Ankara Üniv Vet Fak Derg, **66**, 297-302, 2019
DOI: 10.33988/auvfd.488253

The effect of centrally and peripherally injected CDP-choline on plasma nesfatin-1 level in rats

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Received date: 27.12.2018- Accepted date: 03.05.2019

Abstract: Nesfatin-1 has a role in appetite control and energy balance. The activity of the cholinergic system also is able to affect feeding behavior. Moreover, the central cholinergic system interacts with central nesfatinergic systems. The main goal of the study was to determine the effect of intracerebroventricular (icv) and intravenous (iv) administrated CDP-choline (0.5 ve 1 µmol; icv ve 250 mg / kg; iv) on levels of plasma nesfatin-1 in the homogeneous number of male and female fasted and the satiated Wistar albino rats. The polyethylene cannula was inserted into the carotid artery and jugular vein of the rats anesthetized with sevoflurane (2–4%/100% O_2) to collect blood samples and to make iv injection, respectively. For icv treatment, the lateral ventricle of rats was cannulated with guide cannula. The basal levels of plasma nesfatin-1 in the satiated rats were higher than those observed in the fasted animals. While 0.5 and 1 µmol dose of icv and/or 250 mg/kg dose of iv injected CDP-choline increased the level of plasma nesfatin-1 in the satiated rats, plasma nesfatin-1 level of the fasted animals decreased after the same dose and route of CDP-choline injection. The current findings show that CDP-choline can influence the level of plasma nesfatin-1 in the rats. The effect of the drug was different according to the food intake of the rats. These data might suggest a potential role in CDP-choline on plasma nesfatin-1 concentration.
Keywords: CDP-choline, intracerebroventricular, intravenous, plasma nesfatin-1.

Merkezi ve periferik olarak enjekte edilen CDP-kolin'in sıçanlarda plazma nesfatin-1 seviyesi üzerine etkisi

Özet: Nesfatin-1, iştah regülasyonunda ve enerji homeostazında rol oynar. Kolinerjik sistemin aktivitesi de beslenme davranışını etkileyebilir. Ayrıca, merkezi kolinerjik ve nesfatinerjik sistemler arasında bir etkileşim vardır. Bu çalışmada homojen sayıda erkek ve dişi aç bırakılmış ve tok Wistar albino sıçanlara, serebral yan ventrikül (syv) ve intravenöz (iv) uygulanmış CDP-kolinin (0.5 ve 1 µmol; syv ve 250 mg / kg; iv) plazma nesfatin-1 düzeylerine etkisinin belirlenmesi amaçlanmıştır. Sevofluran (% 2-4 /% 100 O_2) anestezisi altında, sıçanların karotis arteri kan örnekleri toplamak için ve juguler veni ise iv enjeksiyon yapmak için kanüle edildi. Syv enjeksiyonlar için ise sıçanların lateral ventrikülüne kılavuz kanül yerleştirildi. Tok sıçanların bazal plazma nesfatin-1 seviyeleri, aç bırakılan hayvanlardakinden daha yüksek olarak gözlendi. 0.5 ve 1 µmol dozunda syv ve / veya 250 mg / kg dozunda iv enjekte edilen CDP-kolin, tok sıçanlarda plazma nesfatin-1 seviyelerini artırırken, aynı doz ve yolla CDP-kolin uygulaması, aç bırakılan hayvanların plazma nesfatin-1 düzeylerini azalttı. Sonuçlar, CDP-kolinin sıçanlarda plazma nesfatin-1 seviyelerini etkileyebileceğini göstermektedir. İlacın etkisi sıçanların besin alımına göre farklılık göstermektedir. Bu veriler, CDP-kolinin plazma nesfatin-1 konsantrasyonu üzerinde potansiyel etkisi olabileceğini göstermektedir.
Anahtar sözcükler: CDP-kolin, serebral yan ventrikül, intravenöz, plazma nesfatin-1.

