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NEÜ YAYINEVİ

# Evaluation of Lateral Hypothalamic Area Catecholamine Levels Following Intravenous Glucose Administration by Microdialysis Method in Rats

## *Sıçanlarda İntravenöz Glikoz Uygulaması Sonrası Lateral Hipotalamik Alan Katekolamin Düzeylerinin Mikrodiyaliz Yöntemi ile Değerlendirilmesi*

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### Öz

**Amaç:** Lateral hipotalamik alanda (LHA) intravenöz glukoz uygulanmasının noradrenalin, dopamin ve metabolitleri olan dihidroksi fenil glikol (DHPG)- dihidroksifenilasetik asit (DOPAC) düzeylerine etkilerinin beyin mikrodiyaliz yöntemi ile araştırılması amaçlandı.
**Gereçler ve Yöntem:** Normal beslenen 2 grup ve 24 saat besin alımı kısıtlanan 2 grup (serum fizyolojik ve glikoz uygulanan) yetişkin erkek Wistar albino sıçan kullanıldı. Sıçanlara anestezi altında LHA'da mikrodiyaliz işlemleri yapıldı ve örnekler 20'şer dakikalık sürelerde toplandı. İlk örnekler kontrol olarak kaydedildikten sonra, kontrol gruplarına serum fizyolojik, glikoz gruplarına %50'lik glikoz çözeltisi 1.4ml/kg dozunda intravenöz yolla uygulandı. Sonraki 40 dakika boyunca örnekler toplanarak HPLC-ECD sisteminde analiz edildi. İstatiksel değerlendirme için tek yönlü ANOVA kullanıldı.
**Bulgular:** Başlangıçta noradrenalin konsantrasyonu, aç sıçanlarda tok olanlara göre daha yüksek bulunurken 20. dakikadaki noradrenalin seviyeleri tok ve aç grupta kontrol grubuna göre anlamlı olarak azaldı (p=0.01). 40. dakika noradrenalin değerlerinde ve dopamin-DHPG-DOPAC seviyeleri ile kontrol seviyeleri karşılaştırılmasında istatistiksel olarak anlamlı bir fark elde edilmedi.
**Sonuç:** Sistemik glukoz uygulaması aç ve tok sıçanlarda LHA noradrenalin konsantrasyonunu azaltmıştır. Bu sonuçlara göre LHA'daki noradrenerjik nörotransmisyon, plazma glukozu ile modüle edilebilir.

**Anahtar Kelimeler:** Lateral hipotalamik alan, noradrenalin, dopamin, HPLC, mikrodiyaliz, besin alımı

### Abstract

**Aim:** We aimed to investigate the effects of intravenous glucose administration in the lateral hypothalamic area (LHA) on the levels of noradrenaline, dopamine and their metabolites dihydroxyphenylglycol (DHPG)-dihydroxyphenylaceticacid (DOPAC) by brain microdialysis method.
**Materials and Methods:** Adult male Wistar albino rats in 2 normally fed groups and 2 groups of restricted food intake for 24 hours (saline and glucose administered) were used. Microdialysis procedures were performed on the rats in LHA under anesthesia and samples were collected in 20 minutes. After the first samples were recorded as control, 0.9% saline was administered to the control groups and 50% glucose solution was administered intravenously to the glucose groups at a dose of 1.4 ml/kg. During the next 40 minutes, samples were collected and analyzed on the HPLC-ECD system. One-way ANOVA was used for statistical evaluation.
**Results:** Noradrenaline concentration was higher in fasted rats than in satiated animals at baseline. Noradrenaline levels at the 20th minute were significantly decreased in both fasted-satiated groups compared to control group (p=0.01). There was no statistically significant difference in the 40th minute noradrenaline values and dopamine-DHPG-DOPAC levels compared to control.
**Conclusion:** Systemic glucose administration decreased LHA noradrenaline concentration in fasted and satiated rats. It can be mentioned that noradrenergic neurotransmission in LHA can be modulated by plasma glucose.

