ARAŞTIRMA

F.Ü.Sağ.Bil.Tıp Derg.
2010: 24 (1): 25 - 30
http://www.fusabil.org

Melatonin and Ghrelin Differentially Modulate Plasma Oxytocin Level and Noradrenalin Release in Hypothalamic Paraventricular Nucleus in Female Rats

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Geliş Tarihi : 29.12.2009
Kabul Tarihi : 11.03.2010

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Oxytocin secretion, playing key role in parturition and lactation process, is regulated by neuroendocrine mechanisms. Cholecystokinin (CCK)-induced noradrenalin release in hypothalamus stimulates oxytocin secretion to the circulation. The effect of melatonin and ghrelin hormones on noradrenalin concentration in hypothalamic paraventricular nucleus (PVN) and oxytocin level in plasma were assessed in this study. Anaesthetized virgin female Wistar rats were set at a sterotaxic frame after carotid artery cannulation. Melatonin vehicle to the vehicle group, intra-arterial CCK to the CCK group and CCK plus melatonin intracerebroventricularly injected to the melatonin group. In the other procedures, artificial cerebrospinal fluid (control group) and ghrelin were intracerebroventricularly injected. Microdialysis was performed into PVN in all animals and noradrenalin concentrations were analyzed by high performance liquid chromatography obtained from dialyzed in 20 min periods for four time. Blood samples were obtained via intra-arterial cannula for same intervals with microdialysis samples and plasma oxytocin levels were analyzed with radioimmunoassay. One-Way ANOVA was used for statistical evaluations. Noradrenalin concentration in PVN and oxytocin level in plasma increased in the second period in CCK group compared to vehicle group ($p<0.001$ and $p<0.05$, respectively). Noradrenalin and oxytocin values decreased compared to CCK group in the second period. Plasma oxytocin level was significantly high in the 4th period in ghrelin group compared to control ($p<0.05$) while noradrenalin concentrations in PVN did not change. The results of present study were brought into light that melatonin have inhibitory effect on CCK-induced oxytocin secretion to the circulation in virgin female rats. Melatonin also inhibits noradrenalin release in PVN. As for ghrelin, this hormone stimulates plasma oxytocin secretion without affecting noradrenalin release in PVN. These results reveal that melatonin and ghrelin have different modulatory effects on oxytocin secretion.

Key Words: *Melatonin, ghrelin, oxytocin secretion, noradrenalin, microdialysis, rat.*

Melatonin ve Grelin Dişi Sıçanlarda Plazma Oksitosin Düzeyi ve Hipotalamik Paraventriküler Nükleustaki Noradrenalin Salıverilmesini Farklı Olarak Modüle Ederler

Doğum ve laktasyon süreçlerinde rol oynayan oksitosin sekresyonu bazı nöroendokrin mekanizmalar tarafından düzenlenmektedir. Kolesistokinin (CCK) tarafından indüklenen hipotalamik noradrenalin salıverilmesi oksitosin sekresyonunu uyarmaktadır. Bu çalışmada intraserebroventriküler yolla enjekte edilen melatonin ve grelin hormonlarının oksitosin sekresyonu ve paraventriküler nükleustaki noradrenalin konsantrasyonu üzerindeki olası etkileri araştırılmıştır. Anestezi altındaki Wistar türü dişi sıçanlar karotid arter kanulasyonunu takiben sterotaksik cihaza yerleştirilmiştir. Çözücü grubuna melatonin çözücüsü, diğer gruba intrarateryel yolla CCK ve CCK+Melatonin grubuna da CCK'e ilaveten intraserebroventriküler yolla melatonin uygulanmıştır. Diğer prosedürde ise intrasebroventriküler yolla yapay beyin omurilik sıvısı (kontrol) ve grelin enjeksiyonları yapılmıştır. Bütün hayvanların hipotalamik paraventriküler nucleus (PVN)'una mikrodiyaliz uygulanarak 20'şer dakikalık peryotlar halinde toplam 4 defa toplanan diyalizarlardan sıvı kromatografisinde noradrenalin düzeyleri dedekte edilmiştir. Aynı peryotlarda kanülden kan örnekleri alınarak radyoimmünoölçüm yöntemiyle oksitosin analizi yapılmıştır. Istatistiksel analiz iin One-Way ANOVA kullanılmıştır. Çözücü grubuyla karşılaştırıldığında CCK grubunda 2. peryotta PVN noradrenalin ($p<0.001$) ve kan oksitosin düzeyleri ($p<0.05$) artmıştır. Melatonin grubunda ise aynı peryottaki noradrenalin ve oksitosin değerleri CCK grubuyla karşılaştırıldığında daha düşük bulunmuştur ($p<0.05$). Grelin grubunda noradrenalin konsantrasyonları değişmezken 4. peryotta plazma oksitosin düzeyi artmıştır ($p<0.05$). Bu çalışmanın sonuçları, dişi sıçanlarda melatoninin CCK'le indüklenmiş oksitosin sekresyonunu inhibe ettiğini göstermiştir. Bu inhibisyona PVN'daki noadrenalin salıverilmesinin baskılanması da katılmaktadır. Grelin ise PVN'daki noadrenalin konsantrasyonunu etkilemeden plazma oksitosin sekresyonunu stimüle etmiştir. Bu sonuçlar oksitosin sekresyonu üzerinde melatoninin ve grelinin farklı etkilere sahip olabileceğini göstermiştir.