Introduction

Nesfatin-1 is an anorectic neuropeptide with 82-amino acid and it is generated from a precursor protein, nucleobindin-2 (NUCB2) (23). Food intake affects nesfatin-1 expression (23). Significantly decrease in food intake following centrally nesfatin-1 injection in rodents was reported. Moreover, nesfatin-1 also decreased food intake when it was delivered peripherally (9, 23, 26).

CDP-choline is a nucleotide-synthesized endogenously. It has a number of cellular effects in various experimental conditions (34). When CDP-choline is administered, it is able to create cardiovascular, respiratory, neuroendocrine and neuroprotective responses. It also has beneficial effects in the treatment of some neurodegenerative and neurovascular diseases (6, 11, 30, 32, 34). CDP-choline in the body is rapidly metabolized to its metabolites such as choline (18, 35).

CDP-choline raised choline levels in the body and then enhanced cholinergic transmission by increasing the synthesis of acetylcholine (4, 33).

CDP-choline also is related to food intake behavior. In a previous study, CDP-choline-induced significantly declines in appetite ratings were shown (14). It is also known that centrally injected CDP-choline decreased levels of serum ghrelin but increased levels of serum leptin in rats (15). But there is no data on whether CDP-choline can affect plasma nesfatin-1 level or if there is any correlation between the effect of CDP-choline on plasma nesfatin-1 level and CDP-choline-evoked appetite. It is very well known that the hypothalamus has a dense cholinergic innervation as well as an intense nesfatinergic neuron. In consideration of the dense cholinergic innervation of hypothalamus (21, 22, 23), and the effect of CDP-choline on the vagal nerve, CDP-choline treatment could have a potential to affect on plasma nesfatin-1 levels in different food intake condition. Therefore, it was contemplated to indicate the effect of centrally or peripherally delivered CDP-choline on plasma nesfatin-1 levels in satiated and fasted rats in the present study.

Materials and Methods

Animals: A total of 63, 3 months old, female (n=30) and male (n=33) Wistar albino rats (250–300 g) were provided from Experimental Animals Breeding and Research Center, Uludağ University, Bursa, Turkey to use for the current study experiments. Animals housed in controlled conditions (20–22 ^{0}C room temperature, 60–70 % humidity and 12 h light/dark cycle), as four or five rats in per cage. Satiated rats were fed *ad libitum* with free access to food and water. Fasted rats were housed without food but free access water for 12 h overnight. The Animal Care and Use Committee of Uludag University, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, approved all experimental procedures (2014-08/04). Each group has 7 animals, and each rat was used in a single experiment.

Surgical procedures: The animals anesthetized with sevoflurane (2–4% / 100% O_2). Under the anesthesia, to collect the blood sample and to make the intravenous injection, PE 50 tubing containing 100 U/ml heparin was used to cannulate the left carotid artery and jugular vein of rats, respectively. For central treatment, the guide cannula was inserted to the lateral cerebral ventricle. Briefly, a burr hole was drilled the skull according to 1.5 mm lateral to the midline and 1.0 mm posterior to bregma. The guide cannula was inserted into the hole and lowered 4.5 mm below the surface of the skull. And then the guide cannula was fixed to the skull with acrylic cement. Before the experiments, the rats were rested in separate cages for 4–5 h to be allowed to recover from anesthesia.

Drug and icv injection: CDP-choline was bought from Sigma-Aldrich Co. (Deisenhofen, Germany) the desired concentration of the CDP-choline dissolved in saline on the day of the experiment. Icv doses of CDP-choline were chosen from the study reporting the effect of central injected CDP-choline on serum ghrelin and leptin levels in rat (15) and iv dose of CDP-choline was chosen from the study showing the effect of CDP-choline on plasma vasopressin and catecholamines levels, and renin activity (28).

