OXYTOCIN CONTENT IN THE VENOUS BLOOD OUTFLOWING FROM THE VICINITY OF THE CAVERNOUS SINUS AND FROM THE FEMORAL VEIN

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Objective. Magnocellular neuroendocrine cells of the supraoptic nucleus of the hypothalamus produce and release the hormones vasopressin and oxytocin in response to a variety of stimuli to regulate body water and salt as well as and parturition and lactation. The aim of the present study was to estimate oxytocin release into the blood dialysate outflowing from the vicinity of the cavernous sinus and from the femoral vein after NMDA (N-methyl-D-aspartic acid) infusion or acute hypoxia.

Methods. The samples of dialysates of venous blood outflowing from the vicinity of the cavernous sinus and, for comparison, from the femoral vein were collected in anesthetized rats. Oxytocin was determined in the sample of dialysates by radioimmunoassay.

Results. NMDA acid infusion or acute hypoxia caused an increase of oxytocin concentration in the blood dialysate outflowing from the vicinity of the cavernous sinus of the sella turcica and from the femoral vein. A blockade of the NMDA receptors by specific and non-specific antagonists significantly inhibited the increase in the blood dialysate oxytocin concentration.

Conclusion. The results indicate the involvement of excitatory amino acid or acute hypoxia in the control of oxytocin release into the blood.

Key words: Oxytocin - Cavernous sinus - Blood dialysates

The neuropeptides vasopressin and oxytocin are predominantly synthesized in magnocellular neurons of hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei and are released from the axon terminals in the posterior pituitary directly into the systemic circulation where they exert their multiple hormonal effects (HATTON 1990). The secretion of oxytocin (OXY) is finely controlled by osmotic and nonosmotic regulatory factor. The release of OXY and vasopressin (AVP) from the supraoptic nucleus neurons is also tonically regulated by excitatory glutaminergic and inhbitory GABAergic synaptic inputs. LI and PAN (2001) indicated that activation of presynaptic nicotinic, but not muscarinic receptors located in the glutaminergic terminals increase the excitatory synaptic input to the SON neurons of the hypothalamus. Glutamate is a key modulator of the electric activity of both OXY and AVP neurons and receptors for aminopropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) mediate glutaminergic influences on these neurons (STERN et al. 1999).

Animal studies have demonstrated that hypoxia induces A1 cell c-fos expression in the rat (ERICKSON and MILLHORN 1994) and excites SON-projecting caudal ventrolateral medulla (VLM) cells, presumed to be A1 cells, in rabbit (LI et al. 1992). Moreover, it has been demonstrated that A1 noradrenergic cell group of the VLM projections to SON and PVN contribute to neurosecretory cell responses to hypoxia. However, the A1 projections were more significant in the control of AVP than OXY cell activity (DAY et al. 1995). SMITH et al. (1995) indicated that hypoxia activates both OXY and AVP neurosecretory cells, significant recruitment first

occurring at a 10 % level of oxygen in the inspired air. With increasing hypoxic severity the number of activated supraoptic AVP and OXY cells was not significantly different to that observed at the 10 % level (Smith at al. 1995).

The aim of the present study was to estimate simultaneously oxytocin release into the blood dialysate outflowing from the vicinity of the cavernous sinus and from femoral vein during NMDA infusion or acute hypoxia.

Materials and Methods

Animals. The research project was approved by the Ethical Committee. The experiments were performed in male rats weighing 300-360 g and aged 5-9 months, the F1 generation cross-strains of male August and female Wistar from the Institute of Oncology in Warsaw. The animals were anesthetized by i.p. injection of 6 mg chloralose (Roth) and 60 urethane (Fluka, Bucks, Switzerland) per 100 g body weight. After inducing anesthesia, the trachea was exposed and cannulated by polyethylene tubing of 50 mm length and 3 mm diameter. Polyethylene tubing was also inserted into the femoral vein to collect blood samples for determination of the acid-base equilibrium.

