Fos-expressing neurons elicited by refeeding declined in the ipsilateral but remained high in the contralateral DMH suggesting that the solitary-hypothalamic projections may represent the neuronal route through which PrRP and GLP-1 neurons of the NTS activate DMH neurons by conveying information on satiety.

- In contrast, Fos response in the ventral DMH was not attenuated following chemical lesion (neonatal monosodium-glutamate treatment) of the hypothalamic arcuate nucleus, another possible source of DMH inputs.

- Using a combination of immunocytochemistry and in situ hybridization histochemistry, we demonstrated GLP-1 receptors in Fos-expressing neurons of the DMH suggesting that these receptors may play a role in the effect of GLP-1 on DMH neurons in response to satiation.
6. List of publications

6.1. Publications related to the theses

   Impakt faktor: **2,522**

   Impakt faktor: **3,658**

6.2. Abstracts related to the theses


medullary A1 and A2 cell groups. 8th IBRO World Congress of Neuroscience, Florence, Italy


6.3. Publications not related to the theses

   Impakt faktor: 7,837

   Impakt faktor: 3,730
6.4. Abstracts not related to the theses


6. Papp R.S., Ádori C., Balázs T., Könczöl K., Lourmet G., Renner E., Palkovits M. (2011) Functional heterogeneity of orexin-expressing neurons in the hypothalamus of rats. 8th IBRO World Congress of Neuroscience, Florence, Italy


mice during fasting and after refeeding. International IBRO Workshop, Budapest.
Clinical Neuroscience 59. Suppl. 1. 46.

Acknowledgements

First I would like to express my gratitude to my supervisor, Prof. Miklós Palkovits for his outstanding support and guidance during my work.

I would also like to thank to all members of the Laboratory of the Neuromorphology without whom this work could not have been completed. I thank for Judit Hellferich Frigyesné, who thought me histological techniques. I am grateful for Dr. Katalin Gallatz, Dr. Árpád Dobolyi, Dr. Zsuzsanna Tóth, Magdolna Toronyay-Kasztnner, Melitta Kiss, Melinda Cservenák, Rebeka Éva Szabó, Sugárka Rege Papp, Tamás Balázs, Dorottya Kézdi, Nikolett Hanák, Katalin Könczöl, Judit Kerti, Szilvia Deák, Viktória Dellaszéga-Lábas and Kinga Ibolya Szabó-Meltzer for their support and the atmosphere that characterized the Palkovits laboratory.

I also thank for Prof. András Csillag, the Head of the Department of Anatomy, Histology and Embryology that I could perform my work in the institute.

Finally, I am grateful to my family, especially to my husband, Árpád and our son Daniel and daughter Zsófia for their continuous love and patience I can experience day by day.