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INCREASE IN VASOPRESSIN RELEASE INTO THE HYPOPHYSIAL PORTAL BLOOD 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 may influence vasopressin and oxytocin release into the hypophysial portal blood. In urethane-chloralose anaesthesia the pituitary gland was exposed by transpharyngeal approach in rats. The hypophysial portal vessels were transsected in the narrowing between glandular portion of the hypophysis and the infundibulum. The 15 min blood samples from the cut portal vessels were collected before and during electrical stimulation of the superior cervical ganglion. Vasopressin and oxytocin content in the plasma were determined by radioimmunoassay. In the control samples the vasopressin content amounted to 3.2 ± 1.03 ng/mL and oxytocin 0.75 ± 0.3 ng/mL. Stimulation of the superior cervical ganglion evoked an increase (9.6-fold) in vasopressin concentration but not in oxytocin in the blood plasma of hypophysial portal vessels. On the basis of the results obtained, it may be presumed that the sympathetic efferents arising from the superior cervical ganglion induced only vasopressin but not in oxytocin release into the hypophysial portal blood.

K e y w o r d s: *vasopressin, oxytocin, superior cervical ganglion, hypophysial portal blood.*

INTRODUCTION

The majority of vasopressinergic and oxytocinergic neurons of the supraoptic and paraventricular nuclei origin project to the neural lobe of the pituitary. However, vasopressinergic and oxytocinergic axons project also to other structures of the cental nervous system, among them to the median eminence where both vasopressinergic and oxytocinergic axons terminated on the pituitary primary portal vessels (1—3). This fact associated with the finding of high concentration of vasopressin and oxytocin in hypophysial portal blood (4—6) suggests the participation of these peptides in the release of anterior

pituitary hormones. It has been established that vasopressin and oxytocin play an important role in the control of adrenocorticotropic hormone secretion (8—11). They may also be important as stimulators of thyrotropin-releasing hormone and growth hormone release (12, 13). Although earlier studies eliminated vasopressin as a corticotropin-releasing factor (10), recent evidence suggests that oxytocin may augment the response of corticotropes to corticotropin-releasing hormone (8, 14). Oxytocin is also known to be a stimulator of prolactin and thyrotropin-releasing hormone (12, 15, 16) and inhibitor of growth hormone release (12). It was also shown that vasopressin and oxytocin influenced gonadotropins secretion (17, 18).

Only scarce information has been available so far on the mechanism of the regulation of neurohormones release into hypophysial portal blood. Hypothalamic catecholamine depletion had no effect on the release of vasopressin into hypopysial portal blood (19). The noradrenergic innervation of the hypothalamo-hypophysial system has been postulated to converge impulses of the central and autonomic origin (20). The autonomic innervation derives from the superior cervical ganglia (20). Our previous data indicate that stimulation of the superior cervical ganglion caused an increase in the release of vasopressin and oxytocin from the posterior pituitary lobe incubated "*in situ*" (21, 22).

The aim of the present study was to investigate whether the stimulation of the superior cervical ganglion may influence vasopressin and oxytocin simultaneous release into hypophysial portal blood.

MATERIAL AND METHODS

The experiments were performed on 24 males 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, Switzerland) 50 mg/kg b.w. together with chloralose (Roth, Germany) 6 mg/kg b.w.

Exposure of the hypophysial portal vessels

As soon as anaesthesia occurred the animal was immobilized on a special operating board by introducing ear bars into the external ears and fixing the upper jaw with a clamp. The skin and the subcutaneus tissue in the left inguinal region were infiltrated with 4% Polocaine (Polfa, Poland), the left femoral vein was exposed, cut and through it fine polyetylene tubing was introduced into the vena cava inferior. Through the tubing 1.5—2.0 ml of 3% solution of dextran (110.000 molecular weight) in 0.9% NaCl solution was slowly injected until the end of preparation.

The skin, subcutaneus tissues and the muscles on the neck were infiltrated with 4% Polocaine solution in midline and along both mandibular rami. The skin was cut in the midline, the trachea

was exposed and polyetylene tube was placed in it and tied. The mandible was cut in the midline and the bones of the base cranium were reached according to the technique described in detail by Worthington (23) using a binocular dissecting stereomicroscope. With a dental drill, a longitudinal hole was made in the sphenoid bone to expose the dura mater between the posterior margin of the optic chiasma and the pons. The dura was cut longitudinally with a special fine sharp angular knife. Through a polyethylene tube inserted into the femoral vein, 1000 i.u. of heparin (Polfa, Poland) was injected.