**Key words:** Lateral hypothalamic area, noradrenaline, dopamine, HPLC, microdialysis, food intake

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## INTRODUCTION

Nutrition and metabolic events are of great importance for the survival of living things (1). Regulation of food intake is one of the most complex regulatory mechanisms in the organism (2). The most important neural centers that regulate the amount of nutrients to be taken into the body and appetite are located in the hypothalamus (3). So it is a common coordinator in the central nervous system, in which feeding behaviors and nutritional processes are controlled. The paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), arcuate nucleus (ARC) which are the nuclei of the hypothalamus and lateral hypothalamic area (LHA) play a role in regulating food intake (4, 5) ARC is the energy core that plays a role in the regulation of nutritional intake and energy metabolism of the hypothalamus and the perception and evaluation of energy signals (6, 7). The neuron groups of ARC origin are called orexigenic (increasing nutrient intake) and anorexigenic (reducing nutrient intake). There are many orexigenic and anorexigenic effective molecules that play a role in energy balance, central and peripheral control of nutrition. Orexigenic peptides are neuropeptide Y (NPY), agouti related peptide (AgRP), ghrelin, orexin A and B, melanin condensing hormone (MCH). Anorexigenic peptides are leptin, cocaine amphetamine-associated peptide (CART), cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) (2, 8).

LHA acts as a hunger center. LHA is a nutritional center that contains glucose sensitive neurons stimulated through hypoglycemia and plays an important role in hypoglycemia-induced hyperphagia. LHA lesions cause hypophagia and weight loss (9). Dopamine (DA) and noradrenaline (NA) systems originating from ventral tegmental area and locus seruleus are related to the forebrain. DA and NA are the most important neurotransmitters involved in the regulation of food intake (10, 11) Its effect on food intake in DA is related to the desire to eat (5). DA release is triggered in relation to the rate of human beings want to eat their own. DA is also concerned with demonstrating the behaviors necessary to supply and consume nutrients (10). Chemical and electrical lesions in noradrenergic fibers from the hypothalamus line lead to overeating and obesity. Administration of NA into the rat periphornical hypothalamus reduces food intake. These results show that noradrenaline may play a role in satiety (5).

Obesity occurs when the amount of energy taken with nutrients is higher than the amount consumed with metabolism and physical activity and must be treated. The increase in consumption of high-calorie foods and beverages, as well as a general decrease in physical activity, facilitated the development of obesity and obesity has become a rapidly increasing health problem. Diseases such as coronary heart disease, hypertension, type 2 diabetes and cancer have increased due to weight gain (5). Due to excessive weight loss (anorexia), some types of cancer, such as inflammatory bowel diseases, or viruses that cause the immune system to collapse in humans, are caused by extremely debilitating diseases (10).

The detailed mechanisms that play a role in the regulation of food intake are important for the development of effective treatment methods of diseases such as obesity-anorexia. Some areas, especially in the hypothalamus, play an important role in nutritional behavior and metabolic activities. LHA is known as starvation center and VMN is known as satiety center. While electrical stimulation of LHA induces hunger and food intake in experimental animals, stimulation of VMN results in a feeling of satiety and termination of nutrition (12).

Microdialysis studies on the effects of catecholamines on LHA are very limited. NA application to LHA in rats induced food intake. Adrenaline and DA administration did not affect food intake behavior (13). It is not known what kind of changes food intake creates on catecholamines in hypothalamic LHA. Concentration changes at the level of noradrenaline and dopamine in LHA during food intake may initiate or terminate the feeling of hunger in experimental animals.

The aim of our study was to demonstrate how catecholamine levels in LHA change before and after administration by simulating food intake through intravenous (iv) glucose loading in fed and fasted animals and contributing to understanding the importance of catecholaminergic innervation in the hunger center.

## MATERIALS AND METHODS

The protocols of animal experiments were approved by the Local Ethics Committee of Application and Research Center of Experimental Medicine, (2014-040). Adult (12 months old, 350-400 grams) male Wistar Albino rats were used for the study. A total of 40 rats were randomly divided into 4 groups. 2 groups of normal feeding (n=10) and 2 groups of restricted feeding for 24 hours (n=10). Animals were

given intraperitoneal 50 mg/kg ketamine (ketamine hydrochloride 50 mg / ml: Ketas, Pfizer) for anesthesia. The depth of anesthesia and reflex responses were checked by looking at the painful stimulus response, and an additional dose of anesthetic was given if necessary.