Anahtar Kelimeler: *Melatonin, grelin, oksitosin sekresyonu, noradrenalin, mikrodiyaliz, sıçan.*

Introduction

Oxytocin is a peptide synthesized in magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei in the hypothalamus, transported to the posterior pituitary and released into the circulation. Oxytocin is produced also in some neurons of the parvocellular subdivision of the PVN (pPVN), which project to other brain regions

such as the brain stem, medulla, and cortex (1, 2). Within the brain, oxytocin is known to act as a neuromodulator or neurotransmitter (3).

Oxytocin production in the hypothalamus and its secretion from the posterior pituitary is under the control of several brain neurotransmitters. It is well established that brain catecholamines and particularly noradrenalin, exert a stimulatory action on magnocellular oxytocin neurons in the SON and PVN. For example, injection of noradrenalin into the lateral ventricle activates oxytocin neurons in the PVN (4). In whole cell recordings, noradrenalin administration was found to induce depolarization of magnocellular neurons via α1-adrenergic receptors and to increase the excitatory postsynaptic potential *in vitro* (5). The A2 noradrenergic cell group in the nucleus tractus solitarius (NTS) projects directly to oxytocin neurons in the hypothalamus (6), and stimulation of these cells led to specific activation of oxytocin neurons (7).

The release of oxytocin is increased not only in parturition and lactation, but also during stress (8) and next to its unambiguous role in processes related to reproduction, oxytocin is nowadays known to influence several other physiological functions, such as the activity of the cardiovascular system (9, 10). Moreover, there is accumulating evidence on the involvement of oxytocin in the control of food intake. As an anorexigenic peptide, oxytocin is thought to participate in neuroendocrine mechanisms leading to hyperphagia during pregnancy (11). Oxytocin influences the food intake in concert with the action of several other peptides involved in central, satiety and/or adiposity signals (12), such as leptin and ghrelin.

Melatonin is known to have important regulatory effect on hypothalamic and reproductive functions. This hormone may restrict hypotlamo-hypophyseal gonadal axis by inhibiting gonadotrophin release from hypophysis (13). Moreover melatonin inhibits testosterone secretion by affecting directly testes via its' own receptors (14). Uterin contractility is inhibited by melatonin in isolated myometrium in virgin and late pregnant rat (15). There are some studies regarding the effect of melatonin on oxytocin secretion in hypothalamo-neurohypophyseal system. Although high doses of melatonin have stimulatory effect (16) on oxytocin secretion, low doses have opposite effects (17). Additionally there is no available evidence about the possible effect of melatonin on the catecholaminergic involving in oxytocin synthesis and secretion processes.

