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INCREASE IN OXYTOCIN AND VASOPRESSIN CONCENTRATION IN THE BLOOD OUTFLOWING FROM SELLA TURCICA REGION AFTER SUPERIOR CERVICAL GANGLION STIMULATION IN RAT

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The aim of the study was to investigate whether the stimulation of the superior cervical ganglion influences the oxytocin and vasopressin release into the blood in condition of the of the sella turcica integrity. The experiments were performed on male rats under urethane-chloralose anaesthesia. Four 0.7 ml samples of the blood from the sella turcica region flowing through a tube inserted in the maxillary interna vein were collected in the 30, 35, 60 and 90 min of the experiments. The animals were divided into three groups: 1) control, 2) after the exposition of superior cervical ganglion. 3) after the collection of the 1-st sample of the blood the superior cervical ganglion was electrically stimulated for 30 min with trains of pulses. Vasopressin (AVP) and oxytocin (OXY) were determined in the blood plasma by radioimmunoassay. Stimulation of the superior cervical ganglion evoked an significant increase of AVP and OXY relase into the blood. The increase of AVP release occurred after 30 min longer latency than the increase of OXY release.

K e y w o r d s: *blood from sella turcica region, oxytocin, vasopressin, superior cervical ganglion*

INTRODUCTION

Superior cervical ganglion participates in the release of hypothalamic releasing hormones (1—3) and hormones of the anterior pituitary lobe (2, 4—6). The posterior pituitary lobe partially remains also under the influence of impulsation of the superior cervical ganglion, as after its bilateral removal a decrease of noradrenaline (7—9), vasopressin (9) and oxytocin (10, 11) contents is observed in the posterior lobe. The removal of the superior cervical ganglia prevented the formation of "a miniature neurohypophysis" on the central end of transsected pituitary stalk (12) and was followed by degeneration of neurosecretory nuclei of the hypothalamus (13). The above data may point to the stimulatory character of the superior cervical ganglia in the function of

the posterior pituitary lobe. However, some authors have reported its inhibitory effect, as after the superior cervical ganglionectomy in the phase corresponding to the increased release of noradrenaline from degenerating post-ganglionic ends the concentration of plasma vasopressin decreased (9).

Our earlier studies point to the stimulatory influence of the superior cervical ganglia in the function of the posterior pituitary lobe, because after electric stimulation of preganglionic fibres of the superior cervical ganglia the release of vasopressin (14) and oxytocin into the fluid incubating the posterior pituitary lobe in situ (15, 16) increased.

Due to contradictory literature reports, the subject of the present work is to reveal how the stimulation of the superior cervical ganglia influences the release of vasopressin and oxytocin into blood in condition of the sella turcica integrity.

MATERIAL AND METHODS

The experiments were performed on 76 male rats 300—340 g of body weight, being the F_1 generation of cross-breeding of August strain males and Wistar strain females from the stock of the Institute of Oncology in Warsaw. The animals were kept under constant temperature, 14:10 h light : dark cycle and received standard rat pellets (LSM) and water *ad libitum.*

The anaesthesia was induced by intraperitoneal injection of urethane (Fluka AG, CH-9470 Bucks) 50 mg/kg b.w. together with chloralose (Roth) 6 mg/kg b.w.

The experiments we performed on the three groups of animals:

(1) control group-intact animals,

(2) sham operated group — after the exposition of the superior cervical ganglion,

(3) electrically stimulated the superior cervical ganglion group — after the collection of the 1-st sample of blood

Blood sampling

Polyethylene tubing was inserted in the vicinity of cavernous sinus through, the internal maxillary vein and 0.5 ml of 0.9% NaCl with heparin (1000 UJ/ml) was injected into the internal maxillary vein at the beginning of the blood collection. Four 0.7 ml samples of the blood (in the time 1 min) were collected in the 30th, 35th, 60th, 90th min of the experiments. The blood was immediately centrifuged, plasma was frozen and the cells were resuspended in 0.9% NaCl with heparin and returned to the animal *via* the cut central end of internal maxillary vein before the next samples was collected.