Blood collection from hypophysial portal vessels

The hypophysial portal vessels were transsected superficially using irs scissors in the narrowing between the glandular portion of the hypophysis and the infundibulum. The cut vessels were constantly washed with 0.9% NaCl solution and aspirated together with blood from hypopysial portal vessels. Six 25-min samples of blood diluted with 0.9% NaCl (1 mL/15 min) were collected and centrifuged. In the supernatant the vasopressin and oxytocin concentration were determined. Diluted hypophysial portal blood was filled up to the volume of 2 mL and hemoglobin concentration was determined spectroscopically according to cyanmethemoglobin method. The volume of blood outflowing from cut portal vessels was calculated on the basis of the hemoglobin concentration as described previously (27).

Electrical stimulation of the superior cervical ganglion

The tissues around the left common carotid artery were drawn laterally and the left 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 stimulator Type 13 G04. Stimulation parameters were monitored on a Kabid oscilloscope ST 509A. For stimulation of the superior cervical ganglion monophasic electric pulses of the following parameters were applied: frequency 20 Hz, duration 3 msec, amplitude 10 V, (30 sec stimulation on and 30 sec stimulation off). During the stimulation of the superior cervical ganglion ipsilateral dilatation of the palpebral fissure was observed.

Radioimmunoassay of oxytocin and vasopressin

Arginine vasopressin (Lot 1987-08-24) and oxytocin (Lot 1988-02-03) (synthesized in the Institute of Organic Chemistry, Technical University of Lodz) were conjugated with high-molecular ligands by the carboimide method (24). The emulsion was injected intradermally, in the dose of 100 μg, into multiple sitea on the back of New Zeland rabbits at one week intervals, during four weeks. A booster injection of arginine vasopressin was administred 12 weeks after the 4th injection. 10 days later blood was collected and serum was separated.

Characteristic of vasopressin and oxytocin antisera

The titer to be used in the radioimmunoassay for anti-arginine vasopressin antibody No 1228/1987-08-24 was 1:2400 and for anti-oxytocin antibody No 1232/1988-02-03 was 1:80000 (both final dilutions). Cross reactivity with oxytocin for anti-arginin vasopressin antibodies was 0.016%, with lysin vasopressin — 2.7%, with gonadotropin-releasing hormone, thyrotropin releasing hormone, leucine enkephalin, angiotensin II, Substance P (SP), SP hexapeptide (pyr-Glu^6-Tyr^8)SP$_{6-11}$ and SP hexapeptide (Tyr^8)SP$_{6-11}$ it was 0.002%. Cross reactivity with

arginine vasopressin for oxytocin antiserum was 1.12%, with luliberin, thyrotropin releasing hormone, leucine enkephalin and angiotensin II it was 0.002%. The sensitivity of arginine vasopressin antiserum was 1.73 pg per tube that of oxytocin antiserum — 3.56 pg per tube.

Iodination of arginine vasopressin and oxytocin

Arginine vasopressin, Peninsula Laboratorie, Inc Lot No 01115907 (Lot) and oxytocin Peninsula Laboratorie, Inc. Lot No 027179 were iodinated with ^{125}I using the chloramine-T method (25). Unreacted iodide was removed by mixing the reaction mixture with Amberlite (ion Exachange Resin, type CG-400, Serva). Further purification was carried out on column of Sephadex G-25 Fine (Pharmacia) pre-equilibrated and eluated with 0.05% mol/L acetic acid. Labeled vasopressin and oxytocin were identified in the third peak by their ability to bind to the corresponding antibodies (26). The effectivenes of the iodination procedure was 70—90%. The top or the 1 st descending portion of this peak was used as the tracer in radioimmunoassay. Intra and interassay coefficients of variation for the vasopressin assay were 3.9 and 6.4, respectively; for the oxytocin assay the coefficients of variation were 4.8% and 8.4, respectively.

Extraction of vasopressin and oxytocin from diluted blood plasma

0.4 mL acetone was added to 0.8 mL of diluted blood plasma with 0.9% NaCl. The mixture was strirred on Micro-Shacker 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 lyophilized.

For the estimation of recovery of known quantities of the added hormone through the extraction procedure, unlabeled vasopressin and oxytocin were added to the plasma to give concentration of 2.2—35.7 pg/tube, extraction was performed; vasopressin and 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.

Statistical evaluation of the results

The vasopressin and oxytocin concentration were finally expressed in pg/min of blood plasma as mean $\pm$ standard error of the mean (SEM). Analysis of variance followed by Duncan's multiple range test was used to establish the significance of difference. Differences were considered to be significant at $p < 0.05$.

RESULTS

The hypophysial-portal blood flow was 3.0 ± 0.1 μL/min. Electrical stimulation of the superior cervical ganglion did not change hypophysial portal blood flow (*Fig. 1*).

In the control group of animals the oxytocin release into hypophysial portal blood did not differ in consecutive samples and amounted to 2.48 ± 0.93 pg/min (*Fig. 2*). Stimulation of the superior cervical ganglia did not change the oxytocin release into the hypophysial portal blood (*Fig. 3*).