Experimental protocol. The experiments were carried out in 5 groups. In group 1 (n=10), six 30 min blood dialysate samples were collected from the vicinity of caveronous sinus and from the femoral vein. In group 2 (n=10), NMDA at the dose of 0.53 mg/kg was injected into the internal carotic artery at the beginning of the second dialysate sample collection. In groups 3 and 4 (n=10 each), NMDA competitive receptor antagonist DL-2-amino-5-phosphonopetanoic acid (AP-5) or NMDA noncompetitive receptor antagonist 6,7-dinitroquinoxaline-2,3 (1H,4 H)-dione (DNQX) at the dose of 0.53 mg/kg was administered at the beginning of the second dialysate sampling which was 1 min prior to NMDA infusion into the internal carotic artery. In the 5th group (n=10), at the time of collecting the second dialysate sample, acute hypoxia due to increased respiratory dead space was induced for 30 min. A tube previously inserted into the trachea was connected to additional polyethylene tubing of 5 mm diameter, 150 mm length and total volume of 3 cm^3 for the period of 30 min. In ordered to determine the extent of hypoxia and hypercapnia, venous blood samples were drawn: sample 1-30 min after inducing general anesthesia (control), sample 2-60 min after inducing general anesthesia and after 30 min of respiration under increased dead space conditions, sample 3-120 min after inducing anesthesia and after 60 min of respiration under increased dead space conditions.

These samples were stored on ice and analysed within 1 hour. Blood acid/base parameters, oxygen pressure (PvO2), carbon dioxide pressure (PvCO2), blood pH, HCO3 content, saturation of oxygen (Sat O2) were determined using an acid-base equilibrum analyser (Corning –248, CIBA).

Blood dialysate sampling. Venous blood dialysis was carried out according to the previously described method (Goraca and Traczyk 1997; Goraca 1998). In brief, in order to obtain blood dialysate samples from the vicinity of the pituitary, one polyethylene cannula was inserted into the heart end of the internal maxillary vein and the second cannula into the maxillary vein in the vicinity of cavernous sinus of the sella turcica. Two cannulas were inserted into the femoral vein in the same manner. One cannula was inserted into the peripheral end of the femoral vein and the other one into the central end of the femoral vein. Blood was drawn from the region of sella turcica through the polyethylene cannula (and a tube) to a minidialyser with the use of a peristaltic pump. It was then returned to the circulation through the cannula inserted into the heart end of the maxillary vein. Simultaneously, blood was drawn from the peripheral end of the femoral vein through the polyethylene cannula (and tube) to the minidialyser by means of a peristaltic pump. Then, it returned to the rat organism through the cannula inserted into the central end of the femoral vein. At the beginning of the experiments, 2 ml of Locke solution with heparin (400 U/mL) was injected into the internal maxillary vein.

The whole amount of dialysing fluid was exchanged every 30 min for 2.5 h by draining it directly into a test tube. Six samples of one ml dialysate were obtained in this way. Before refilling the minidialyser with dialysing fluid, its housing was rinsed with the dialysing fluid. The dialysates were lyophilized and used for the determination of oxytocin by radioimmunoassay (Ciosek et al. 1993).

At the end of each experiment, 1 % solution of trypan blue was injected through a cannula inserted into the internal maxillary vein. The brains were than removed from the skulls and the dye in the posterior pituitary lobes was verified under a stereomicroscope. Only such dialysate samples which were collected from animals showing the staining of the posterior pituitary lobe were included into the results. Staining of the posterior pituitary lobe proved proper insertion of the

cannula into the vicinity of the cavernous sinus of the sella turcica, and proper blood collection.

Results

Oxytocin concentration concentrations were detected in blood dialysate samples from the region of the sella turcica and from the femoral vein. The basaline of blood vasopressin from the femoral vein (24.2±3.1 pg/ml; n=10) were lower than from the region of the sella turcica (38.1±6.1 pg/ml; n=10) (Fig.1). Oxytocin content did not change in the course of dialysis. Infusion of NMDA caused a significant increase in OXY concentration of both dialysates from the region of the sella turcica (to 80.7±5.9 pg/ml, $p<0.05$; n=10 compared with the initial value) and from the femoral vein (to 48±5.3 pg/ml, $p< 0.05$, n=10 compared with the initial value (Fig.2). The blockade of NMDA receptors by a competitive receptor antagonist AP-5 and by a noncompetitive NMDA antagonist DNQX significantly reduced oxytocin concentration in blood dialysates from the vicinity of the cavernous sinus and from the femoral vein (27.1±6 pg/ml and 16.1±5.1 pg/ml, respectively, for competentive blockade ($p<0.05$, n=10) and to 26.3±8 pg/ml and 15.9±5 pg/ml, respectively, after non-competentive blockade ($p<0.05$, n=10) (Fig.3 and 4).