To perform icv delivery, the injection cannula was connected to a microsyringe with polyethylene tubing. The drug or its vehicle was delivered icv in a volume of 5 µl over a 60s time period. To ensure the drug was entirety delivered, the air bubble moving in the polyethylene tubing was tracked.

Experimental procedure: Firstly, to test how central injection of CDP-choline affected the plasma nesfatin-1 levels in both sexes of the fasted or the satiated rats, 0.5 (n=7 for fasted group; n=6 satiated group) and 1 µmol (n=7 for fasted group; n=6 satiated group) doses of CDP-choline or saline (5 µl; n=6 for fasted group; n=6 satiated group) were icv injected. 250 µl blood samples for plasma nesfatin-1 levels were collected via the arterial catheter before injection and at the 5, 10, 20, 40 and 60th min of injection.

Secondly, it was determined if peripherally injected CDP-choline had a role in the plasma nesfatin-1 levels in both sexes of the fasted or the satiated rats. For this reason, CDP-choline (250 mg/kg, iv; n=7 for fasted group; n=6 satiated group) or saline (1 ml/kg, iv; n=6 for fasted group; n=6 satiated group) was administered to the rats through venous catheter. Again 250 µl blood samples for levels of plasma nesfatin-1 were collected via the arterial catheter before injection and at the 5, 10, 20, 40 and 60th min of injection. Table 1 shows female and male animal number for each injection line.

Table 1. Female and male animal number for each injection line.
Tablo 1. Her enjeksiyon için dişi ve erkek hayvan sayısı

		Fasted rats number		Satiated rats number	
		Female	**Male**	**Female**	**Male**
Icv	Saline	3	3	3	3
	0.5 µmol	3	4	3	3
	1 µmol	3	4	3	3
Iv	Saline	3	3	3	3
	250 mg/kg	3	4	3	3

Determination of plasma nesfatin-1 level: To separate the plasmas, the blood samples in tubes containing EDTA were centrifuged at +4 ^{0}C, 1800 rpm for 20 min, and then plasma samples were kept at -80 ^{0}C up to plasma nesfatin-1 level measurements. Microplate enzyme immunoassay (ELISA), which was purchased from Phoenix Pharmaceuticals Inc. (CA, USA) was used to measure the plasma concentration of nesfatin-1. The measurements were performed according to the kit instruction. Level of plasma nesfatin-1 was expressed as ng/ml. Each sample was used for once to measure plasma nesfatin-1 level.

Statistical analysis: The results are given as mean ± standard error of the mean (S.E.M.). $P<0.05$ was considered as the level of significance. The repeated-measures two-way analysis of variance (RM-ANOVA; two-way) and the post-ANOVA test of Bonferroni were used for statistical evaluation.

Results

The fasted rats had 7.44 ± 0.82 ng/ml (7.38 ± 0.73 ng/ml for female rats and 7.50 ± 0.94 ng/ml for male rats) the basal levels of plasma nesfatin-1 (Fig 1). Levels of plasma nesfatin-1 were diminished significantly at 60 min following 0.5 and 1 µmol dose of icv (Fig 1A) or 250 mg/kg dose of iv (Fig 1B) CDP-choline injection in the fasted rats compared with saline-injected group. Both icv and iv injected CDP-choline-induced decreasing in plasma nesfatin-1 level started suddenly and lasted up to 60 min after the injections (Fig 1A, B).

The satiated rats had 13.37 ± 1.78 ng/ml (12.98 ± 1.24 ng/ml for female rats and 13.76 ± 2.32 ng/ml for male rats) the basal plasma nesfatin-1 levels (Fig 2). Administration of 0.5 and 1 µmol dose of icv (Fig 2A) or 250 mg/kg dose of iv (Fig 2B) CDP-choline significantly increased plasma nesfatin-1 levels of the satiated rats according to saline injected satiated rats' levels, respectively. While the increase in plasma nesfatin-1 level of the icv CDP-choline injected satiated rats continued up to 60 min after the injection (Fig 2A), the increase in plasma nesfatin-1 level of the iv CDP-choline injected satiated rats was observed only at 5th min of the injection (Fig 2B).