After anesthesia, the experimental animals were fixed to the stereotaxy instrument (RWD Life Science CHINA). The microdialysis probe (CMA 12, CMA microdialysis, Sweden) was vertically implanted into the right holder of the sterotaxic device. Antero-posterior and medio-lateral coordinates of the marked bregma point were determined. The antero-posterior (1.80 mm) medio-lateral coordinates obtained from the rat brain atlas (-1.90 mm) were marked on the right temporal bone with reference to the bregma point on the right temporal bone. The microdialysis probe was inserted into the guide cannula and the probe tip with a 1 mm perfusion membrane was contacted to the LHA. According to the coordinates in the Atlas, the right LHA point (8.2 mm if the protective cannula is referenced, 9.2 mm if the probe is referenced) was entered into the brain tissue in the vertical direction and the right lateral hypothalamic area was reached. The microdialysis process started with the insertion of the guide cannula and the probe into it. By the manual glucometer (Optium Xceed), blood glucose values were determined 20 and 40 minutes after administration by taking one drop of blood from all animals just before and after iv PSS and glucose administration. The supernatant collected in the first 20 minutes after the 60th minute was considered as the 0th sample. After the supernatants collected in the next 40 minutes period were accepted as the control group, a 50% glucose solution (14) was infused into the glucose group and the other group was subjected to a saline infusion at a dose of 1.4 ml/kg in the 40th minute. The subsequent microdialysis periods were also injected into the HPLC system as samples 1, 2 and 3 for 20 minutes, and catecholamine analyzes were performed.

***Catecholamine Analysis in HPLC System***

Agilent Technologies 1260 brand high pressure liquid chromatography (HPLC) was used for catecholamine analysis (250x4.6mm C18 ODS analytical HPLC column). HPLC analysis range is set to 5. The temperature of the column oven was fixed at 40°C. Flow rate in HPLC was set to 1ml/min. Electrochemical Detector Waters 2465 was used. Injections were carried out in a volume of 20 µl (Hamilton). Before the microdialysis samples were analyzed for the experiments, standard curves were prepared for each catecholamine. Curves were drawn by applying 5 different doses between DHPG 0.1-10 ppb / 20µl, NA 0.1-10 ppb / 20µl, DOPAC 0.1-10 ppb / 20µl, DA 0.1-10 ppb / 20µl.

***HPLC Images of Catecholamines***

The administrations at the doses specified on the table were applied for all standards and the areas revealed in HPLC were calculated by the Agilent HPLC software program. The concentration of the samples whose areas were calculated was found by the formula y= 90,976x-16,471 in ppb for each catecholamine. By writing the area values where y is located, x concentration amount was calculated (Fig 1. and Fig 2.)

***Statistical Analysis***

Before intravenous administration, the DHPG, NA, DOPAC, DA levels of each animal were measured as '0' and the values of '0' were accepted as 100% on average. After iv glucose and PSS infusions of the samples, statistical analysis was performed by normalizing the catecholamine values according to the values of '0' in their group. SPSS 20.0 package program was used during the analyzes and the descriptive statistics of the variables were expressed as arithmetic mean (AM) ± standard error (SE). Normal distribution control was not performed because of the low number of observations. Nonparametric methods were preferred for the analyzes. Mann-Whitney

