Volume 56(1) 75-92, 2012
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

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

Gyöngyi Karcsúné Kis

Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged, Hungary

Orexins have been described before a decade, from the lateral hypothalamic area. Orexin neurons project to multiple region in the brain, including the hypothalamic paraventricular and supraoptic nucleus, which are the main vasopressin (VP) producing cells in the central nervous system. Positive immunostaning of the orexin receptors (OX_1R and OX_2R) were also observed in these magnocellular regions. The influence of the orexins on the water metabolism has been proved, but the possible role of VP release in connection with polydipsia and polyuria has not been clarified.

The effects of the centrally administered neuropeptides orexin-A and -B on water intake and VP secretion after hyperosmotic and histamine (HA)-induced stimulus were studied *in vivo* in male Wistar rats; and the effects of monoamine (dopamine (DA), serotonin (5-HT), HA, adrenaline (ADR), noradrenaline (NADR)) and K^+ administration on VP secretion were studied *in vitro* in 13-14-day cell cultures from rat neurohypophysis (NH), and it was examined whether orexins can modify the induced VP release enhancement.

Increased water consumption was observed after the administration of both orexin-A or orexin-B. There were no changes in basal VP concentration of the plasma after the administration of different doses of the orexins. A significant increase in VP secretion was detected following HA and 2.5% NaCl administration, a moderate VP level enhancement was detected in the latter case. Centrally administered orexin-A blocked the VP level increases induced by HA or hyperosmosis. The inhibitory effects of orexin-A were prevented by specific OX_1R antagonist.

Following administration of orexin-A or orexin-B in increasing doses, significant changes were not observed in the VP levels of the supernatant media of the cell cultures from isolated rat NH. VP level substantially increased after NADR, ADR or 5-HT treatment, while the enhancing effects of DA, HA or K^+ administration were more moderate. Preincubation with orexin-A or orexin-B reduced the monamine-induced VP level increases, except in the case of DA. The decreases were significant, but the VP concentrations of the supernatant media remained high above the control level. There was no significant difference in the decreasing effect between orexin-A and orexin-B. Orexins had no influence on the VP level increase induced by K^+, which causes non-specific, receptor-independent hormone secretion. Orexin-A or -B did not induce any changes in VP release when administered after the monoamine-treatments. OX_1R antagonist treatment avoided the effects of the orexin-A preincubation on monoamine-induced VP level enhancements.

According to our results we concluded that: 1. Orexin-A or orexin-B can cause polydipsia. 2. Orexin-A (when administered i.c.v. *in vivo*) and both orexin-A or orexin-B (by preincubation in cell cultures) can reduce the induced VP release enhancement. 3. The effects of orexin-A on the water metabolism or on the VP level increases (either histamine- or osmotic-induced *in vivo*, or monoamine-induced in NH cell cultures) are mediated through the OX_1R. 4. The interactions of the orexin systems regarding VP secretion occur both at hypothalamic and at the level of the posterior pituitary.

Supported by SROP 4.2.2.-08/1-2008-0006, TÁMOP 4.2.1/B-09/1/KONV-2010-0005, HURO/0901/037/2.2.2.

Supervisors: Ferenc A. László, Csaba Varga
E-mail: karcsukis@gmail.com

Genetic analysis of the FMRFamide-related neuropeptides and their specific receptors in *Drosophila melanogaster*

Brigitta Kiss

Developmental Genetics Unit, Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Neuropeptides are produced and secreted by specific neurons in all Metazoan organisms. They steer important processes like reproduction, feeding, behaviour, circadian rhythm, etc. from worms to humans. They are also very important regulators of insect life. *Drosophila melanogaster* has 45 known neuropeptides. We use the excellent genetic system of the fruitfly to perform a systematic genetic analysis of the functions exerted by the FMRFa-related (FaRP) group of peptides (FMRFa, Dms, Dsk, NPF, sNPF) and their GPCR receptors (FR, Dms-R1 and -2, Dsk-R1 and -2, NPFR, sNPFR).