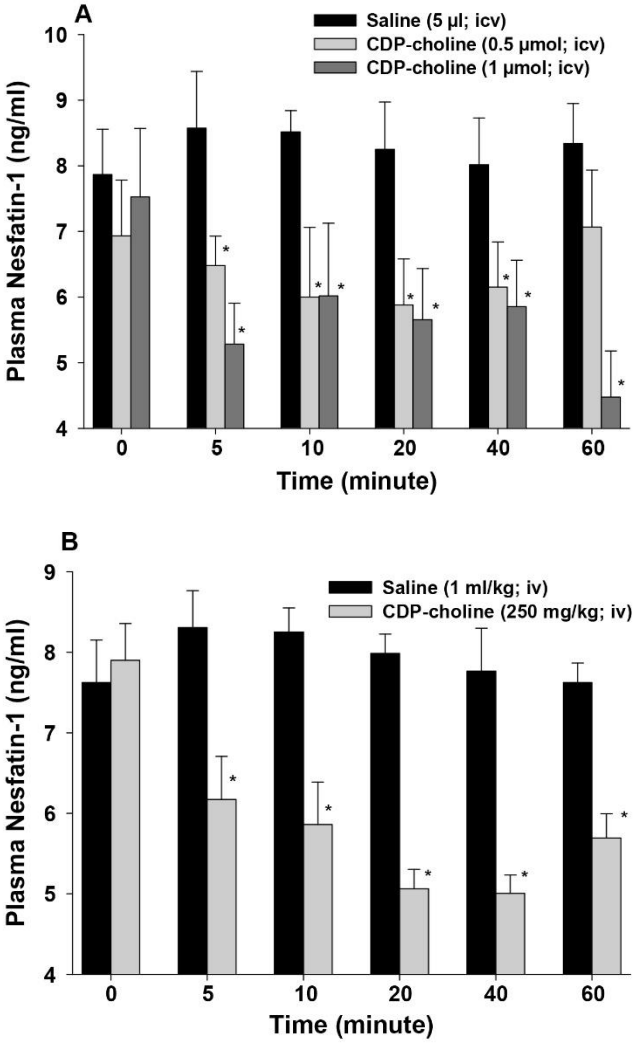

Figure 1. Effects of icv (A) and iv (B) administrated CDP-choline on plasma nesfatin-1 levels of the fasted rats. Data are given as means ± S.E.M. *P<0.05 significantly different from the saline group.

Şekil 1. Syv (A) ve iv (B) uygulanan CDP-kolinin aç bırakılmış sıçanlarda plazma nesfatin-1 seviyeleri üzerine etkileri. Veriler ortalama ± standart hata olarak verilmiştir. * P<0.05 tuzlu su grubuna göre anlamlı farkı göstermektedir.