Ghrelin, an endogenous ligand of the growth hormone secretagogue (GHS) receptor, is mainly secreted from gastric endocrine cells (18). It exerts a strong stimulatory action on growth hormone secretion (19) and food intake (20). Ghrelin can cross the blood-brain barrier (21). GHS receptors are expressed in various brain areas (22). It has been demonstrated that plasma ghrelin levels sharply increase at the end of pregnancy compared to those in non-pregnant rats (23). In line with this finding, administration of ghrelin to the lateral ventricle has been shown to activate oxytocin neurons in the PVN (24).

The neural control and mutual interrelationships among individual factors involved in the regulation of food intake and simultaneously related to reproduction are far from being understood. We have suggested that at least some of the effects of orexigenic and anorexigenic peptides might be mediated via noradrenalin release in the PVN. In non-pregnant female rats, we have tested the following hypotheses: (I) melatonin has an inhibitory action on oxytocin secretion mediated via hypothalamic release of noradrenalin; (II) ghrelin has a stimulatory action on both circulating oxytocin concentrations and hypothalamic release of noradrenalin. To bring to light the potential inhibitory action of melatonin, we have decided to use a model of cholecystokinin-8 (CCK) induced oxytocin release as it is well known that systemic administration of CCK increases electrical activity of oxytocin cells (25) and induces oxytocin release into the circulation (26, 27). CCK-induced excitation of oxytocin cells is accompanied by concomitant increase in noradrenalin release within the PVN (28, 29).

Materials and Methods

Animals: The local ethics committee approved our study protocol. Virgin female Wistar rats (200-220g) obtained from Firat University Biomedical Unit were used in this study. They were housed under controlled temperature (22 ± 1°C) and light conditions (lights on from 07.00-19.00 h). Food and water were freely available. Daily vaginal smear was performed and only animals on diestrus were included in the experiments.

Experimental Design: In chloral hydrate anesthesia (400 mg/kg, Botafarma Lab, Istanbul, Turkey), a carotid artery was cannulated and the animals were placed in a stereotaxic frame. A microdialysis guide cannula (CMA/Microdialysis, Stockholm, Sweden) and a microdialysis perfusion probe (CMA/Microdialysis, Stockholm, Sweden) were set into the right PVN with guidance of the rat stereotaxic atlas (30). Artificial cerebrospinal fluid was run through a micropump (Harward App, Holliston, MA, USA) throughout the experimental period. The flow rate of artificial cerebrospinal fluid was set at 1.5μl/min. After a settling period (at least 1h), microdialysis samples were collected at 20 min intervals for a period of 80 min. In the first series of experiments, 50μg/kg CCK-8 (Sigma, St Louis, MO, USA), was administered to rats of the CCK group via the carotid artery cannula (n=8) after the collection of the first sample. In addition to CCK, melatonin (10μg/5μl; Sigma, St Louis, MO, USA) was infused into the left lateral ventricle to rats of the melatonin group (n=8). Animals of the vehicle (n=10) and CCK groups received intracerebroventricular (ICV) infusions of the vehicle. In the second series of experiments, the collection of the first microdialysis sample was followed by ICV infusion of

5µl artificial cerebrospinal fluid in the control, vehicle treated rats (n=8) and by infusion of ghrelin (1µg/5µl; Sigma, St Louis, MO, USA) in rats of the ghrelin group (n=8).

In all experimental groups, blood samples were collected via the carotid artery cannula concomitantly with the microdialysis sample at four time intervals. After collection of the last microdialysis and blood samples, rats were decapitated and brain tissues gently removed. Coronal brain slices were cut according to the rat stereotaxic atlas for verification of the location of the probes.

Chromatography: Catecholamine concentrations in microdialysis samples collected from the PVN were analyzed by a high performance liquid chromatography with electrochemical detector system (HPLC-ECD, Waters Corp., Milford, MA, USA). A 20µl aliquots of the samples were injected onto the HPLC column (ODS2, 4.6X250mm C18, Waters Corp., Milford, MA, USA) coupled to ECD. The concentrations of noradrenalin and its metabolite (3,4-dihydroxyphenylglycol, DHPG) were simultaneously detected. The method has been described previously (31, 32).