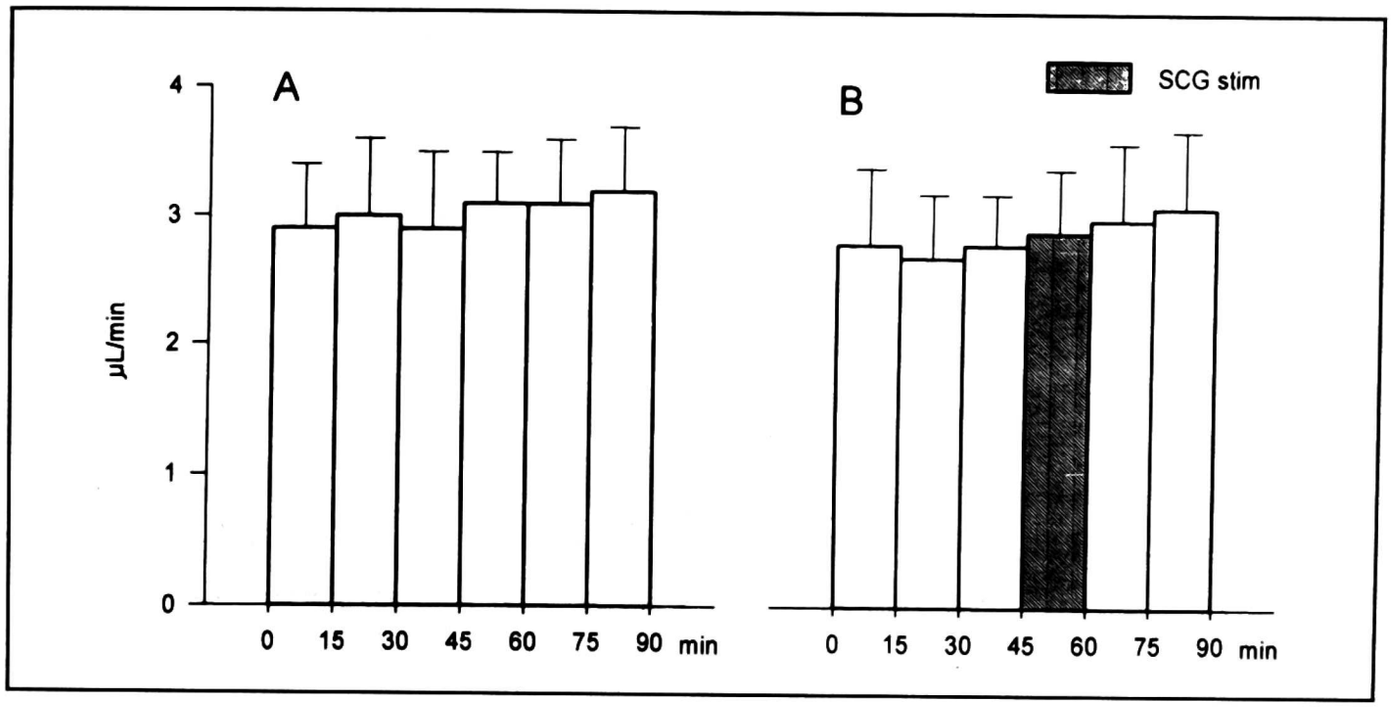

Fig. 1. Hypophysial portal blood flow in µL/min (mean ± SE). A — control; B — during collection 4-th sample the superior cervical ganglion (SCG) was stimulation (10 V, 20 Hz, 3 ms).

Vasopressin release into the hypophysial portal blood in the control group was 9.6 ± 3.1 pg/min (*Fig. 2*). Stimulation of the superior cervical ganglia evoked an increase (9.6-fold) in vasopressin release into the blood hypophysial portal vessels (*Fig. 3*).