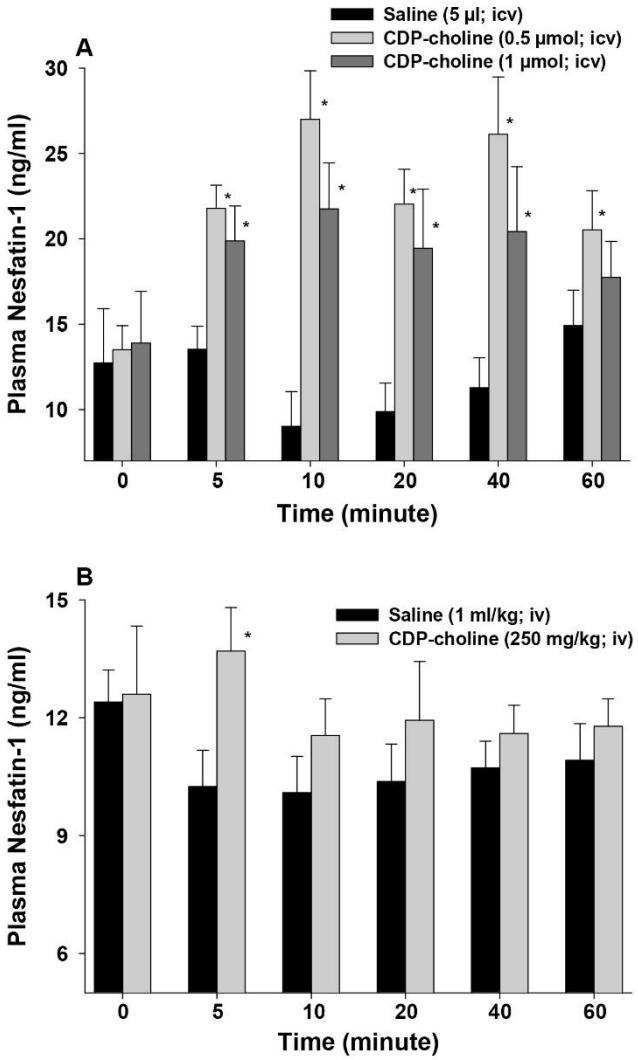

Figure 2. Effects of icv (A) and iv (B) injected CDP-choline on plasma nesfatin-1 levels of the satiated rats. Data are given as means ± S.E.M. * P<0.05 significantly different from the saline group.
Şekil 2. Syv (A) ve iv (B) uygulanan CDP-kolinin tok sıçanlarda plazma nesfatin-1 seviyeleri üzerine etkileri. Veriler ortalama ± standart hata olarak verilmiştir. * P<0.05 tuzlu su grubuna göre anlamlı farkı göstermektedir.

Discussion and Conclusion

The current findings report that CDP-choline differently affected plasma nesfatin-1 levels in fasted or satiated rats. The drug considerably enhanced levels of plasma nesfatin-1 in satiated conditions however it significantly suppressed the levels of plasma nesfatin-1 in fasted animals. Interestingly, basal levels of plasma nesfatin-1 were higher in satiated rats than those observed in fasted animals.

The data clearly show that 12 h fasting caused to decrease in levels of plasma nesfatin-1 in rats. The levels of plasma nesfatin-1 in the satiated rats were observed almost 2 times more than the fasted rats. It was explained that the hypothalamic NUCB2 mRNA expression was significantly decreased after the rats fasted for 24 h (23). On the other hand, refeeding after 48 h fasting period resulted in an increase in nesfatin-1 immunoreactivity in the hypothalamic area (16). It was also reported that gastric mucosa expressed NUCB2 mRNA almost 10 times more than the brain (31). Again 24 h fasting in rats caused decreasing in gastric mucosal NUCB2 mRNA expression. Those reports evidently indicate that the fasting led to suppress in the releasing of nesfatin-1 in central and peripheral and also those reports support our findings showing 12 h overnight fasting causing the decrease in plasma nesfatin-1 level. Moreover, in the current study, both sexes of rats were used in all groups and levels of plasma nesfatin-1 were quite similar in both sexes of rats. There is evidence supporting our current findings, which there are no differences between healthy male and female subjects in levels of plasma nesfatin-1 (17).

In the current study, the data show that the effect of CDP-choline on levels of plasma nesfatin-1 depends on satiety or fasting conditions. Although levels of plasma nesfatin-1 were suppressed by icv or iv CDP-choline injection in 12 h fasted rats, they were elevated by same routes and doses administered CDP-choline in satiated rats. CDP-choline is a highly bioavailable compound and it rapidly metabolized and increased acetylcholine concentration in the brain and, plasma and tissue, when it was injected (18, 25, 27, 35). Also, CDP-choline can cross the blood-brain barrier (29). Hence when it injected