Oxytocin assay: Iodination of oxytocin was performed using chloramine-T. Material was chromatographed on a column of Sephadex G-25 fine (Fluka Chemie, Buchs, Switzerland). Radioimmunoassay (RIA) procedure per se was performed according to Robinson (33) with 3 day incubation and separation of bound from free 125I oxytocin by addition of 900µl ethanol. Standard curves were obtained using 200µl dilutions of standard oxytocin (Peninsula-Bachem, Switzerland). 200µl samples of unextracted plasma were measured by RIA in duplicates with highly specific antibody kindly provided by Prof. Robinson (I.C., England). Intra-assay coefficient of variation was about 4.7%.

Statistics: Catecholamine data were evaluated and expressed as percentages of the baseline levels. Noradrenalin levels were normalized by nominating the control level as 100%, and its levels in 20-mins samples were expressed as percentage of these values. Data were statistically analyzed by two-way repeated measures ANOVA with post-hoc testing by using the Student-Newman-Keuls multiple range tests. $p<0.05$ was considered statistically significant.

Results

Noradrenalin concentrations in the microdialysis samples were significantly higher in the CCK group compared to those in the vehicle-treated group at 20 min following CCK injection ($p<0.001$, Figure 1). There were no significant differences between the values in these two groups at the subsequent time intervals. Melatonin administration didn't cause any difference on catecholamine concentration in PVN compared to CCK group. DHPG concentrations did not differ significantly between any of the treatment groups studied and therefore the values are not presented.

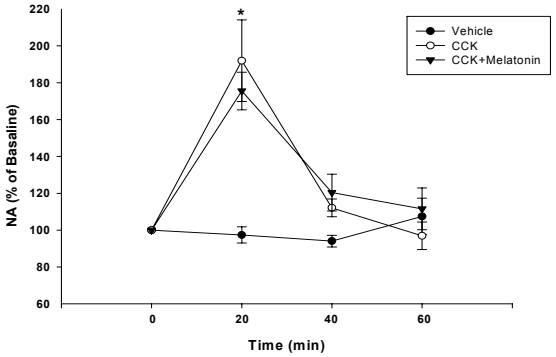

Figure 1. Noradrenalin (NA, percentage of baseline) values of vehicle, cholecystokinin (CCK) and CCK+Melatonin groups (Mean±SEM).

*$p<0.05$ compared to vehicle group by using One-Way ANOVA followed by Student-Newman-Keuls-Test.

Treatment with CCK resulted in increased oxytocin secretion throughout the whole experimental period (Figure 2). The differences between oxytocin concentrations measured in plasma of the CCK- and vehicle-treated rats reached statistical significance at 20 and 40 min time intervals ($p<0.05$, Figure 2). Central melatonin injection significantly reduced CCK induced oxytocin levels in the second experimental period compared to CCK group.

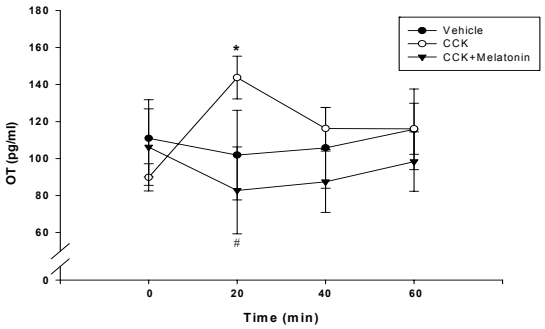

Figure 2. Oxytocin (OT, pg/ml) values of vehicle, cholecystokinin (CCK) and CCK+Melatonin groups (Mean±SEM). *$p<0.05$ compared to vehicle group.

#$p<0.05$ compared to CCK group, by using One-Way ANOVA followed by Student-Newman-Keuls-Test.

Central administration of ghrelin led to a significant increase in oxytocin concentrations in plasma compared to the baseline levels at 60 min ($p<0.05$; Figure 3). There were no significant differences at the other time intervals following ghrelin infusion. Ghrelin did not significantly alter noradrenalin and DHPG concentrations in microdialysis samples collected from the PVN at any time interval studied (data not shown).