At the end of each experiment 1% solution of trypan blue (Chemapol, Prague) was injected in the vicinity of cavernous sinus of the sella turcica *via* a cannula inserted into the internal maxillary vein. The heads of the animals were cut off and kept in 10% formalin for several days. The brains were then removed from the skull and the posterior pituitary lobes were verified under stereomicroscope. Only animals showing the staining of the posterior pituitary lobe were included in our results. Staining of the posterior pituitary lobe has proved proper insertion of the cannula into the vicinity of the cavernous sinus of the sella turcica, and proper blood collection.

Electrical stimulation of the superior cervical ganglion

The tissues around the left common carotid artery were drawn laterally and the superior cervical ganglion was exposed. Bipolar platinum electrodes were slipped under the ganglion so that the electrodes did not come into contact with adjacent tissues. Electrodes were connected to a Disa stimular Type 13 G04. Stimulation parameters were monitored on a Cossor oscilloscope ST 509A. For stimulation of the superior cervical ganglion monophasic electric pulses of the following parameters were applied: frequency 20 Hz, duration 3 msc, amplitude 10 V, (30 sec stimulation on and 30 sec stimulation off) for 30 min. Ipsilateral dilatation of the palpebral fissure was observed during the stimulation.

Extraction of vasopressin and oxytocim from blood plasma

0.4 ml of acetone was added to 0.4 ml of plasma. The mixture was strirred on Micro-Shaker 326 m for 15 min and then centrifuged. The precipitate was discarded and the supernatant was gently mixed with 0.8 ml of benzene. The top benzene phase was then removed and discarded. The remaining delipidated lower aqueous acetone phase was blown to dryness by nitrogen at 35—40°C and stored at −20°C until assayed.

For the estimation of recovery of known quantities of the added hormone trough the extraction procedure, unlabeled vasopressin and oxytocin was added to the plasma to give concentration of 2.2—35.7 pg/tube, extraction was performed and vasopressin or oxytocin was determined. The recovery was estimated to approximate 56% for vasopressin and 50% for oxytocin. Values given for plasma vasopressin and oxytocin in this paper have not been corrected for losses during extraction.

Radioimmunoassay

Anti-AVP and anti-OXY antibodies were raised in rabbits according to More et. al. (17). Arginine vasopressin and oxytocin used for immunization, iodination and as standard was synthesized in Institute of Organic Chemistry, Technical University of Lodz. OXY and AVP was jodinated with ^{125}I-using the chloramine-T method (18). The sensitivity of anti-AVP serum was 1.73 pg per tube and that of anti-OXY serum-3.56 pg per tube. Characteristics of anti-AVP and anti-OXY antiserum obtained from rabbits and used in radioimmunoassay were described previously (19).

Statistical evaluation of the results

The vasopressin or oxytocin concentration was finally expressed in pg per ml of blood plasma as mean ± standard error of the mean (SEM). For statistical evaluations Student's t-test was used.

RESULTS AND DISCUSSION

Vasopressin and oxytocin release in the control blood (1st experimental group) of the examinated rats exceed 10-fold the values known from the literature data (20—22). It is probably due to the fact that in our experiments

neurohormones were determined in blood flowing directly from the area of the sella turcica, as the cannula taking blood was inserted into the vicinity of cavernous sinus. Blood obtained this way contained more neurohormones than peripheral blood (21).

Exposure of the superior cervical ganglia (2nd experimental group) like the electrical stimulation of the superior cervical ganglion (3rd experimental group) caused 2.5-fold increase in oxytocin release into the blood during 60 min (*Fig. 1*). Vasopressin release increased 6-fold, the most in 30th min after the superior cervical ganglion electrical stimulation similarly as after exposure of the superior cervical ganglion (*Fig. 2*).