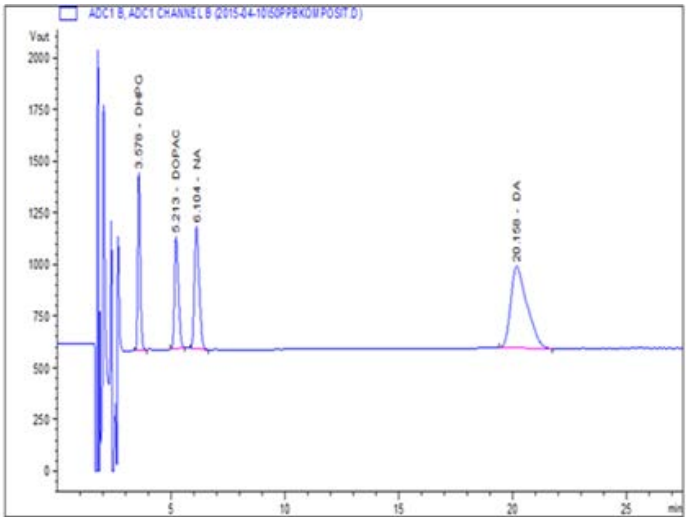


**Figure 1.** Standard catecholamine 50 ppb composite traces. 2-4. min. solvent front between, 3.578 min. DHPG, 5.213 min. DOPAC, 6.104 min. NA and 20.158 min. Peaks of DA

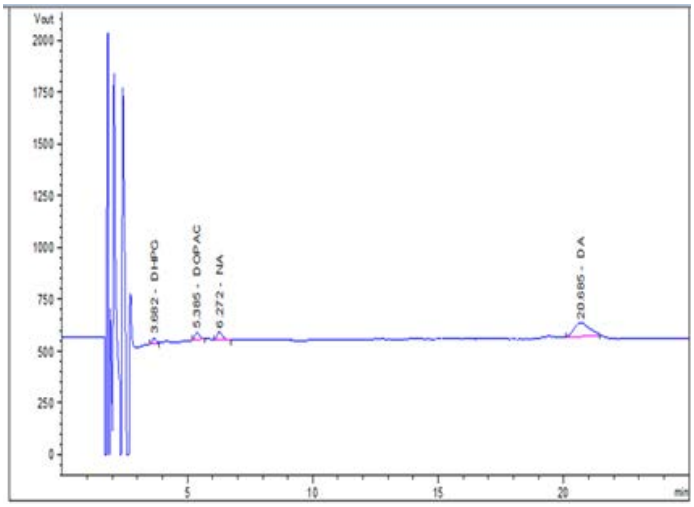


**Figure 2.** Catecholamine traces in HPLC of samples obtained by microdialysis

U test was used for the independent two-group comparisons, and Friedman's two-way analysis of variance was used to compare multiple groups with repeated measures. In cases where the overall result was significant, binary comparisons were made. Meaningful results were visualized with tables and related graphics. In all analyzes, $p<0.05$ value was accepted as statistically significant.

## RESULTS

### *Evaluation of Blood Glucose Levels*

The blood glucose levels of the rats were measured by a glucometer (Optium Xceed) from the rat tails. During the experiment, the blood glucose levels of the fasted and fed animals were measured at 0 minutes before application and at 20th minutes and 40th minutes after glucose and PSS adaptation. While blood glucose levels did not change in PSS group, it increased in the glucose group (Table 1).

**Table 1.** Evaluation of Blood Glucose Levels

| Groups | Blood Glucose Levels |
|---|---|
| Fasted / Saline AM±SE | 67.96±2.69 |
| Fasted / Glucose AM±SE | 173.43±13.98 |
| Satiated / Saline AM±SE | 133.53±2.85 |
| Satiated / Glucose AM±SE | 209.30±10.45 |

### *Monoamine Findings*

Absolute catecholamine values at 0, 20th and 40th minutes calculated as a result of the analysis of microdialysis supernatants obtained from experiments in HPLC are shown in the table as arithmetic mean (AM) ± standard error (SE). As a result of PSS and glucose administration in fasted and satiated animals, the percentage change rates determined by normalizing them according to the values of '0' in their group are specified. As a result of the statistical evaluation of the catecholamines with percent change values, no statistically significant difference was observed in the rats left for 24 hours when iv PSS was applied to LHA when the first 20 minutes and 40 minutes were compared with the NA values in the control group ($p>0.05$), (Fig 3). When compared with the control NA values in the LHA and 50% glucose