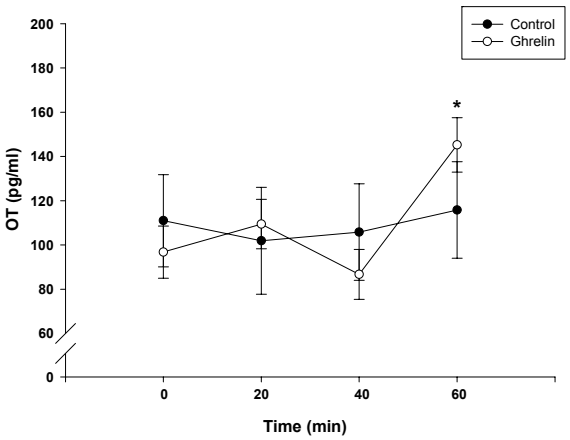

Figure 3. Oxytocin (OT, pg/ml) values of control and ghrelin groups (Mean±SEM).

*p<0.05 compared to control group by using Repeated Measures One-Way ANOVA followed by Student-Newman-Keuls-Test.

Discussion

Data from the present study reveal that melatonin inhibits CCK-induced oxytocin secretion to the circulation. This evidence was consistent with the some investigations. Forsling et al show that pinelaectomy increases oxytocin secretion (34). Melatonin inhibits pup-suckling induced oxytocin decretion in lactating rats (17). In another study, Juszczak and Stempniak (35) observed melatonin diminished substance P induced oxytocin release from the rat hypothalamo-neurohypophysial system: in vitro. According to our results, hypothalamic noradrenergic mediation is not involved in inhibition of oxytocin secretion by melatonin. In a study, the effect of melatonin on oxytocin neurons may occur through the dopaminergic mechanism rather than noradrenalin (36).

Oxytocin is an anorexigenic neuropeptide and its central administration reduces food intake (37, 38). NTS appears to be a critical brain area for the regulation of appetite control (39, 40) and there are pPVN oxytocin neurons, which project directly to the NTS (41). Oxytocin cells located in the pPVN are particularly promising candidates for mediating leptin's anorectic signals to the brain stem because leptin administration was found to activate fos expression in oxytocin neurons in the pPVN (42). Our results neither support nor contradict this hypothesis as oxytocin concentrations in plasma do not reflect the pattern of oxytocin production in the pPVN.

Present experiments in female rats revealed also a mild stimulatory action of centrally administered ghrelin on oxytocin secretion. Our results are in agreement with those of Olszewski et al (24) who have demonstrated that ICV injection of ghrelin activate fos expression in oxytocin cells in the PVN. Observed lack of effect of ghrelin on noradrenergic neurotransmission in the PVN allows us to suggest that noradrenalin is not involved in the stimulation of oxytocin release by ghrelin. As to the possible physiological significance, plasma ghrelin concentrations were found to be low during the initial periods and to increase towards the end of pregnancy in rats (23). Therefore, ghrelin may have a role in the activation of oxytocin neurons prior to parturition. Stimulation of parvocellular oxytocin neurons with ghrelin may provide an intuitive insight related to anorexigenic effect of oxytocin. It is speculated that oxytocin can exert a negative feedback role to limit the food intake induced by ghrelin because pretreatment with an oxytocin receptor antagonist elevated orexigenic response to ghrelin (24). It is clear that additional studies are necessary to elucidate the physiological outcomes of ghrelin-oxytocin interactions.

In conclusion, CCK stimulates oxytocin secretion by inducing noradrenergic neurotransmission in the PVN. Melatonin inhibited CCK induced oxytocin concentration in plasma. As for ghrelin, we have shown that it may stimulate oxytocin secretion without affecting noradrenalin concentrations in the PVN.

Acknowledgement: This study was supported by Turkish Scientific and Technical Research Council (TUBITAK Project No: 104S486) and by a grant of VEGA 0098.

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