Significance of orexin in the water metabolism and the regulation of vasopressin secretion

Ph.D. Thesis

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List of abbreviations

5-HT	serotonine
ADR	adrenalin
DA	dopamine
HA	histamine
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
ISH	*in situ* hybridization
LHA	lateral hypothalamus
NH	neurohypophysis
NADR	noradrenalin
OT	oxytocin
OX_1R	orexin-1 receptor
OX_2R	orexin-2 receptor
PCR	polymerase chain reaction
PVN	nucleus paraventricularis
RIA	radioimmunoassay
SON	nucleus supraopticus
VP	vasopressin

Introduction

The neuropeptides expressed by a specific population of neurons in the LHA, are called orexins (earlier: hypocretins). Orexin-A contains 33 amino-acids with two intramolecular disulfide bonds, while orexin-B is a linear 28-residue peptide. Orexin-A and orexin-B bind and activate the G-protein-coupled receptors, OX_1R and OX_2R, which are generated from two separate genes. Orexin-A has a 10-fold higher affinity to OX_1R than orexin-B. SB 408124 is a selective antagonist for OX_1R.

The orexins act as neurotransmitters in the regulation of the hormonal system and the maintenance of the energy balance via the food intake. Other observation have indicated that, either stimulation of LHA or following i.c.v. administration of orexins increases water intake and plays a physiological role in the regulation of drinking behaviour. Orexin-positive fibres and orexin receptor mRNAs coexist with NH hormones in the PVN and SON, such as VP and OT.

VP, this 9-amino-acid hormone is synthesized in the hypothalamic SON and PVN neurons and transported in packed neurosecretory granules into the NH by axons. Then VP is secreted into the circulation through the local portal veins. In contrast with the opinion that NH is only a storage site of the VP, it has been showed that dispersed cell cultures of isolated NH obtained from adult rats are able to synthesize and release VP. These observations are based on the immunological and spectrometrical identification of VP from the cell cultures. It has been also proved with ISH and PCR methods that the pituicytes of NH could be the sites of VP mRNS expression.

The monoaminerg compounds of the brain, which are localized in the magnocellular portion of the hypothalamus and which are secreted from nerve terminals into the posterior lobe of the hypophysis, play an important role in the regulation of VP secretion. These neuroactive compounds (HA, DA, ADR, NADR, 5-HT) enhance the VP secretion from pituicytes in isolated NH cell cultures *in vitro* and in rats *in vivo*. It is general that hyperosmotic stimulation, such as hyperosmotic NaCl i.p injection causes increased VP level in blood.

It appeared worthwhile to investigate whether the orexins exert their polydipsic and polyuric effects through alterations in VP secretion.

Aims

The anatomical localization and physiological roles of the above-mentioned neurotransmitters suggest that they are in functional connections with each other. We therefore designed our experiments to investigate the interrelation of VP secretion and water metabolism with the orexins *in vitro* and *in vivo*.

The following questions were examined by *in vivo* studies:

1. Are there any differences between the effects of either ic.v. administered orexin-A or orexin-B on the food or water intake in rats?
2. How can orexin neuropeptide modify the increased VP secretion in rats after osmotic stimulus (i.p. administration of 2.5% NaCl)?
3. How can orexin neuropeptide influence the non-osmotically-induced (i.p. administration of HA) VP level increase?
4. Can the OX_1R antagonist prevent the orexin-A-induced VP secretion changes in rats?

The following questions were examined by *in vitro* studies:

1. In isolated rat NH cell cultures is there any direct effect of the orexins on the VP release?
2. Do these two neuropeptides, orexin-A or orexin-B, modify the monoaminerg-induced VP release changes in the supernatant media of NH cell cultures?
3. How can orexin-A or orexin-B modify the VP release changes induced in the supernatant media of isolated NH cell cultures after aspecific osmotic stimulus (K^+ treatment)?
4. Can the OX_1R antagonist prevent the monoaminerg-induced VP secretion changes of the supernatant media of isolated NH cell cultures?