peripherally, it could enhance cholinergic transmission in the brain and peripheral tissues. It was well known that activation of the hypothalamus or afferent/efferent vagal stimulus with cholinergic innervation has an important role in feeding control. Those hypothalamic or vagal innervations with icv or iv CDP-choline injection might cause the increase or decrease in plasma nesfatin-1 concentrations in the satiated or fasted rats, respectively. It has not been shown whether gastric mucosa is the source of the plasma nesfatin-1. However, there is a possibility that plasma nesfatin-1, at least in part, may be derived from gastric mucosa, because the abundance of the NUCB2 mRNAs (31) and the mRNAs for prohormone converting enzymes (20) in gastric mucosa have been described. Also, it was demonstrated that the nesfatin-1 concentrations in serum were raised after vagus nerve stimulation in high-fat-fed rats (8). This report is well compatible with the data showing an increase in levels of plasma nesfatin-1 after icv or iv injection of CDP-choline in satiated rats. Since icv or iv injection of CDP-choline causes to stimulate vagal nerve by activating cholinergic system it may increase in plasma nesfatin-1 levels, particularly in satiety condition.

Central and peripheral CDP-choline and choline treatments have some roles on glucose metabolism such as increase in plasma insulin and hyperglycemia depended on an increase in plasma levels of catecholamines (3, 10, 12, 13). Previous studies indicate that high levels of plasma glucose and insulin stimulated hypothalamic nesfatin-1 neurons so that meal ingestion was stimulated by nesfatin-1 neurons in the hypothalamus and thereby produces satiety (7). It was also reported that diabetic patients had lower plasma nesfatin-1 than healthy subjects (1). Considered the role of CDP-choline on glucose metabolism, the effect of CDP-choline on plasma nesfatin-1 concentrations might be secondarily effect depended on CDP-choline-induced glucose metabolism changes.

Hypothalamus plays vital roles in the control of appetite and energy metabolism. It is well known that hypothalamus has dense cholinergic innervations and is rich from nesfatinergic (23) and cholinergic neurons (21-23). It has been shown that both CDP-choline (14) and nesfatin-1 (23) have the anorexigenic effect. These results indicate that both cholinergic and nesfatinergic systems neuroanatomically originate from the same brain area and they have the same effect on food intake behavior. The central nesfatinergic and cholinergic systems interact with each other (2). Because centrally applied nesfatin-1 produced an increase in levels of the hypothalamic acetylcholine and choline and also central cholinergic receptors mediated the nesfatin-1-induced cardiovascular responses (2). Recently it has been reported that central injection of CDP-choline suppressed levels of serum ghrelin but increased levels of serum leptin in fed *ad libitum* rats (15). It was also demonstrated that leptin directly activated nesfatinergic neurons in the hypothalamus (5). It was known that obese subjects had high plasma nesfatin-1 concentration but low plasma ghrelin levels (24). When the role of CDP-choline on levels of plasma leptin and ghrelin, and its anorexigenic effect are taken consideration, CDP-choline might have a role on plasma nesfatin-1 level depends on satiety and fasting conditions. The effects of CDP-choline on plasma nesfatin-1 concentrations in the current study also support an earlier report showing that cholinergic mechanisms play a role in food intake behavior (14).

In conclusion, the current data demonstrate that the level of plasma nesfatin-1 was significantly lower in 12 h fasted rats than satiated rats. Furthermore, centrally and/or peripherally applied CDP-choline caused the additional decrease in plasma nesfatin-1 concentration of the rats fasted 12 h. In contrast, icv and/or iv CDP-choline injection increased extra the plasma nesfatin-1 levels in the satiety condition. Nevertheless, the current data did not explain which mechanism mediated CDP-choline related plasma nesfatin-1 levels change. However, the data might be conceded that the cholinergic system has a physiological role in nesfatin-1 release. The results may give an idea about CDP-choline usage for obesity treatment but future investigations are needed on the topic.