**Table 2.** Catecholamine levels in the lateral hypothalamic area

| Groups | Control (0. Min) (AM±SE) | 20. min. (AM±SE) | 40. dak (AM±SE) | p değeri |
|---|---|---|---|---|
| Fasted PSS NA | 0.50±0.16 | 0.69±0.40 | 0.51±0.23 | p=0.325 |
| Fasted Glucose NA | 0.80±0.33 | 0.39±0.17 | 0.48±0.18 | p=0.013* |
| Satiated PSS NA | 0.38±0.12 | 0.44±0.26 | 0.17±0.79 | p=0.846 |
| Satiated Glucose NA | 0.38±0.14 | 0.18±0.17 | 0.07±0.04 | p=0.06 |
| Fasted PSS DHPG | 0.82±0.14 | 0.30±0.18 | 0.3±0.29 | p=1.00 |
| Fasted Glucose DHPG | 0.31±0.16 | 0.32±0.24 | 0.32±0.30 | p=0.71 |
| Satiated PSS DHPG | 0.34±0.19 | 0.36±0.25 | 0.34±0.20 | p=0.68 |
| Satiated Glucose DHPG | 0.32±0.03 | 0.34±0.28 | 0.32±0.32 | p=0.71 |
| Fasted PSS DA | 0.09±0.4 | 0.18±0.12 | 0.09±0.66 | p=0.07 |
| Fasted Glucose DA | 0.10±0.04 | 0.34±0.15 | 0.21±0.10 | p=0.15 |
| Satiated PSS DA | 0.07±0.01 | 0.05±0.04 | 0.36±0.36 | p=0.36 |
| Satiated Glucose DA | 0.11±0.06 | 0.06±0.03 | 0.04±0.03 | p=0.89 |
| Fasted PSS DOPAC | 0.30±0.54 | 0.25±0.88 | 0.36±0.10 | p=0.67 |
| Fasted Glucose DOPAC | 0.62±0.26 | 0.54±0.27 | 1.18±0.62 | p=0.49 |
| Satiated PSS DOPAC | 0.27±0.72 | 0.27±0.67 | 0.32±0.16 | p=0.68 |
| Satiated GlucoseDOPAC | 0.25±0.85 | 0.21±0.71 | 0.34±0.14 | p=0.36 |

Abbreviations: DA: Dopamine, DHPG: Dihydroxyphenylglycol, NA: Noradrenaline, PSS: Physiological saline solution
Mann-Whitney U test, *($p<0.05$),

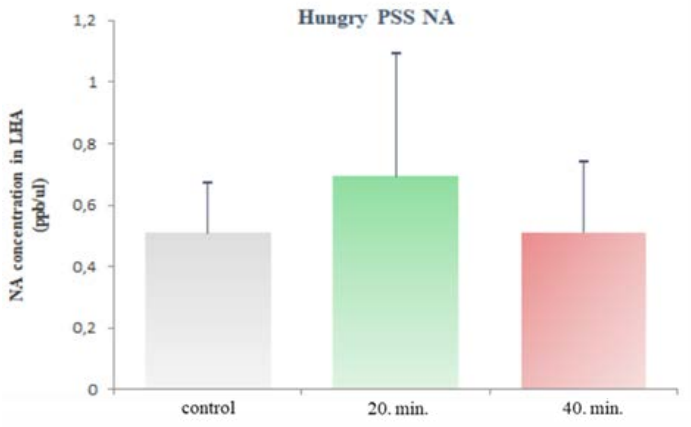


**Figure 3.** NA concentration change in LHA by iv PSS administration in the fasted animals (Control= 0. minute, 20 minutes after iv saline administration, 40. minute), (p = 0.325)

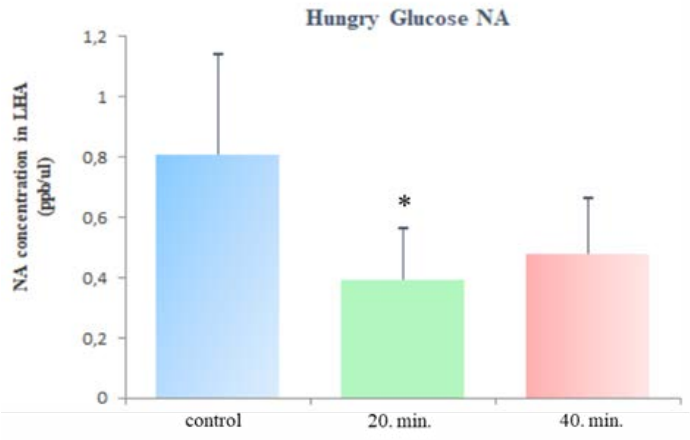


**Figure 4.** NA concentration change in LHA by iv glucose administration in fasted animals (Control= 0. minute, 20 minutes after iv glucose administration, 40. minute), (p <0.013)