Materials and methods

In vivo experiments

180-230 g male Wistar were used. The rats were randomized before the experiments and housed for 1 week, and cannulated (into the right lateral ventricle) under ether anaesthesia 7 days before the studies.

Water consumption and food intake were measured for 6 and 4 h following i.c.v. injections.

Blood was obtained following decapitation 30 min after orexin administration and 15 min after hyperosmotic or HA stimulation. The plasma VP level was measured by RIA.

Statistical analysis was performed by using the Turkey-Kramer multiple comparison test.

The following compounds were used during the experiments:

1. Orexin-A or orexin-B (i.c.v. 10-30-90 µg/10 µl/animal)

2. 2.5% NaCl solution (i.p. 1ml/100g animal, 15 min after orexin administration)

3. HA (i.p. 1 mg/100g animal; 15 min after orexin administration)

4. OX_1R antagonist: SB408124 (N-(6,8-Difluoro-2-methyl-4-quinolinyl)-N′-[4-(dimethylamino)phenyl]urea) (i.c.v. 30 µg/10µl/animal, simultaneously administered with the orexin)

The animal care and research protocols were in accordance with the guidelines of our university and had been approved in advance by the appropriate ethical committee.

***In vitro* experiments**

The pituitary of male Wistar rats weighing 180-230 g was removed under sterile conditions immediately after decapitation under anaesthesia.

The posterior lobe was carefully separated and digested enzimatically (trypsin, collagenase, DN-ase I-II). The viability was 99-100%, and the cell count was determinate to be $2x10^6$/dish. The cell cultures were maintained in 24-well plastic plates with collagen at 37 °C and humified atmosphere of 5% CO_2 in air.

The VP level of the supernatant media was measured by RIA on days 13 or 14, the hormone content of the medium had become constant by this time.

For the study of the interactions of the orexins and the monoaminergic system, the compounds were administered in 60 min differences.

A modified Lowry method was used for the determination of total protein content.

Statistical analysis of VP concentrations was performed with the Kruskal-Wallis test.

The following compounds were used during the experiments:

1. orexin-A or orexin-B (10^{-10}-10^{-4} M)

2. HA, DA, ADR, NADR, K^+ (10^{-6} M)

3. SB408124 (10^{-6} M)

Results

In vivo experiments

Effects of orexins on water and food intake:

Our observations confirm that feeding behaviour can be stimulated by the orexins: the total food consumption during 1 h after orexin administration increased in a dose-dependent manner. One aim of our study was to investigate whether orexins can modulate drinking behaviour and thereby influence VP secretion. Orexin-A administered i.c.v. resulted in a significant increase in water consumption 4-6 h after the injection at all concentrations, but the elevation proved much higher for the 30 µg/10 µl dose. To reveal the manner in which the orexins possibly influence drinking behaviour, we carried out examinations with a specific OX_1R antagonist. SB 408124 in the same concentration as orexin-A considerably reduced this increase, but the water intake still remained significantly higher as compared with the controls.

Effects of orexin-A on plasma VP level:

Following the i.c.v. administration of orexin-A or SB 408124 in different concentrations (10-30-90 µg/10 µl, n= 8-10), the plasma VP level did not change relative to the untreated controls. Hyperosmotic stimulation with 2.5% NaCl solution injected i.p. resulted in a high increase in plasma VP concentration; this was reduced by previous orexin administration. The highest effect was observed for the 30 µg/10 µl orexin dose, but the VP level was not as low as that of the control. I.p. HA injection significantly increased the plasma VP concentration, to a much higher level than that following the hyperosmotic stimulus. Orexin administered i.c.v. before the HA injection in each case reduced the HA-induced increase in plasma VP concentration, but the VP levels still remained above those of the vehicle controls. At the most effective dosage (30 µg/10 µl), treatment with the specific OX_1R antagonist SB 408124 together with orexin-A after HA or hyperosmotic saline injection prevented the reduction in VP level increase.