Acknowledgement

This study was supported by the Commission of Scientific Research Projects of Uludağ University, Project number HDP (T2012/10). This paper is the result of a Master thesis performed by Hikmet Aysin Usta at Bursa Uludağ University in Bursa.

References

1. **Algul S, Ozkan Y, Ozcelik O** (2016): *Serum nesfatin-1 levels in patients with different glucose tolerance levels*. Physiol Res, **65**, 979-985.
2. **Aydin B, Guvenc G, Altinbas B, et al.** (2018): *Modulation of nesfatin-1-induced cardiovascular effects by the central cholinergic system.* Neuropeptides, **70**, 9-15.
3. **Cansev M, Ilcol YO, Yilmaz MS, et al.** (2008): *Peripheral administration of CDP-choline, phosphocholine or choline increases plasma adrenaline and noradrenaline concentrations.* Auton Autacoid Pharmacol, **28**, 41-58.
4. **Cohen EL, Wurtman RJ** (1976): *Brain acetylcholine: control by dietary choline.* Science, **191**, 561–562.
5. **Darambazar G, Nakata M, Okada T, et al.** (2015): *Paraventricular NUCB2/nesfatin-1 is directly targeted by leptin and mediates its anorexigenic effect.* Biochem Biophys Res Commun, **456**, 913-918.
6. **Davalos A, Secades J** (2011): *Citicoline preclinical and clinical update 2009-2010.* Stroke, **42**, 36-39.
7. **Gantulga D, Maejima Y, Nakata M, et al.** (2012): *Glucose and insulin induce Ca^{2+} signaling in nesfatin-1*

neurons in the hypothalamic paraventricular nucleus. Biochem Biophys Res Commun, **420**, 811-815.