from the tail vein in the first 20 minutes and 40 minutes after the administration of 50% glucose in the rats left for 24 hours, a statistically significant decrease in the NA level was observed in the first 20 minutes after the glucose administration (p>0.01) (Fig 4). No statistical significance was found in satiated animals (Fig 5-6), table 2. According to this result, administering glucose in fasted animals by IV route affects the level of NA in LHA. There was no statistical significance in DHPG values in fasted and satiated animals (p>0.05). In DA levels, although there was a little decrease in fasted animals compared with satiated animals, no statistically significant change was observed (p>0.05) (Table 2).

***DHPG Levels After Physiological Saline Solution and Glucose Administration in Fasted and Satiated Animals***

The animals fasted for 24 hours underwent brain microdialysis under anesthesia at 09.30 am. When intravenous physiological saline solution (PSS)

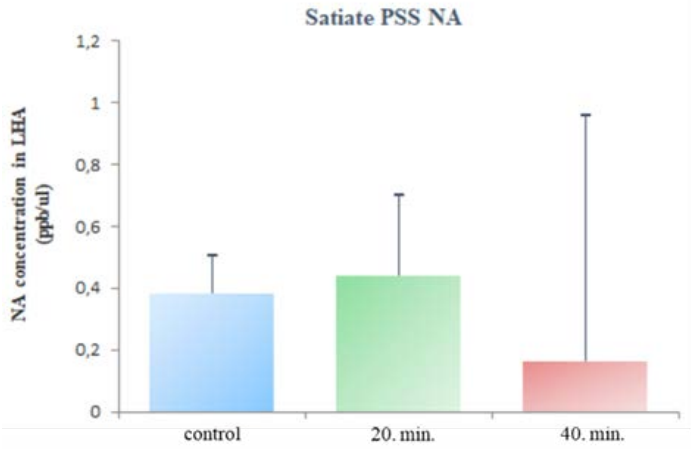


**Figure 5.** NA concentration change in LHA with ıv PSS administration in satiated animals (Control= 0. minute, 20 minutes after iv PSS administration, 40. minute), (p = 0.846)

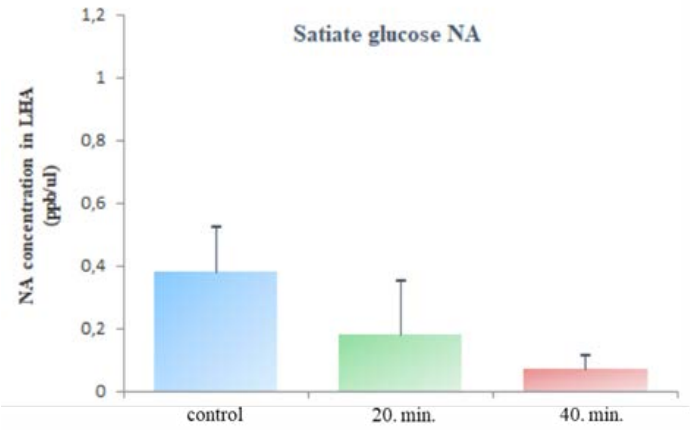


**Figure 6.** NA concentration change in LHA with iv glucose administration in satiated animals (Control= 0. minute, 20. minutes after iv glucose administration, 40. minute), (p = 0.06)

administration from tail vein was compared with the first 20th minute and 40th minute DHPG values and the DHPG value in the control group, there was no statistically significant difference was observed (p=1.00). When the first 20 minutes and 40 minutes of DHPG values were compared in the glucose treated group, no statistically significant difference was observed after glucose administration (p=0.71). When the same process is applied to satiated animals kept under normal feeding conditions, DHPG levels in PSS group (p=0.68) and DHPG levels in the glucose group (p=0.71) were not statistically significant (p>0.05).

