Arcuate nucleus revisited; the role of dopamine and leptin in physiological homeostasis

Manoj G Tyagi, Tarun Shah, Deepak G Shewade and Sumith K Mathew

Department of Pharmacology,Mahatma Gandhi Institute of Dental SciencesJaipur, Rajasthan and JIPMER, PondicherryIndia
Correspondence:Manoj G Tyagi, PhD

Abstract:*Arcuate nucleus (ARN) consisting of neurons is located in the hypothalamus and plays an important role in integrating the hormonal and neuronal signals. In females it seems to regulate the secretion of sex steroids and affect the physiological and metabolic processes. More specifically, injections of leptin or insulin into the ARN, or nonspecific activation of the ARN, increase sympathetic nerve activity (SNA) in part via suppressionof tonic NPY inhibition of PVN presympathetic neurons. Because the ARN is a primary site for metabolic integration and the coordination of metabolic, activity, and endocrine rhythms is essential for this function, it is likely that the ARNmay play an important role in the disruptive effects of desynchrony.Because the ARN is critical for the integration of signals important to feeding and metabolism, the ARN may also be critical for food anticipatory elevation of activity and temperature, which are integral to these functions. This review article investigates the role of ARN in locomotor, feeding behavior and hormonal regulation.*

Keywords: *Estrogens, arcuatenucleus, glucocorticoid receptor, obesity, negative feedback, paraventricular nucleus*

I. Introduction

Discrete brain regions and in particular the hypothalamic nuclei are connected neurally with specific areas of the brain in regulating intricate physiological functions some which have been delineated and others which have not been well understood. In the discrete brain sites, the arcuate nucleus (ARN) stands as a pivotal center in integrating and distributing the peripheral information from hormonal to neuronal signals that reflect the metabolic status (1-2).The ARN group of nerves is known to share extensive connections with the paraventricular nucleus and the lateral hypothalamic and perifornical areas are important pathways of neuropeptide transport for the control of energy balance (3-4) (Refer Fig.1). It has been shown that ARN neurons abundantly express estrogen receptor alpha (ERα) (5-6) that co-localizeswithneuropeptide Y (NPY) and POMC (7).Estradiol action through ERα regulates the expression of neuropeptides that affect food intake, body weight, and adiposity (8-10).It has been shown that, in the ARN, estradiol modulates feeding behavior by decreasing the levels of orexigenic and increasing the levels of anorexigenic peptides. Data from recent studies shows, for the first time with stereological methods, that EB prompts a reduction in the total number of ARN neurons that express NPY and, in parallel, an increase in the total number of neurons that express α-MSH (11). It is known that approximately 20% of NPY-ir neurons in the ARN accumulate estradiol and that the ERα subtype, whose activation inhibits NPY secretion, is predominantly related with NPY role in hypothalamic feeding circuits. It is also a fact that the increase in NPY levels in the ARN after the estradiol decline, occurring after ovariectomy or at menopause, is correlated with an increase in food consumption and in body weight gain (12).The microgliosisthatoccurs in the ARN nucleus is induced by the HF diet,thus indicating hypothalamus inflammation and consequent disruptionof the neuronal network involved in energy balance control andeven death of POMC neurons.It has also been demonstrated that ARN connections to anteroventralparaventricular nucleus (AVPV) regulate the central biological clock interactions with optimal reproductive functionality and kisspeptin neurons may contribute effectively in these processes (13). This review examines the role of ARN and its neural network in regulating feeding behavior, circadian rhythm, and hormonal homeostasis.

Arcuate Nucleus and Leptin:The homeostatic systems in the ARN have been proposed to regulate the drive to eat under conditions of food restriction or fasting, and are considered to be the molecular basis of the phenomenon of hunger. In the ARN nucleus, leptin activates the anorexigenic pathways andinduces a negative energy balance, but in obese animals this negativefeedback system is interrupted, resulting in central leptinresistance. One of the mechanisms underlying central leptin resistance ishyperleptinemia, which occurs in

---

obese animals, with a consequent attenuation of anorectic response to exogenous leptin. The expression of leptin receptors and leptin mRNAs is well documented in the mouse brain and notably in the main neurogenic niches, the subventricular zone of the lateral ventricles, and the dentate gyrus of the hippocampus. In homeostatic conditions, leptin inhibits food intake, and in extra-hypothalamic sites, leptin acts on neurogenesis, synaptogenesis, neuronal excitability, and neuroprotection (14). Leptin was also shown to improve cognition and mood in depressed and anxious animal models, notably by improving long-term potentiation. there are also published positive correlations between plasma levels of leptin and body weight (15-16).