8. **Gil K, Bugajski A, Kurnik M, et al.** (2012): *Chronic vagus nerve stimulation reduces body fat, blood cholesterol and triglyceride levels in rats fed a high-fat diet.* Folia Med Cracov, **52**, 79-96.
9. **Goebel-Stengel M, Wang L, Stengel A, et al.** (2011): *Localization of nesfatin-1 neurons in the mouse brain and functional implication.* Brain Res, **1396**, 20-34.
10. **Gurun MS, Ilcol YO, Taga Y, et al.** (2002): *Hyperglycemia induced by intracerebroventricular choline: involvement of the sympatho-adrenal system.* Eur J Pharmacol, **438**, 197-205.
11. **Hurtado O, Lizasoain I, Moro MA** (2011): *Neuroprotection and recovery: recent data at the bench on citicoline*. Stroke, **42**, 33-35.
12. **Ilcol YO, Cansev M, Yilmaz MS, et al.** (2007): *Intraperitoneal administration of CDP-choline and its cholinergic and pyrimidinergic metabolites induce hyperglycemia in rats: involvement of the sympathoadrenal system.* Arch Physiol Biochem, **113**, 186-201.
13. **Ilcol YO, Gurun MS, Taga Y, et al.** (2002): *Intraperitoneal administration of choline increases serum glucose in rat: involvement of the sympathoadrenal system.* Horm Metab Res, **34**, 341-347.
14. **Killgore WD, Ross AJ, Kamiya T, et al.** (2010): *Citicoline affects appetite and cortico-limbic responses to images of high17 calorie foods*. Int J Eat Disord, **43**, 6-13.
15. **Kiyici S, Basaran NF, Cavun S, et al.** (2015): *Central injection of CDP-choline suppresses serum ghrelin levels while increasing serum leptin levels in rats.* Eur J Pharmacol, **764**, 264-270.
16. **Kohno D, Nakata M, Maejima Y, et al.** (2008): *Nesfatin-1 neurons in paraventricular and supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding.* Endocrinology, **149**, 1295-1301.
17. **Li QC, Wang HY, Chen X, et al.** (2010): *Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans.* Regul Pept, **59**, 72-77.
18. **Lopez G-Coviella I, Agut J, Von Borstel R, et al.** (1987): *Metabolism of cytidine (5')-diphosphocholine (CDP-choline) following oral and intravenous administiration to the human and the rat.* Neurochem Int, **11**, 293-297.
19. **Lopez-Coviella I, Agut J, Savci V, et al.** (1995): *Evidence that 5'-cytidinediphosphocholine can affect brain phospholipid composition by increasing choline and cytidine plasma levels.* Neurochem, **65**, 889-894.
20. **Macro JA, Dimaline R, Dockray GJ** (1996): *Identification and expression of prohormoneconverting enzymes in the rat stomach.* Am J Physiol, **270**, 87-93.
21. **Mason WT** (1985): *Staining of the magnocellular nuclei of the rat hypothalamus by a monoclonal antibody directed against the alpha-subunit of the nicotinic cholinergic receptor.* Neurosci Lett, **59**, 89-95.
22. **Michels KM, Meeker RB, Hayward JN** (1986): *Differential distribution of muscarinic cholinergic and putative nicotinic cholinergic receptors within the hypothalamo-neurohypophysial system of the rat.* Neuroendocrinology, **44**, 498-507.
23. **Oh-I S, Shimizu H, Satoh T, et al.** (2006): *Identification of nesfatin-1 as a satiety molecule in the hypothalamus.* Nature, **12**, 709-712.
24. **Ozkan Y, Timurkan ES, Aydin S, et al.** (2013): *Acylated and desacylated ghrelin, preptin, leptin, and nesfatin-1 Peptide changes related to the body mass index.* Int J Endocrinol, **2013**, 236085.
25. **Paroni R, Cighetti G, Del Puppo M, et al.** (1985): *Evidence for a different metabolic behaviour of cytidine diphosphate choline after oral and intravenous administration to rats.* Pharmacol. Res Commun, **17**, 805-829.
26. **Ramanjaneya M, Chen J, Brown JE, et al.** (2010): *Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity*. Endocrinology, **151**, 3169-3180.
27. **Savci V, Cavun S, Goktalay G, et al.** (2002): *Cardiovascular effects of intracerebroventricularly injected CDP-choline in normotensive and hypotensive animals: the involvement of cholinergic system.* Naunyn Schmiedebergs Arch Pharmacol, **365**, 388-398.
28. **Savci V, Goktalay G, Cansev M, et al.** (2003): *Intravenously injected CDP-choline increases blood pressure and reverses hypotension in haemorrhagic shock: effect is mediated by central cholinergic activation.* Eur J Pharmacol, **468**, 129-139.
29. **Secades JJ, Frontera G** (1995): *CDP-choline: pharmacological and clinical review.* Methods Find Exp Clin Pharmacol, **17**, 1-54.
30. **Sevim Ç, Altinbaş B, Yalçın M, et al.** (2017): *Protective effect of CDP-choline on hypotension and tissue injury in septic shock model.* Ankara Üniv Vet Fak Derg, **64**, 103-110.
31. **Stengel A, Goebel M, Yakubov I, et al.** (2009): *Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa*. Endocrinology, **150**, 232-238.
32. **Topuz BB, Altinbas B, Yilmaz MS, et al.** (2014): *The effect of centrally injected CDP-choline on respiratory system; involvement of phospholipase to thromboxane signaling pathway.* Respir Physiol Neurobiol, **195**, 50-58.
33. **Ulus IH, Wurtman RJ, Mauron C, et al.** (1989): *Choline increases acetylcholine release and protects against the stimulation-induced decrease in phosphatide levels within membranes of rat corpus striatum.* Brain Res, **484**, 217-227.
34. **Weiss GB** (1995): *Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline*. Life Sci, **56**, 637-660.
35. **Wurtman RJ, Regan M, Ulus I, et al.** (2000): *Effect of oral CDP-choline on plasma choline and uridine levels in humans.* Biochem Pharmacol, **60**, 989-992.