***DA and its metabolite DOPAC Levels as a Result of SF and Glucose Administration in Fasted and Satiated Animals***

Animals fasted for 24 hours were subjected to brain microdialysis under anesthesia at 09.30 am. A statistically significant difference was not observed when the DA and its metabolite DOPAC values in the first 20th and 40th minutes with iv SF administration through the tail vein and the value in the control group (p=0.74), (p=0.67). When DA and its metabolite DOPAC values were compared in the first 20th and 40th minutes after glucose administration in the iv glucose group, no statistically significant difference was observed after glucose administration (p=0.15), (p=0.49).

When the same procedure was applied to satiated animals kept under normal feeding conditions, the levels of DA and its metabolite DOPAC in the SF group (p=0.36), (p=0.68), and the levels in the glucose group (p=0.89) (p=0.36) were not found to be statistically significant. Although DA levels decreased slightly in the glucose injected group in fasted animals compared to satiated animals, no statistically significant change was observed (p>0.05).

## DISCUSSION

The most important neural centers that regulate the amount of nutrients to be taken into the body and appetite are located in the hypothalamus. In humans and animals, the ventromedial nucleus is known as the center of satiety, while the lateral hypothalamic area is defined as the center that receives the hunger signals. The hypothalamus is the most critical region involved in nutrition and body weight regulation (3). ARC is the energy nucleus that plays a role in the regulation of nutritional intake and energy metabolism of the hypothalamus and the perception and evaluation of energy signals (15, 6). The α-melanin-stimulating hormone precursor, known for its ARC, NPY, AgRP and appetite suppressing action contains the population of neurons expressed in proopiomelanocortin (POMC) (2). Of these, NPY/AgRP neurons are orexigenic effective, while POMC/CART neurons are anorexigenic effective. NPY/AgRP and POMC/CART neurons are projected from ARC to other regions of the hypothalamus, PVN, DMN, VMN and LHA (16).

LHA is a nutritional center containing glucose sensitive neurons stimulated through hypoglycemia (9). A drop in blood sugar level may indicate the onset of food intake. It contains glucose sensitive neurons activated via LHA glucopenia, thereby making a positive regulation in nutrition and energy consumption in a short time. All or some of the orexin's neurons may be glucose sensitive neurons or receive projections that stimulate glucose sensitive neurons (17). Orexigenic neurons are stimulated when blood sugar drops and the stomach is empty. Increased mRNA expression of hypothalamic preproorexin has been shown in hypoglycemia due to insulin or food restriction. It has long been known that glucose sensitive nerves mediate nutrition associated with hypoglycemia. In an electrophysiological study, it was shown that some of the glucose-sensitive nerves that were stimulated with low or high glucose synapse with orexigenic neurons. In addition, OXA has been shown to stimulate glucose sensitive nerves in LHA and suppress glucose sensitive nerves in VMN. Therefore, it is claimed that hypoglycemia stimulates nutrition by partially activating orexigenic nerves (18).

In LHA, food intake is altered through catecholamines. Administration of NA and DA from LHA increased food intake in rats. The increase in NA and DA release from the medial hypothalamic nucleus stimulates food intake. This effect on monoamine release indicates coordination between the lateral and medial hypothalamic areas, which are particularly related to food intake, meal size and number of meals (10). In our study, we observed the change in LHA catecholamine levels, which has a regulatory effect on food intake, when we increase the blood glucose level in animals fasted for 24 hours and animals that are fed normally by microdialysis method. When we administered 50% 0.5 / 0.8 cc iv glucose through the tail vein to animals fasted for 24 hours, there was a statistically significant decrease in LHA NA level in the first 20 minutes (p= 0.001). NA values are considerably higher than that of its metabolite, DHPG. We could not find a statistically significant change at the DA level (p> 0.05).

It was investigated that NPY can alter the stimulating effect of food intake and the effect of reducing food intake of leptin by increasing or decreasing the extracellular NA level (19). Central NA levels in the brain regulate leptin release. Leptin and NA are in an opposite relationship. Leptin reduces the effect of NA in order to reduce food intake, it can decrease food intake by decreasing the level of NA in the brain. In our study, when iv glucose was administered in fasted animals, a decrease in NA level was observed. Leptin, an anorexigenic peptide, inhibits glucose sensitive ARC and LHA neurons as a result of increased blood glucose level. It stimulates glucose sensitive VMN neurons with increased leptin receptors in these cells. With the stimulation of the satiety center, food intake stops (6). The administration of leptin to the lateral ventricle causes a significant decrease in DA release in the nucleus accumbens (20).