Suprachiasmatic nucleus and connections to arcuate nucleus and influence on hormones : Many of the compounds also have a clear 24-hour rhythm directly or indirectly driven by the SCN with receptors in the ARC (17-18), indicating that the temporal ARC activation can also be promoted by humoral pathways or via the multisynaptic neuronal relays such as through the dorsomedial hypothalamic nucleus (19-20). An indication for alternative control mechanisms for Corticosteroid (Cort) secretion came after polysynaptic retrograde neuronal tracing from the adrenal cortex, identifying the first-order neurons in the intermediolateral column followed by neurons in the PVN, and third-order neurons in the arcuate nucleus (ARN). Based on these observations, it was hypothesized that the identified projections from the ARN to PVN neurons projecting to the adrenal gland may serve to integrate information about circulating Cort levels to fine-tune endocrine function (21). In the ARC, type I and type II glucocorticoid receptors are highly expressed (22). ARN type II glucocorticoid receptor concentration is one of the highest within the brain together with the hippocampus and PVN (23). In the ARN, two major subsets of neurons have been reported to be responsive to Cort with electrophysiological and transcriptional changes (24), as follows: one co-expresses the orexigenic agouti-related peptide (AGRP) and neuropeptide Y (NPY), while melanocyte stimulating hormone, an anorexigenic peptide, is present in the other population together with the cocaine- and amphetamine-related transcript (25). AVP release from the SCN starts during the late dark period and increases further at the beginning of the light phase. Conversely, recently it was observed that an opposite pattern of MSH neuronal activity with increased c-Fos expression during the dark phase. In addition, it has been demonstrated that alpha-MSH activity in the dark phase disappears after light exposure (26). These alternating patterns are in tandem with the temperature changes induced by alpha-MSH and AVP antagonists. This demonstrates that the circadian release pattern of AVP and alpha-MSH is related to the diurnal temperature modulations (Refer Fig.2 & 3).

Arcuate nucleus and glucose metabolism links with insulin hormone:

The hypothalamic ARN is also designated as infundibular nucleus in the human brain. Alterations in the concentrations of insulin and glucose modify the activity of several neuronal populations within these areas. These nutrient/hormone sensitive neurons project to pre-autonomic neurons located in the hypothalamus and brainstem and modulate the autonomic output to liver and pancreas. In both areas, neurons were identified that are either activated or inhibited by changes in glucose availability (27-28). At present, the ARN is mainly characterized by two antagonistic neuronal groups: the Neuropeptide Y (NPY) and proopiomelanocortin (POMC) expressing cells. In experimental animals, NPY neurons in the ARN are activated by negative metabolic challenges, such as fasting and hypoglycemia (29) and NPY signaling is required to stimulate hepatic glucose production in response to a decrease in plasma glucose levels (30). In the rodents it has been depicted that a variation in glucose and insulin signaling to NPY neurons of the ARN results in the generation of physiological responses that culminate in the regulation of hepatic glucose production and lipid metabolism via the autonomic system (31-33). Specifically, an increase in plasma glucose and insulin levels is followed by suppression of sympathetic activity to the liver resulting in lower glucose production. Type-2 diabetes patients, instead, display inappropriately high hepatic glucose production, in spite of their hyperglycemic, hyperinsulinemic condition. This high hepatic glucose production is shown to be caused by increased sympathetic tone, which stimulates both glycogenolysis and gluconeogenesis (34-35).