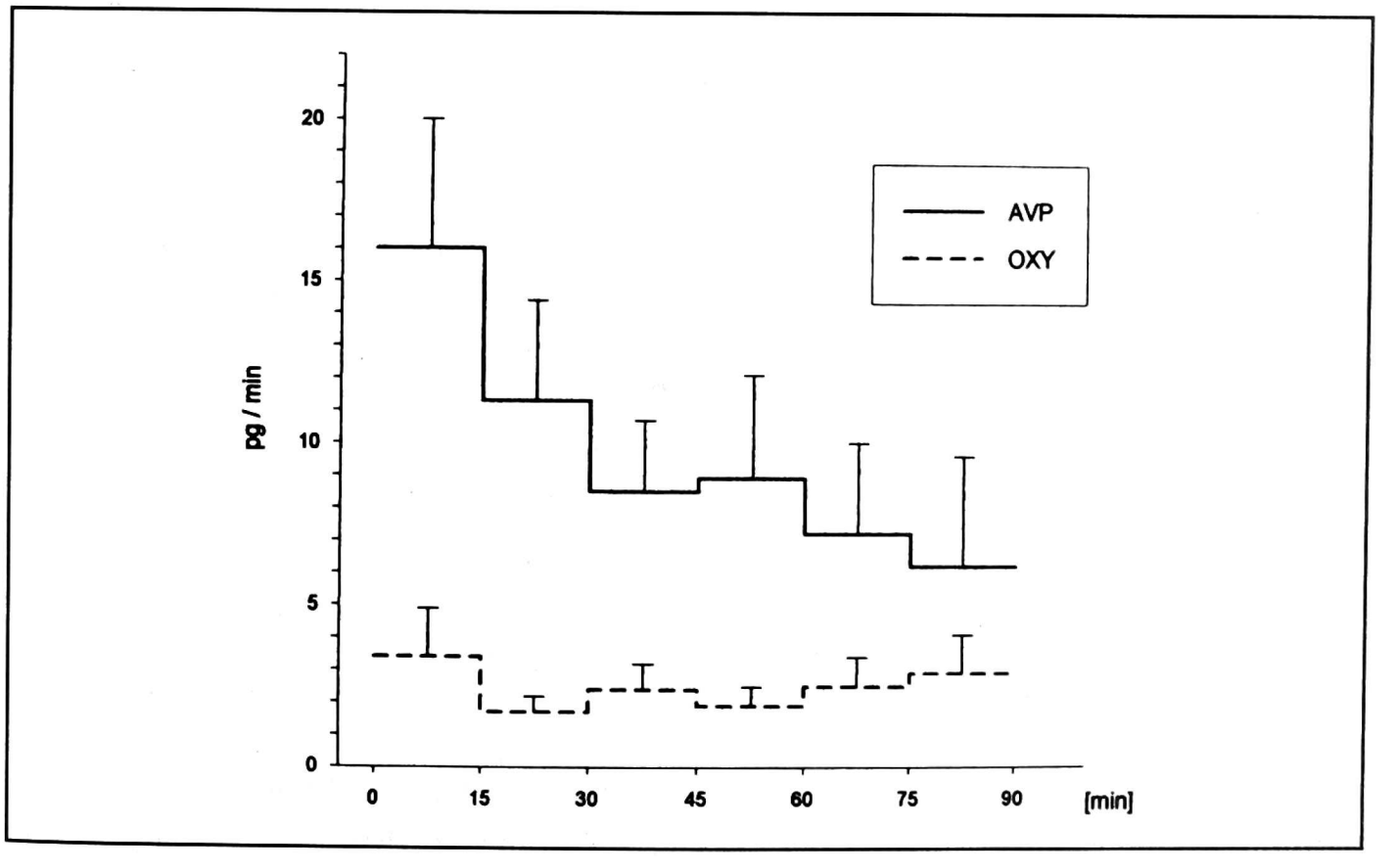

Fig. 2. Vasopressin and oxytocin release into the hypophysial portal blood. mean ± SE, n = 12. Hypophysial portal blood flow in the same animals is presented on *Fig. 1–A*.