In vitro experiments:

Following the administration of orexin-A or orexin-B, significant changes in the basal VP concentration were not observed in the cell culture medium.
E, NE or DA administration increased VP secretion in a similar manner. Preincubation with orexin-A or orexin-B reduced the E or NE-induced increases in VP level. DA

administration proved to be ineffective in this respect. Following E, NE or DA treatment, orexin-A or orexin-B did not induce changes in VP release. Preincubation with orexin-A or orexin-B reduced the HA or 5-HT-induced VP level increases, but the VP concentrations of the supernatant media remained above the control level. Substantial differences in decreasing effect were not observed between orexin-A or orexin-B. Neither orexin-A nor orexin-B induced changes in VP release following monoaminergic treatment. Orexins had no influence on the VP level increase induced by K^+, which causes non-specific hormone secretion.

Conclusions

1. Orexin-A or orexin-B increased the water consumption. The 30 µg/ 10 µl dosage resulted the highest effect. After orexin-A administration, the polydipsia was more pronounced. The OX_1R antagonist decreased the polydipsia significantly.
2. Histamine or hyperosmotic VP release enhancement was reduced by i.c.v. orexin administration. This inhibition was not observed following OX_1R antagonist administration.
3. Our results suggest that the effect of orexin on the water consumption or blockade of the histamine and osmotic-induced VP level increase is mediated by the OX_1R.
4. The changes in VP secretion of isolated rat NH cell cultures induced by the monoaminergic system can be directly influenced by the orexin system.
5. The interactions between the monoaminergic and orexin systems regarding VP secretion occur at the level of the posterior pituitary.

Summary

Our results show that the orexins stimulate food intake and water consumption, acting through the orexin receptors, and resulted polydipsia and polyuria. We have now demonstrated that the orexins are physiologically involved in the regulation of VP release: although the orexins do not cause a VP level enhancement themselves, they can clearly reduce the osmotically induced elevation of the plasma VP concentration both *in vitro* and *in vivo*, and the reduction is prevented by the OX_1R antagonist SB 408124 in the case of orexin-A treatment, which furnishes further evidence of the receptor-mediated pathway.
Via the results of *in vitro* studies we demonstrated that the interactions between the monoaminerg and orexin system occur both at the levels of hypothalamus and the posterior pituitary.

List of publications:

Total impact factor: *21,222*

Publications on the topic of the dissertation (IF: 7,924):

The osmotically and histamine-induced enhancement of the plasma vasopressin level is diminished by intracerebroventricularly administered orexin in rats
Gyöngyi K. Kis, Andor H. Molnár, Lejla Daruka, János Gardi, Kinga Rákosi, Ferenc László, Ferenc A. László, Csaba Varga
Pflügers Archiv - European Journal of Physiology (2012) Accepted IF: 3,354

The effects of orexins on monoaminerg-induced changes in vasopressin level in rat neurohypophyseal cell cultures
Gyöngyi K. Kis, Tímea Ocskó, Márta Gálfi, Marianna Radács, Zsolt Molnár, Kinga Rákosi, Andor H. Molnár, Ferenc László, Csaba Varga, Ferenc A. László
Neuropeptides (2011) 45: 385-389. IF: 1,917

Effects of orexin-monoaminergic interactions on oxytocin secretion in rat neurohypophyseal cell cultures
Tímea Ocskó, Márta Gálfi, Mariann Radács, Zsolt Molnár, **Gyöngyi K. Kis**, Kinga Rákosi, Andor H. Molnár, Ferenc László, Csaba Varga, Ferenc A. László
Regulatory Peptides (2012) Accepted IF: 2,473

Refereed abstract on the topic of the dissertation:

Effects of orexins on water intake and vasopressin secretion in rat
G.K. Kis, L. Daruka, K. Rákosi, AH. Molnár, F. László, Cs. Varga, F.A. László
Acta Physiologica (2011) Volume 202, Supplement 684 :P39 IF: 3,138

Publications in other themes:

Gervain Mihály, Vörös Erika, Molnár Andor, **Karcsú-Kis Gyöngyi**, László Ferenc, László A. Ferenc
Combined Treatment with Buserelin+Cabergoline in Patient with Prostate Cancer and Pituitary Macroprolactinoma.
J Cancer Therapy 2010; (1): 214-218. IF: 0,00

Tari I, **Kiss G** , Deer AK , Csiszar J, Erdei L , Galle A, Gemes K , Horvath F, Poor P, Szepesi A, Simon LM

Salicylic acid increased aldose reductase activity and sorbitol accumulation in tomato plants under salt stress.