Increased leptin reduces NA activity to suppress eating behavior, whereas in the absence of leptin, nutrient intake also increases as a result of increased NA release. An increase in NA levels has been observed in the hypothalamus of obese (ob/ob) mice with leptin-producing gene deficiency (19). Ghrelin is a hormone that increases food intake. Ghrelin exerts its appetizing effect by increasing the NPY and AgRP orexigenic peptides in the ARC in the hypothalamus (21). NPY is also a neurotransmitter that stimulates eating behavior. NPY and NA coexist in neurons in the brain and are secreted together from the brainstem. Many studies have shown that NPY increases NA release in the brain (22). With the administration of glucose exogenously, a decrease in the level of ghrelin, an orexigenic peptide that increases food intake, was found (23).

In a study on PVN, the extracellular NA level was determined and food intake was recorded over a 24-hour period. Consuming more than 70% of the food in the dark phase, the extracellular NA level in PVN peaked just before the onset of the dark phase in rats (24). Microdialysis studies have shown a sudden increase in endogenous NA release in PVN at the beginning of the dark phase. The sensitivity of exogenous NA to the effects that trigger nutrition increases at the beginning of the dark phase. Abizaid stated that any mutation or deficiency in the dopamine gene, similar to lesions in the LHA, prevents food intake. Hypothalamic peptides such as NPY, α-MSH, AgRP, orexin and MCH play an important role in regulating the activity of dopaminergic cells in the nucleus accumbens. In ARC, it alters the effect of the mesolimbic dopaminergic system through direct projections of metabolic information from signals such as leptin and ghrelin to nucleus accumbens. Through orexin neurons in ghrelin-sensitive LHA, the ventral tegmental area innervates dopamine cells, and ghrelin directly inhibits DA release (25).

Microdialysis probes were placed in substantia nigra and striatum. During the first 40 minutes of the 50 mM glucose infusion, a 50% increase in DA flow from substansia nigra to the striatum was observed and returned to the baseline after 60 minutes (26). Changing SN glucose levels affects striatal dopamine release. DA decreased due to satiety in samples taken by microdialysis before and after feeding from LHA (9). In our study, when we administered iv glucose, no statistically significant result was found at the level of DA and DA metabolite DOPAC.

Experimental animals should be kept under deep anesthesia while applying the microdialysis method. It is known that ketamine used for anesthesia in the study has a dose-dependent effect on blood glucose levels. Ketamine can produce different effects at low and high doses (27). In a different study, it was reported that there was no clinically significant change in blood glucose level at any dose of ketamine (28). Considering the previous studies and literature of the study team, an optimal dose was applied and it is thought that it did not cause any situation that would change the result.

Microdialysis is thought to be the most appropriate method for observing instantaneous changes in catecholamine levels. For this reason, the microdialysis method was preferred in the study. The limitation of the study is that the animal is kept under anesthesia in a stereotaxic device during microdialysis. This may cause changes in catecholaminergic mechanisms. However, since the same procedures were applied to the groups, the margin of error was tried to be minimized.

## CONCLUSION

According to the findings of the study, systemic glucose administration significantly decreased the level of noradrenaline in LHA in both non-fed and fed animals ($p=0.01$). These results may be modulated in relation to plasma glucose of noradrenergic neurotransmission in LHA. NA values are much higher than the metabolite DHPG. This finding evidences that NA is poorly metabolized by the experimental procedure applied in microdialysis supernatants. No statistical significance was found in DHPG values

in fasted and fed animals (p>0.05). Although DA and its metabolite DOPAC levels decreased slightly in non-fed animals compared to fed animals in our study, no statistically significant change was observed (p=0.15). This may be because there is a lower level of DA concentration than the HPLC-ECD system can analyze. According to our method, we could not analyze the possible changes that glucose administration might cause in DA concentration. We hope to reveal possible changes with systems that we can analyze at lower concentrations in further studies.

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