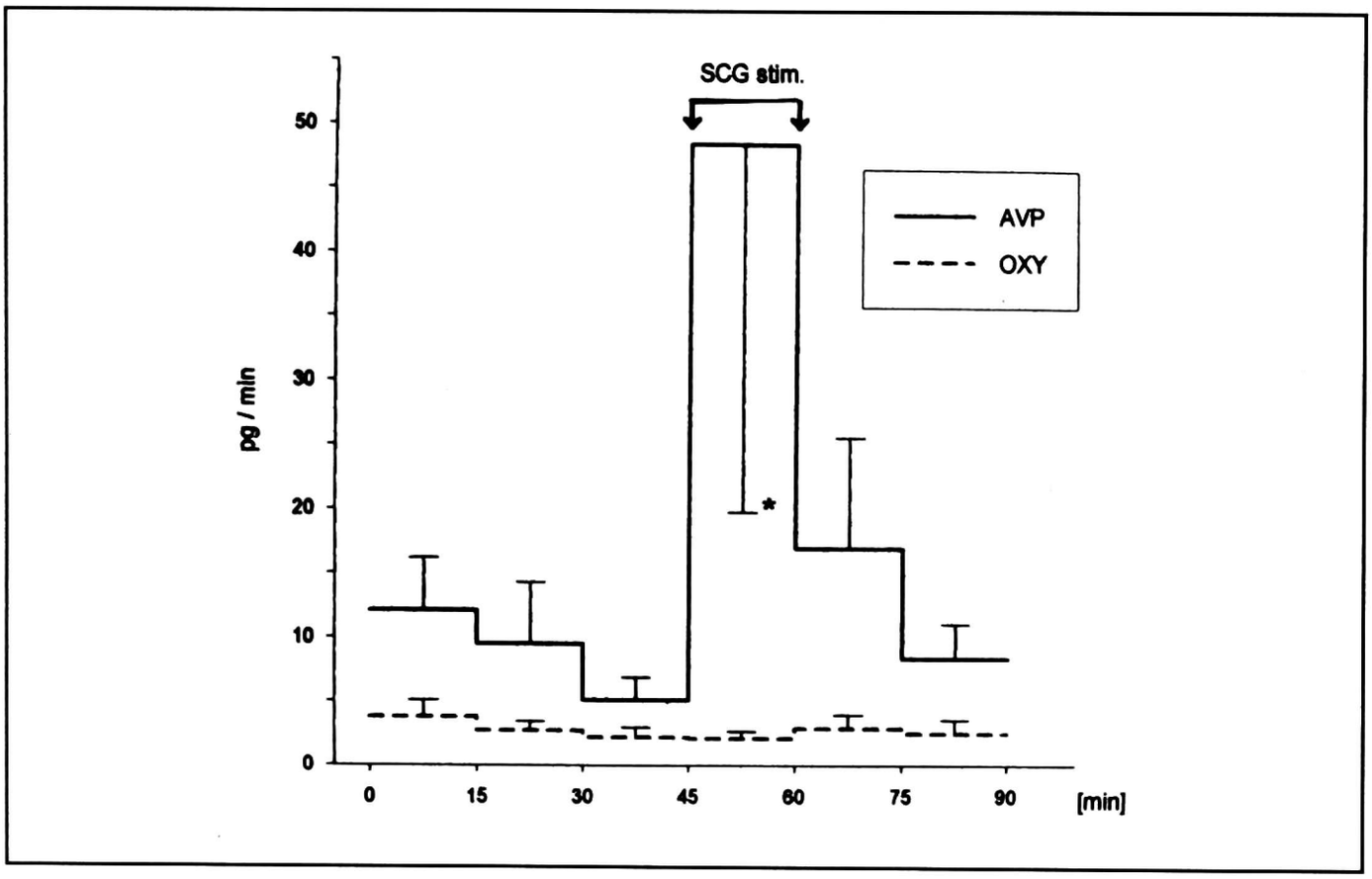

Fig. 3. Vasopressin and oxytocin release into the hypophysial portal blood. During collection 4-th sample the superior cervical ganglion was stimulation (10 V, 20 Hz, 3 ms); mean ± SE, n = 12. *$p < 0.05$. Hypophysial portal blood flow in the same animals is presented on *Fig. 1–B*.

DISCUSSION

The method applied in this study enables collection of the blood from hypophysial portal vessels. Hypophysial portal blood flow was constant during all experiments. In our experiments we have not obtained any changes in hypophysial portal blood flow after electrical stimulation of the superior cervical ganglion. Similar results were obtained in previous studies (27). It has been suggested that sympathetic efferents do not participate in the regulation of hypophysial portal blood flow. Therefore it means that the concentration of the hormones in the hypophysial portal blood does not depend on the quantity of the blood flow.

The obtained data of neurohormones concentration in hypophysial portal blood are consistent with other reports of a high concentration of vasopressin and oxytocin in blood from the same vessels (4—7). Electrical stimulation of the superior cervical ganglion stimulates of the sympathetic system which takes place in many physiological and pathophysiological conditions, e. g. during stress (28, 29). The obtained results indicate that excitation of the sympathetic system by electrical stimulation of the superior cervical ganglion causes a considerable increase (9.6-fold) in vasopressin release, but has no effect on the

release of oxytocin into blood of the hypophysial portal system. In our studies we have demonstrated in the increase in both oxytocin (21) and vasopressin (22) release from the posterior pituitary lobe after superior cervical ganglion stimulation. Romeo *et al.* (28) postulate that stimulation of the sympathetic system by the stimulation of the sympathetic efferents inhibits the release of vasopressin into the blood, as after superior cervical ganglionectomy, in the "walerian 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 decreases. 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 (30). Inhibitory or excitatory effects of noradrenaline on vasopressin and oxytocin release can depend on the adrenoceptor subtype (31—34), and/or peptide modulators (35, 36) involved in mediating mechanism.

Electrophysiological studies have historically supported the role of noradrenaline in the control of supraoptic nucleus of hypothalamus; the initial study using iontophoresis of noradrenaline antidromically identified neurons indicated that noradrenaline was primarily inhibitory in cats, and that the inhibition was mediated by a β-receptor (37). Inhibition of rat supraoptic nucleus neurons was also the predominant effect seen by Arnauld *et al.* (38), but in this study noradrenaline delivered with lower currents was sometimes excitatory to some phasically firing (i.e., putative vasopressin secreting) neurons. More recently, investigators have found that noradrenaline and α_1-receptor agonists consistently excite most phasic supraoptic nucleus neurons in slices (39) and explants of the hypothalamus-neurophypophysial *in vitro* when applied through the bath or by micropressure (40). In acutely prepared explants (i.e., those tested on the day of excision), stimulation of tuberal supraoptic nucleus neurons by noradrenaline and the α_1-agonists phenylephrine was concomitant with a dose-dependent release of vasopressin into the perfusate, whereas β-and α_2-agonists proved ineffective (41). The results from *in vivo* studies suggest that central and β-adrenoceptors mediate inhibitory effects of noradrenaline on vasopressin release, whereas 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 (30).