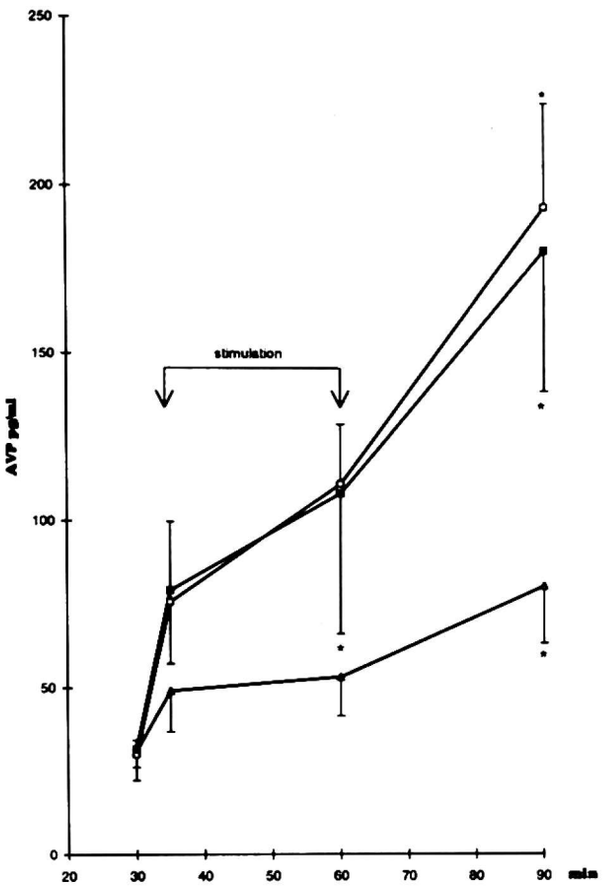

Fig. 1. 1. Oxytocin release into the blood from the vicinity of cavernous sinus sella turcica in 30, 35, 60 and 90 min of experiments [pg/ml]. Mean ± SE. —▲— control n = 12; —□— sham operation n = 12; —■—in 31—60 min of experiments superior cervical ganglion was stimulated: 10 V, 20 Hz, 3 ms [30 s on, 30 s off] n = 16.
Statistical significance calculated against initial values $p < 0.01$.

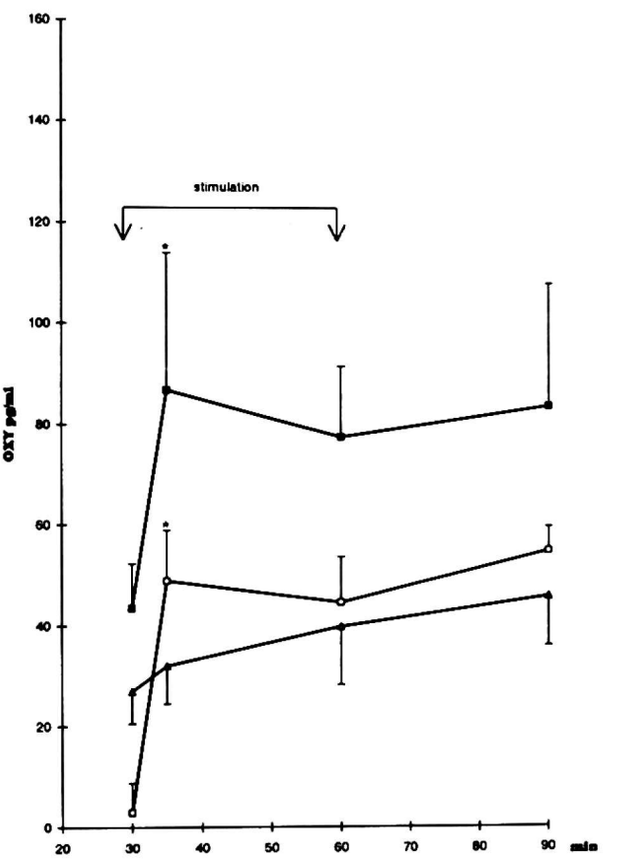

Fig. 2. Vasopressin release into the blood from the vicinity of cavernous sinus sella turcica in 30, 35, 60 and 90 min of experiments [pg/ml]. Mean ± SE. —▲—control n = 12; —□— sham operation n = 12; —■— in 31—60 min of experiments superior cervical ganglion was stimulated: 10 V, 20 Hz, 3 ms [30 s on, 30 s off] n = 12.
Statistical significance calculated against initial values $p < 0.01$.