We have built a RNAi-based genetic system in which the FMRFa-related genes and their specific receptors can be silenced in pairwise combinations. For this we used the RNAi transgenes available from stock centers. The RNAi transgenes can be driven by the Gal4-inducible UAS promoter. Using the Act5-Gal4 „driver" which induced an ubiquitous and continuous expression of the RNAi double-stranded RNA, silencing of the *FMRFa, Dms, Dms-R1* and *Dsk-R2* genes resulted in complete lethality while the others remained viable. The lethal effect

was observed with the KK series of mutants where the silencing construct is inserted into a standard „landing site" in the 2nd chromosome ensuring an uniform strong expression. The same constructs inserted at accidental places (GD series) had no lethal effect, probably due to lower expression.

By P transposon remobilization, we isolated 8 intragenic deletion mutants in the *DMS-R1* gene which abolished the gene function. However, in contradiction to the RNAi results above, the homozygous mutant flies proved to be fully viable and fertile. The contradiction could be explained with the „off-target" effect: if the RNAi construct contains a ≥20-mer sequence motif shared by a non-target gene, both genes will be silenced, and the lethality can come from the non-specific off-target effect. Another possibility is that the lethality is caused by the Act-Gal4 driver activating the gene expression at tissues or organs in which the gene is not normally expressed. These possibilities are investigated now by using more specific drivers, e.g. elav-Gal4 which is restricted to the CNS neurons.

It is known that the 5' upstream DNA sequence regulates the expression pattern of the *FMRFa* gene in the *Drosophila* CNS (Benveniste and Taghert, J. Neurobiol. 38, 507-520), and certain parts of this sequence can reproduce specific parts of the pattern.We amplified by PCR these DNA sequences, cloned them into the pBPGUw vector upstream to the Gal4 coding sequence, and made transgenic stocks carrying these constructs. These new drivers can be used in future experiments to target UAS-dependent expression to the FMRFa-expressing neurons.

In cooperation with Dr.Michal Žurovec (Inst. of Entomology, Czech Acad. Sci., České-Budejovice) we test the above mutant combinations for their basic metabolism (CO_2 production) and moving activity. In CO_2 production there was no difference between the mutant and the wild type adults. However, the mutants showed significantly less moving activity than the wild type. Experiments to test the possible mutant effect on heartbeat frequency are also in progress.

Supervisor: István Kiss
E-mail: kiss.brigitta@brc.mta.hu

Biogas production from protein rich substrates

Etelka Kovács

Department of Biotechnology, University of Szeged, Szeged, Hungary

In the European Union currently there are approximately 6000-7000 biogas plants, none of them processes protein-rich waste. However, in the course of food production and processing many protein-rich by-products and waste are made. These are serious pollutants, their disposal is a costly and energy-consuming task. In the world millions of tons of protein-rich hazardous waste are produced continuously. Biogas is a renewable energy carrier and the production of biogas is associated with double benefits: elimination of environmental pollution problems is coupled with the generation of renewable energy carrier. The digestion effluent is used as fertilizer for agricultural nutrient recovery and eliminates the need for artificial fertilizers, the production of which is a highly energy demanding process. Anaerobic digestion of slaughterhouse waste presents a specific task because this waste stream is rich in proteins. Blood, casein and meat powder have a very low C/N ratio therefore they are not favorable substrates for biogas production. Several earlier attempts corroborated the inhibitory effects of elevated NH_3 – NH_4^+ concentrations on anaerobic digestion.

Anaerobic digestion of animal waste was investigated in batch and continuously stirred tank reactor experiments at 37°C. In all experiments efficient degradation of blood, casein and meat-powder containing samples were observed using a specially adapted microbiological consortium. Contrary to the findings published earlier ammonia did not inhibit the biogas process at concentrations up to 10 g N/dm^3.

As pH raises the free ammonia concentration increases significantly. The experiments were designed to compare the protein hydrolysis potential of substrates that were acclimated and non-acclimated to protein rich media.

Proteinase activities of the consortia were monitored regularly. The changes in acetate and ammonium-nitrogen concentrations were followed during the fermentations. Volatile fatty acid compositions (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic acids) were determined using HPLC to monitor the microbiological activity in the reactors. Total carbon and total organic carbon contents have been measured to determine the C/N ratio of the biomass. Volumetric biogas yields gave information about the efficacy of the anaerobic digestion process. The composition of the evolved gas was determined by gas chromatography.

Supervisor: Kornél L. Kovács
E-mail: kovacs.etelka@brc.mta.hu