The frequency of electrical pulses used in our experiments is an efficient stimulus to release acetylcholine from preganglionic fibers (42), noradrenaline and coexisting peptides from postganglionic fibres (43). The same stimulation as regards frequency, duration, amplitude of electric pulses with the same

lenght of "on" and "off" stimulation evoked also an increase in oxytocin and vasopressin (21, 22) release into the incubation medium of the posterior pituitary lobe incubated "*in situ*" and into the blood outflowing from sella turcica region (44), as well as the decrease of the amount of neurosecretory granules in the posterior pituitary lobe. The decrease of the amount of neurosecretory granules in the neurohypophysis may be caused by an increased release of neurohormones after the superior cervical ganglion stimulation (45).

As it was said in the introduction, vasopressin and oxytocin released into the blood of the pituitary portal system fulfil another physiological role than the same neurohormones released from the posterior pituitary lobe, and therefore, mechanism regulating their release may take various courses. In literature there have been very few reports concerning the regulation of vasopressin and oxytocin release into the pituitary portal blood. The lack of influence of hypothalamic catecholamines and glucocorticoids on the release of vasopressin and oxytocin into the pituitary portal vessels has been proved (46). On the other hand, the removal of the posterior pituitary lobe caused an increase in vasopressin and oxytocin content in hypophysial portal blood (47).

Peripheral noradrenergic innervation arising from the superior cervical ganglion exerts a modulation influence on the release of hypothalamo-hypophysial hormones, which may be accomplished by the effect of mediator and modulators released on postganglionic endings, (34, 25) surrounding hypothalamic neurons secreting hypophysiotropic hormones, on the secretory cells of the anterior lobe of the hypophysis, as well as, indirectly, by the blood of the hypophysial portal system.

One the basis of results obtained, it may be presumed that the sympathetic efferents arising from the superior cervical ganglion induced only vasopressin but not oxytocin release into the hypophysial portal blood.

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REFERENCE

1. Silverman AJ. Ultrastructural studies on the localization of neurohypophysial hormones and their carrier proteins. *J Histochem Cytochem* 1976; 24: 816—827.
2. Vandersnde F, Diereckx K, de Mey J. The origin of vasopressinergic and oxytocinergic fibers of the external region of the medium eminence of the rat hypophysis. *Cell Tissue Res* 1977; 180: 443—452.