General procedure for Immunohistochemistry of the brain regions: Brains are excised and postfixed at room temperature for 4 h in 4.1 % formalin, and cryoprotected overnight in 25% sucrose solution. Coronal cryostat sections (30 μm) through the length of the Arc and SCN are mounted directly. Sections are processed using previously described immunohistochemical techniques. After pretreatment with 50% ethanol for 20 min, slides are washed (3 × 5 min) in 0.1 M Phosphate Buffer and incubated for 1 h in 10% normal horse serum. The blocking solution is removed, and the tissue are incubated in the primary antibody, a specific goat polyclonal antibody (Santa C 1:1,000), and made up in 8 % normal horse serum-TPBS. After 72 h, the primary antibody is removed, the sections washed (3 × 10 min) in TPBS, and then incubated in a secondary antibody (biotinylated anti-goat 1:500; Jackson ImmunoResearch Laboratories, West Grove, PA) made in 1% normal horse serum-TPBS. After 24 h, the slide is washed (3 × 10 min) in TPBS, incubated with extravidin-peroxidase (1:1,200 in TPBS; Sigma-Aldrich) for 3 h, washed again (3 × 10 minutes), and reacted for visualization of immunoreactivity using peroxidase reaction. Slides are than cover-slipped for microscopic evaluation (36).
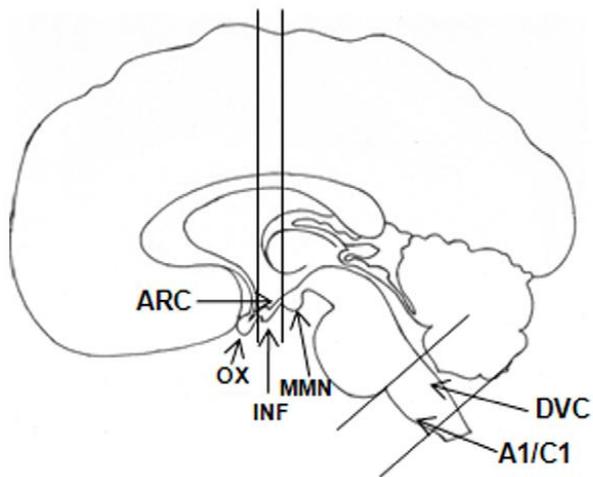
Fig.1 : Anatomical localization of the arcuate nucleus
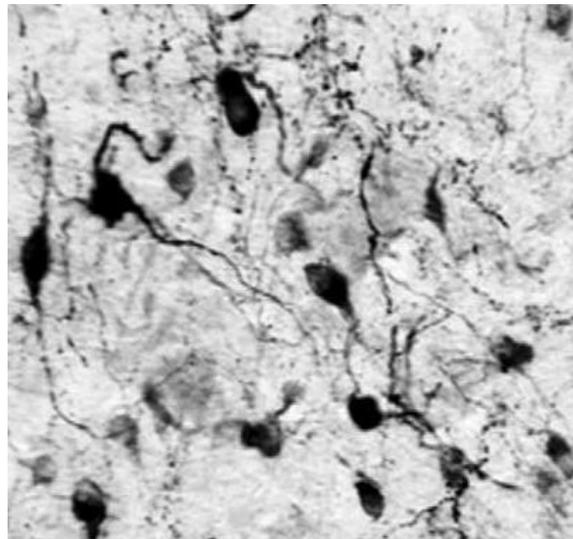
Fig.2 : Alpha MSH neurons seen in the arcuate nucleus
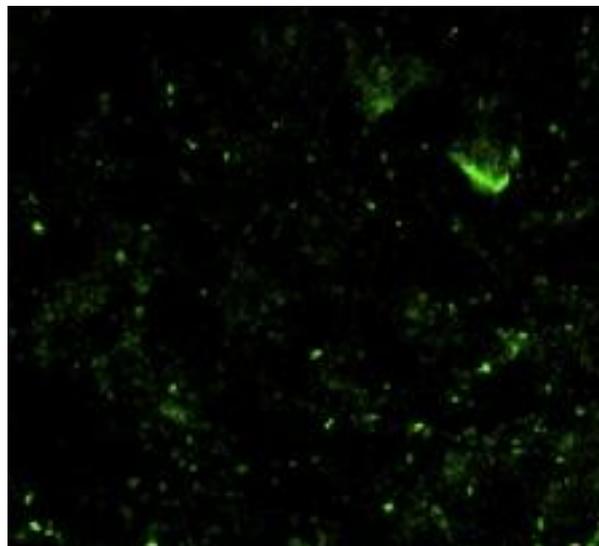
Fig.3: Alpha-MSH immunoreactivity in the arcuate nucleus of the rat