The frequency of electrical pulses used in our experiments is an efficient stimulus to release acetylcholine from preganglionic (22), noradrenaline and coexisting peptides from postganglionic fibres (23). The same stimulation as

regards frequency, duration, amplitude of electric pulses with the same length of on and off stimulation evoked also an increase of oxytocin and vasopressin (14, 15) release into the medium incubation of the posterior pituitary lobe incubated „*in situ*", as well as the decrease of the amount of neurosecretory granules in the posterior pituitary lobe (24). The decrease of the amount of neurosecretory granules in the neurohypophysis may be caused by an increased release of the neurohormones after the superior cervical ganglion stimulation.

The obtained results indicate that electrical and mechanical excitation of the sympathetic system by the stimulation of the superior cervical ganglion increases the release of vasopressin and oxytocin into the blood. Romeo et al. (25) postulate that stimulation of the sympathetic efferents inhibits the release of vasopressin, as after superior cervical ganglionectomy, in the „wallerian degeneration" phase of sympathetic fibres, in which an increased release of noradrenaline from the postganglionic endings (imitating the physiological activation) is due to occur, the plasma concentration of vasopressin decreased. It seems, however, that electric stimulation is closer to physiological conditions of transmitter release than an increased release of noradrenaline from degenerating postganglionic endings caused by superior cervical ganglionectomy (26).

Inhibitory or excitatory effects of noradrenaline on vasopressin and oxytocin release can depend on the adrenoceptor subtype involved in mediating mechanism (27—30). In anesthetized dogs, the inhibition of noradrenaline release to intraventricular infusion of noradrenaline was blocked by the α_2-antagonists, yohimbine, but not by the α_1-antagonists, prazosin (31). In conscious rats, centrally administred noradrenaline and α_1-agonist phenylephrine — increased vasopressin release, whereas α_2- and β-agonists were inhibitory (28). In the latter study, evidence for a tonic inhibitory influence of noradrenaline on vasopressin was provided by showing elevations in plasma vasopressin following the infusion of β- or α_2-antagonists. Intravenous administration of the α_2-agonist, clonidine, also decreased vasopressin release in anaesthetized dogs even when its peripheral pressor effects had been abolished (32). Thus current results from *in vivo* studies would suggest that central α_2- and β-adrenoceptors mediate inhibitory effects of noradrenaline on vasopressin release, wherease the α_1-subtype mediates excitation. The inhibition of vasopressin release in cultured hypothalamus-neurohypophysial explants *in vitro* by noradrenaline and its prevention by nonspecific α-antagonists provides evidence that these receptors are localized within the ventral hypothalamus, perhaps on the supraoptic nucleus neurons themselves (27).

In our previous research the application of short (5 s on and 5 s off) bursts of impulses stimulating the preganglionic fibres of the superior cervical ganglia did not affect the release of vasopressin and oxytocin (14—16) into the fluid

incubating the posterior pituitry lobe *in situ*, while the application of long bursts (30 s) with a long break increased significantly the release of both neurohormones. Lundberg and Hökfelt (31) revealed that the release of the transmitter and/or peptide modulators from the postganglionic endings in the autonomic nervous system due to electric stimulation is dependent on the characteristics of the applied stimulatory impulses. The fact of obtaining convergent results as concerns the release of neurohormones resulting from the stimulation of the sympathetic efferents may be connected with the release of noradrenaline and/or peptide modulators, which is dependent on the way of stimulation of this system, or the kind of receptor with which the separate chemical transmitter will bind (2, 5).

The release of vasopressin after the stimulation of the superior cervical ganglia occurs after a longer period of latent stimulation than the release of oxytocin, which may point to a greater reactivity of oxytocynergic system as compared to the vasopressinergic system. Peptide modulators present, similarly as noradrenaline, in neurons of the superior cervical ganglia (32) may be responsible for this late effect.

To sum up it may be concluded that sympathetic system influences the release of vasopressin and oxytocin. The mechanism of this action, however, still remains an open question and requires further research.

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