3. Zimmerman EA, Stillman MA, Recht LD, Antunes JL, Carmel PW. An axonal pathway to portal capillaries in the zona externa of the median eminence containing vasopressin and its interaction with adrenal corticoids. *Ann NY Acad Sci* 1977; 249: 405—414.
4. Zimmerman EA, Carmel PW, Husain MK *et al.* Vasopressin and neurophysin: High concentration in monkeys hypophysial portal blood. *Science* 1973; 182: 925—927.
5. Gibbs DM. High concentrations of oxytocin in hypophysial portal plasma. *Endocrinology* 1984; 114: 1216—1218.
6. Recht LD, Hoffman DL, Haldar J, Silverman A, Zimmerman EA. Vasopressin concentrations in hypophysial portal plasma: insignificant reduction following removal of the posterior pituitary gland. *Neuroendocrinology* 1981; 33: 88—90.
7. Horn AM, Robinson ICAF, Fink G. Oxytocin and vasopressin in rat hypophysial portal blood: experimental studies in normal and Bratleboro rats. *J Endocrinol* 1985; 104: 211—224.
8. Link H, Dayanishi G, Fohr KJ, Gratzl M. Oxytocin at physiological concentrations evokes adrenocorticotropin (ACTH) release from corticotrophs by increasing intracellular free calcium mobilized mainly from intracellular stores. Oxytocin dispays synergistic or additive effects on ACTH-releasing factor or arginine vasopressin-induced ACTH secretion respectively. *Endocrinology* 1992; 130: 2183—2191.
9. Salata RA, Jarret DB, Verbalis JG, Robinson AG. Vasopressin stimulation of adrenocorticotropin hormone (ACTH) in humans. In vivo bioassay of corticotropin-releasing factor (CRF) which provides evidence for CRF mediation of diurnal rythm of ACTH. *J Clin Invest* 1988; 81: 766—774.
10. River C, Vale W. Interaction of corticotropin releasing factor and arginine vasopressin on adrenocorticotropin secretion in vivo. *Endocrinology* 1983; 113: 939—942.
11. Ecland DJ, Harbuz MS, Jessop DS, Linghtman SL. Corticotropin-releasing factor and arginine vasopressin in the hypothalamo-hypophysial portal blood of rats following high-dose glucocorticoid treatment and withdrawal. *Brain Res* 1991; 568: 311—313.
12. Lumpkin MD, Samson WK, McCann SM. Arginine vasopressin as a thyrotropin-releasing hormone. *Science* 1987; 235: 1070—1073.
13. Franci CR, Anselmo-Franzi JA, Kozlowski GP, McCann SM. Actions of endogenous vasopressin and oxytocin on anterior pituitary hormone secretion. *Neuroendocrinology* 1993; 57: 693—699.
14. Gilles GE, Linton EA, Lowry PJ. Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. *Nature* 1982; 299: 355—357.
15. Lumpkin MD, Samson WK, McCann SM. Hypothalamic and pituitary sites of action of oxytocin to alter prolactin secretion in the rat. *Endocrinology* 1982: 112: 1711—1717.
16. Samson WK, Lumpkin MD, McCann SM. Evidence of a physiological role for oxytocin in the control of prolactin secretion. *Endocrinology* 1986; 119: 554—560.
17. Frawely LS, Leong DA, Neil JD. Oxytocin attenuates TRH-induced TSH release from rat pituitary cells. *Neuroendocrinology* 1985; 40: 201—204.
18. Kirilov G, Saharieva S, Javorska N. The inability of oxytocin to influence the secretion of testosterone, prolactin, luteinizing and follicle-stimulating formone in normal men. *Exp Clin Endocrinol* 1986; 88: 115—118.
19. Eckland DJ, Todd K, Jessop DS, Biswas S, Lightman SL. Differential effects of hypothalamic catecholamine depletion on the release of arginine vasopressin and CRF-41 into hypothalamo-hypophyseal portal blood. *Neurosci Lett* 1988; 90: 292—296.
20. Saavedra JM. Central and peripheral catecholamine innervation of the rat intermediate and posterior pituitary lobes. *Neuroendocrinology* 1985; 40: 281—284.
21. Lipinska S, Orlowska-Majdak M, Rubacha G, Traczyk WZ. Oxytocin release from neurohypophysis, evoked by preganglionic stimulation of the rat superior cervical ganglion. *Endokrinologie* 1982; 79: 385—392.

22. Lipinska S, Orłowska-Majdak M, Traczyk WZ. Vasopressin release from posterior pituitary lobe incubated in situ after preganglionic stimulation of the rat superior cervical ganglion. *J Physiol Pharmacol* 1992; 43: 367—372.
23. Worthington WC. Blood samples from the pituitary stalk of the rat: method of collection and factors determining volume. *Nature* 1966; 210: 710—712.
24. Moore G, Lutterodt A, Burford G, Lederis K. The preparation of ^{131}I-labelled human growth hormone of a high specific radioactivity. *Endocrinology* 1977; 101: 1421—1435.
25. Greenwood FC, Hunter WM, Glower JS. A high specific antiserum for arginine vasopressin. *Biochem J* 1963; 114—123.
26. Ciosek J, Orlowska-Majdak M. Thyrotropin-releasing hormone (TRH) inhibits the release of vasopressin but not that of oxytocin from the hypothalamo-neurohypophysial system in haemorrhaged rats. *Endocrine Regulations* 1995; 29: 47—55.
27. Lipinska S, Szkudlarek U, Traczyk WZ. Hypophysial portal blood flow during preganglionic stimulation of the superior cervical ganglion under condition of systemic arterial blood pressure stabilization in rat. *Acta Physiol Pol* 1990; 41: 53—62.
28. Martin AI, Lopez-Calderon A, Tresquerres JAF, Gonzales-Quijano MI, Cardinali DP. Resistant-induced changes in serum luteinizing hormone and corticosterone levels in rats: Effect of superior cercical ganglionectomy. *Neuroendocrinology* 1995; 61: 173—179.
29. Gibs DM. Dissociation of oxytocin, vasopressin and corticotropin secretion during different types of stress. *Life Sci* 1984; 35: 487—491.
30. Armstrong WE, Gallagher MJ, Sladek CD. Noradrenergic stimulation of supraoptic neuronal activity and vasopressin release *in vitro*: Mediation by α_1-receptor. *Brain Res* 1986; 365: 192—197.
31. Brooks DP, Share L, Crofton JT. Central adrenergic control of vasopressin release. *Neuroendocrinology* 1985; 42: 416—420.
32. Kimura T, Sahoji M, Itake K, Ota K, Matsui K, Yoshinaga K. The role of central α-and β-adrenoceptors in regulation of vasopressin an and the cardiovascular system. *Endocrinology* 1984; 114: 1426—1432.
33. Reid IA, Nolan PP, Wolf JA, Keil LC. Suppresion of vasopressin secretion by clonidine: Effect of α-adrenoceptor antagonists. *Endocrinology* 1979; 104: 1403—1406.
34. Lunberg JM, Hökfelt T. Coexistence of peptides and clasical neurotransmitters. *Trends Neurosci* 1983; 6: 325—333.
35. Ariano MA, Kenny SL. Peptide coincidence in rat superior cervical ganglion. *Brain Res* 1985; 340: 181—185.
36. McLachlan EM. An analisis of the release of acetylocholine from preganglionic nerve terminals. *J Physiol (London)* 1975; 245: 447—466.
37. Barker JL, Crayton JW, Nicoll RA. Supraoptic neurosecretory cells: adrenergic and cholinergic sensitivity. *Science* 1971; 171: (967): 208—210.
38. Arnauld E, Cirino M, Layton BS, Renuald LP. Contrasting actions of amino acids, acetylcholine, noradrenaline and leucine enkephalin on the excitatory of supraoptic vasopressin-secreting neurons. A microiontophoretic study in the rat. *Neuroendocrinology* 1983; 36: 187—196.
39. Wakerlay JB, Noble R, Clarke G. In vitro studies of the control of phasic discharge in neurosecretory cells of the supraoptic nucleus. *Prog Brain Res* 1983; 60: 53—59.
40. Randle JC, Bourque CW, Renaud LP. Alpha-adrenergic activation of rat hypothalamic supraoptic neurons maintained in vitro. *Brain Res* 1984; 307: 374—378.
41. Armstrong WE, Sladek CD, Sladek JR Jr. Characterization of noradrenergic control of vasopressin release by the organ-cultured rat hypothalamo-neurohypophyseal system. *Endocrinology* 1982; 111: 273—279.