Link between Locomotor activity and feeding behavior; role of leptin and dopamine in the arcuate nucleus: Circadian rhythms in clock gene expression are observed in many brain regions including those with roles in motivational and emotional state, hormone release and feeding. The perception that feeding is a strong synchronizer as light/dark cycles relies on many empirical observations on laboratory rodents. Feeding can affect the clock controlled genes in peripheral oscillations via hypocaloric diets, glucose, insulin etc,. The fact that ARN is connected to SCN, and other regions in the hypothalamus and the brain *per se* and via the spinal cord translates oscillating neural signals from the master clock to various neural and humoral signals that can reach the tissues and organs across the body. Does an increase in locomotor activity affect the feeding behavior in animals and human and if it does, than what is the role of leptin and dopamine in the arcuate nucleus and its interaction with other areas such as the suprachiasmatic nucleus. ARN contains two distinct neuronal populations, proopiomelanocortin/cocaine and amphetamineregulated transcript-containing neurons (POMC/CART neurons) and neuropeptide Y/agouti-related peptide containing neurons (NPY/AGRP neurons) (37-39). Activation of POMC/CART neuronsresults in suppression of appetite (anorexigenic) and increase in energy expenditure and activation of NPY/AGRP neurons results in hyperphagia (orexigenic) and suppression of energy expenditure. Retrograde trans-synaptic tracing studies using pseudorabies virus have shown an anatomical link of the hypothalamic ARN, PVN, and DMN nuclei tointerscapular BAT (IBAT) (40-41). Lesions induced in the ARN block the feeding and anorexic effects of exogenous leptin, produce severe hyperphagia and obesification (42), produce rest-activity disturbances, and result in circadian arrhythmia for ad libitum feeding. Diet-induced obesity decreases food anticipatory activity, suggesting that the ARN leptin/NPY circuits are critical to the anticipation of scheduled food availability, inhibiting activity when leptin levels are increased by obesity and facilitating activity when leptin signaling is absent (43-44). It appears that the increased dopaminergic turnover in ARN may affect the cardiovascular activity and alter locomotor and feeding behaviour. There is also a possibility of zeitgeber neural cells which may be interacting with arcuate nucleus.

II. Conclusions

The ARN in conjunction with neural connections to discrete brain regions seems to be the regulator of hormonal secretion, locomotor and feeding behaviour and it appears that biological clock and circadian rhythms manifest these actions and with ARN centrally at the helm of affairs.

Acknowledgements

References

[1]   Buijs JS, MG Ruiz, LL Mercado, MC Basualdo, C Escobar, AKalsbeek, RM Buijs. Suprachiasmatic nucleus interaction with arcuate nucleus; essential for organizing physiological rhythms. eNeuro. 2017, 4(2)0028

[2]   Kawabe T, Kawabe K, and Sapru HN. Cardiovascular responses to chemical stimulation of the hypothalamic arcuate nucleus in the rat; role of the hypothalamic PVN. Plos one, 2012, 7 (9), e 43180

[3]   Mene´ndezJA, McGregor IS, Healey PA, Atrens DM, Leibowitz SF (1990). Metabolic effects of neuropeptide Y injections into the paraventricular nucleus ofthe hypothalamus. Brain Res 516(1): 8–14.

[4]   Ritter RC, Slusser PG, Stone S (1981) Glucoreceptors controlling feeding andblood glucose: location in the hindbrain. Science 213(4506): 451–2.

[5]   Clegg DJ, Brown LM, Zigman JM, Kemp CJ, Strader AD, Benoit SC, et al. Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats. Diabetes. 2007;56:1051–8.

[6]   Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An *in situ* hybridization study. J Comp Neurol. 1990;294:76–95.

[7]   Pilcher WH, Joseph SA, McDonald JV (1988) Immunocytochemical localizationof pro-opiomelanocortin neurons in human brain areas subserving stimulationanalgesia. J Neurosurg 68(4): 621–9.

[8]   Shughrue PJ, Komm B, Merchenthaler I. The distribution of estrogen receptor-beta mRNA in the rat hypothalamus. Steroids. 1996;61:678– 81.