Biologia Plantarum 2010; 54(4): 677-683. IF: 1,656

Mihály András, **Karcsú-Kis Gyöngyi**, Bakos Mónika, Bálint Erika

Cellular distribution of B-Raf protein kinase in the brainstem of the adult rat. A fluorescent immunohistichemical study.

Acta Biol. Szeged. 2007; 51; 7-15. IF: 0,000

Bereczki L, **Kis G**, Bagdi E, Krenacs L.

Optimization of PCR amplification for B- and T-cell clonality analysis on formalin-fixed and paraffin-embedded samples.

Pathol Oncol Res. 2007;13(3):209-14. IF: 1,272

Krenacs L, Schaerli P, **Kis G**, Bagdi E.

Phenotype of neoplastic cells in angioimmunoblastic T-cell lymphoma is consistent with activated follicular B helper T cells.

Blood. 2006; 1;108(3):1110-1. IF: 10,370

Poster presentations on the topic of the dissertation:

GK Kis, M Gálfi, M Radács, K Kádár, Z Molnár, AH Molnar, F László, C Varga, FA László

Effects of orexin-monaminergic interactions on vasopressin secretion in rat neurohypophyseal cell cultures

13th European Congress of Endocrinology

30 April – 4 May 2011, Rotterdam, The Netherlands

Karcsúné Kis Gyöngyi, Daruka Leila, Rákosi Kinga, Molnár H. Andor, Varga Csaba, László Ferenc, László A. Ferenc

Orexinek hatásának vizsgálata a vízháztartásra és a plazma vazopresszin szintjére patkányban

Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája, 2011. június 8-11., Pécs

Poster presentations on other topics:

Molnár Andor, **Karcsúné Kis Gyöngyi**, Rákosi Kinga, Miski Scerif, Korbonits Márta
Orexin-A hatása a bőr alatti és hasi zsírszövet AMPK aktivitására
„Sport- Kultúra- Életminőség" Nemzetközi Sporttudományi Konferencia, Pécs, 2010. október 27-29.

Ferenc A. László, Mihály Gervain, Erika Vörös, Andor H. Molnár, **Gyöngyi Karcsú-Kis**, Ferenc László
Macroprolactinoma after chronic buserelin treatment in patient with prostate cancer
12th European Congress of Endocrinology; 24-28 April 2010; Prague, Czech Republic

Karcsúné Kis Gyöngyi, Weiczner Roland, Krecsmarik Mónika, Mihály András
Raf-protein kinázok sejtszintű immunhisztokémiai lokalizációja patkány agytörzsben.
XI. MITT Konferencia, Szeged, 2007. január 24-27.
Ideggyógyászati Szemle 2007; 60(S1):31p.

Kis Gyöngyi, Bereczki László, DR. Bagdi Enikő, DR. Krenács László
In situ hibridizáció alkalmazása a tumordiagnosztikában
V. Magyar Sejtanalitikai Konferencia, Budapest, 2006. május 4-6.

Tari I, Simon LM, Deér KA, Csistár J, Bajkán Sz, **Kis Gy**, Szepesi Á
Influence of Salicylic Acid on Salt Stress Acclimatization of Tomato Plants: Oxidative Stress Responses and Osmotic Adaptation
Federation of European Societies of Plant Biology: The 14th FESPB Congress, August 23-27., 2004., Cracow, Poland

Hencová Mária, Kissné Deér Aranka, **Kis Gyöngyi**, Ábrahámné Gulyás Magdolna
Kadmium hatása busa és harcsa máj citokróm P450-függő enzimrendszerére
Szegedi Akadémiai Bizottság Kémiai Szakbizottság Környezetvédelmi és Analitikai Munkabizottság: „The 10th Symposium on Analytical and Environmental Problems",
Szeged, 2003. szeptember 29.