42. Haefely W, Hurliman A, Thoenen H. Relation between the rate of stimulation and quantity of noradrenaline liberated from sympathetic nerve endings in isolated perfused spleen of the cat. *J Physiol (London)* 1965; 181: 48—54.
43. Lipińska S, Orłowska-Majdak M, Traczyk WZ. Increase in oxytocin and vasopressin concentration in the blood outflowing from sella turcica region after superior cervical ganglion stimulation in rat. *J Physiol Pharmacol* 1996; 47: 281—288.
44. Karasek M, Traczyk WZ, Orłowska-Majdak M, Rubacha G. Quantitative changes of the neurosecretory granules in the rat neurohypophysis after preganglionic stimulation of the superior cervical ganglion. *Acta Med Pol* 1980; 21: 351—352.
45. Eckland DJ, Todd K, Jessop DS, Bisways S, Lightman SL. Differential effects of hypothalamic catecholamine depletion on the release of arginine wasopressin and CRF-41 into hypothalamo-hypophyseal portal blood. *Neurosci Lett* 1988; 90: 292—296.
46. Makara GB, Sutton S, Otto S, Plotsky PM. Marked changes of arginine vasopressin, oxytocin and corticotropin releasing hormone in hypophysial portal plasma after pituitary stalk damage in the rat. *Endocrinology* 1995; 136: 1864—1868.
47. Cardinali DP, Boado RJ, Vacas MI, Deza S. Changes in growth hormone and prolactin release after superior cervical ganglionectomy of rats. *Brain Res* 1986; 36: 350—353.
48. Cardinali DP, Esquifino AI, Arce A, Vara E, Ariznavarreta C, Tresquerres JAF. Changes in serum growth hormone and prolactin levels, and in hypothalamic growth hormone-releasing hormone and thyrotropin-releasing hormone and somatostatin content, after superior cervical sympathectomy in rats. *Neuroendocrinology* 1994; 59: 42—48.
49. Romeo HE, Spinedi E, Vacas MI, Estvaritz F, Cardinali DP. Increase in adrenocorticotropin release during wallerian degeneration of peripheral sympathetic neurons after superior cervical ganglionectomy od rats. *Neuroendocrinology* 1990; 51: 213—218.
50. Romeo HE, Arias P, Szwarcfarb B, Moquilevsky JA, Cardinali DP. Hypothalamic luteinizing hormone-releasing hormone content and serum luteinizing hormone levels in male rats during wallerian degeneration of sympathetic nerve terminals after superior cervical ganglionectomy. *J Neural Transm Gen Set* 1991; 85: 41—49.
51. Cardinalil DP, Romeo, HE, Ochatt CM, Moquilevsky JA. Estrous cycle delay and inhibition of gonadotropin and prolactin release during sympathetic nerve degeneration after superior cervical ganglionectomy of rats. *Neuroendocrinology* 1989; 50: 59—65.

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