[9]   Hirosawa M, Minata M, Harada KH, Hitomi T, Krust A, Koizumi A. Ablation of estrogen receptor alpha (ERα) prevents upregulation of POMC by leptin and insulin. BiochBiophy Res Communic. 2008;371:320–3

[10]   Geary N, Asarian L, Korach KS, Pfaff DW, Ogawa S. Deficits in E2-dependent control of feeding, weight gain, and cholecystokinin satiation in ER-alpha null mice. Endocrinology. 2001;142:4751–7.

[11]   Sar M, Sahu A, Crowley WR, Kalra SP. Localization of neuropeptide-Y immunoreactivity in estradiol-concentrating cells in the hypothalamus. Endocrinoloy. 1990;127:2752–6.

[12]   Bauer-Dantoin AC, Urban JH, Levine JE. Neuropeptide Y gene expression in the arcuate nucleus is increased during preovulatory luteinizing hormone surges. Endocrinology. 1992;131:2953–8.

[13]   M Putteeraj, T Soga, T Ubuka, IS Parhar.A "timed' Kiss is essential for reproduction: Lessons from mammalian studies. Front.Endocrinol. (Lausanne), 2016; 7: 121

[14]   Pocai A, Obici S, Schwartz GJ, Rossetti L (2005) A brain-liver circuit regulatesglucose homeostasis. Cell Metab 1(1): 53–61.

[15]   Smith JA, Das A, Ray SK, Banik NL. Role of pro-inflammatory cytokines releasedfrom microglia in neurodegenerative diseases. Brain Res Bull 2012;87:10-20.

[16]   Dube MG, Xu B, Kalra PS, Sninsky CA, Kalra SP. Disruption in neuropeptide Y and leptin signaling in obese ventromedial hypothalamic-lesioned rats. Brain Res. 1999;816:38–46.

[17]   Balfour RH, Hansen AM, Trapp S (2006) Neuronal responses to transienthypoglycaemia in the dorsal vagal complex of the rat brainstem. J Physiol 570 (Pt3): 469–84.
[18]   Han F, Ozawa H, Matsuda K, Nishi M, Kawata M (2005) Colocalization of mineralocorticoid receptor and glucocorticoid receptor in the hippocampus and hypothalamus. Neurosci Res 51:371–381
[19]   Nishi M, Tanaka M, Matsuda K, Sunaguchi M, Kawata M (2004) Visualization of glucocorticoid receptor and mineralocorticoid receptor interactions in living cells with GFP-based fluorescence resonance energy transfer. J Neurosci 24:4918–4927.
[20]   MG Tyagi and Divya V. Central nervous regulation of spleen function: New insights from animal studies. Int.Jr.Appl.Biol.Pharma.Tech. 3, 2, 162-168, 2012
[21]   Morimoto M, Morita N, Ozawa H, Yokoyama MK, Morimoto M, Morita N, Ozawa H, Yokoyama K, Kawata M (1996) Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: an immunohistochemical and in situ hybridization study.Neurosci Res 26:235–269.
[22]   Buijs RM, Escobar C (2007) Corticosterone and activity: the longarms of the clock talk back. Endocrinology 148:5162–5164.
[23]   Guzma´n-Ruiz M, Saderi N, Cazarez-Ma´rquez F, Guerrero-Vargas NN, Basualdo MC, Acosta-Galva´n G, Buijs RM (2014) The suprachiasmatic nucleus changes the daily activity of the arcuate nucleus alpha-MSH neurons in male rats. Endocrinology 155:525–535
[24]   Shimuzu H, Arima H, Watanabe M, Goto M, Banno R, Sato I, Ozaki N, Nagasaki H, OisoY.Glucorticoids increase NP-Y and agouti related peptide gene expression via adenosine monophosphate activated protein kinase signalingin the arcuate nucleus of rats. Endocrinology 149: 4544-4553
[25]   Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melacortinergic neurons in feeding and the agouti obesity syndrome. Nature 385: 165-168
[26]   Oomura Y, Ono T, Ooyama H, Wayner MJ (1969) Glucose and osmosensitiveneurones of the rat hypothalamus. Nature 222(5190): 282–4.
[27]   Akabayashi A, Zaia CT, Silva I, Chae HJ, Leibowitz SF (1993) Neuropeptide Yin the arcuate nucleus is modulated by alterations in glucose utilization. BrainRes.621(2): 343–8.
[28]   Boden G, Chen X, Stein TP (2001) Gluconeogenesis in moderately and severelyhyperglycemic patients with type 2 diabetes mellitus. Am J PhysiolEndocrinolMetab 280(1): 23–30.
[29]   Mercer JG, Lawrence CB, Beck B, Burlet A, Atkinson T, Barrett P. Hypothalamic NPY and prepro NPY mRNA in Djungarian hamsters: effects of food deprivation and photoperiod. Am.J.Physiol. 1995; 269: R1099-R1106
[30]   Marks JL, Waite K (1996) Some acute effects of intracerebroventricularneuropeptideY on insulin secretion and glucose metabolism in the rat.J Neuroendocrinol 8(7): 507–13.
[31]   Paz-Filho G, Wong ML, Licinio J. The procognitive effects of leptin in the brain and their clinical implications. Int J ClinPract. 2010;64:1808–12.
[32]   Fleisch AF, Agarwal N, Roberts MD, Han JC, Theim KR, Vexler A, *et al*.Influence of serum leptin on weight and body fat growth in children at high risk for adult obesity. J ClinEndocrinolMetab. 2007;92:948–54.
[33]   White JD, Kershaw M (1990) Increased hypothalamic neuropeptide Yexpression following food deprivation. Mol Cell Neurosci 1(1): 41–8.
[34]   Basu R, Basu A, Johnson CM , Schwenk WF, Rizza R (2004) Insulin dose responsecurves for stimulation of splanchnic glucose uptake and suppression of endogenous glucose production differ in nondiabetic humans and are abnormal in people with type 2 diabetes. Diabetes 53(8): 2042–50.
[35]   Mok JS, JS Hutchinson, MK Sim. Effects of dopamine on blood pressure and heart rate after microinjection into the hypothalamus of spontaneously hypertensive and normotensive Wistar Kyoto rats. Clin.Exp.Hypertens.; 12(1): 15-36
[36]   Cano G, Passerin AM, Schiltz JC, Card JP, Morrison SF, SvedAF.Anatomical substrates for the central control of sympathetic outflow tointerscapular adipose tissue during cold exposure. *J Comp Neuro.l*460:303–326, 2003.
[37]   Li C, Chen P, Smith MS. Corticotropin releasing hormone neurons in the paraventricular nucleus are direct targets for neuropeptide Y neurons in the arcuate nucleus: an anterograde tracing study. Brain Res. 2000;854:122–9.
[38]   Olofsson LE, Unger EK, Cheung CC, Xu AW. Modulation of AgRP-neuronal function by SOCS3 as an initiating event in diet-induced hypothalamic leptin resistance. Proc.Natl.Acad.Sci USA 2013;110:E697-E706
[39]   Sukhov RR, Walker LC, Rance NE, Price DL, Young W (1995) Opioidprecursor gene expression in the human hypothalamus. J Comp Neurol 353(4):604–22.
[40]   Thaler JP, Yi CX, Schur EA, *et al*. Obesity is associated with hypothalamic injuryin rodents and humans. J Clin Invest 2012;122:153-162
[41]   L Paeger,IKarakasilioti, J Altmuller, P Frommolt, J Bruning, P Kloppenburg. Antagonistic modulation of NPY/AgRP and POMC neurons in the arcuate nucleus by noradrenalin. Elife.2017; 6: e25770
[42]   Bouret SG. Neurodevelopmental actions of leptin. Brain Res. 2010;1350:2–9.
[43]   Kalra SP, Bagnasco M, Otukonyang EE, Dube MG, Kalra PS. Rhythmic reciprocal ghrelin and leptin signaling; new insight in the development of obesity. Regul.Pept. 111: 1-11, 2003
[44]   Satoh N, Ogawa Y, Katsuura G, Hayase M, Tsuji T, Imagawa K, et al. The arcuate nucleus as a primary site of satiety effect of leptin in rats. Neurosci Letters. 1997;224:149–52.