The 30- min period of acute hypoxia resulted in increase in vasopressin concentration in the blood dialysate from the vicinity of cavernous sinus and from the femoral vein. During acute hypoxia (Fig.5), vasopressin concentration rose from 36.91±7.2 pg/ml to 50.1±6.9 pg/ml and 84.2±8.1 pg/ml after hypoxia ($p<0.05$, n=10 compared with the initial value). In the samples from the femoral vein vasopressin concentration increased from the control value of 22.1±3.45 pg/ml to 28.7±4.9 pg/ml during acute hypoxia and 56.8±4 pg/ml after hypoxia ($p< 0.05$, n=10 compared with the initial values).

Within 30 min hypoxia the venous oxygen pressure (PvO2), fell from 51.78±3.9 mmHg to 45.65 ± 4.5 mmHg (n=7). A further reduction to 39.81 ± 4.5 mmHg was observed 30 min later. Saturation of oxygen decreased from 76.61 ± 8 % to 57.1 ± 10 (n=7) thirty min after hypoxia. After 30 min hypoxia venous carbon dioxide pressure (PvCO2) rose from 66.52 ± 2.2 to 70.25±4 mm Hg (n=7) 30 min later. After 30 min hypoxia, venous pH fell from 7.23±0.017 to 7.19±0,01 and became further reduced to 7.16±0,03 (n=7) thirty min later. After 30 min hypoxia, HCO_3^- concentration fell from 27.44±0.48 to 26.3±0.9 mEq/l and was further reduced to 24.65±1.36 mEq/l (n=7) after 30 min hypoxia.

Discussion

In the current study technique, minidialysis blood in vivo was used to estimate vasopressin release into the blood venous dialysate outflowing from the vicinity of cavernous sinus and from the femoral vein after infusion of NMDA or acute hypoxia. The present study demonstrated that infusion of NMDA or acute hypoxia significantly influenced oxytocin release into the blood outflowing from the cavernous sinus and from the femoral vein. Intraarterial infusion of AP-5 (a selective antagonist for NMDA receptor) or DNQX (a non-selective antagonist for NMDA receptor), caused the reduction in plasma OXY concentration.

It is generally accepted that oxytocin and vasopressin are well-characterized nonapeptides synthetized in magnocellular neurons of the hypothalamic supraoptic nucleus (SON) and paraventricular nuleus (PVN), which project to the neurohypophysis, where the nonapeptides are stored and released into blood (Kasting 1988). It was demonstrated that direct activation of cell bodies in the PVN by L-glutamate (Yang et al. 1995) or NMDA (Jezova and Michajlovskij 1992) caused an increased release of AVP and OXY from the posterior pituitary. In another report, NMDA-R activation induced rhythmic bursting activity in all SON neurons (Hu and Bourgue 1992) and both AVP and OT neurons responded with increased activation from NMDA application (Hussy et al. 1997). Pharmacological studies reported that excitatory amino acids stimulated oxytocin release from the hypothalamic explants (Morsette et al. 2001).

Pampillo et al. (2001) also indicated that the activation of non-NMDA receptors stimulates oxytocin release from the hypothalamic nuclei, while inhibits oxytocin release from the posterior pituitary lobe. Experiments in vitro revealed that physiological concentrations of steroid hormones inhibit NMDA stimulated AVP and OXY release (Sladek et al. 2000; Somponpun and Sladek 2002).

In accordance with previous reports demonstrating a rise in plasma OXY concentration after NMDA administration (Morsette et al. 1999; Somponpun and Sladek 2002), the present data also show an elevation of OXY concentration in blood dialysate outflowing from the vicinity of the cavernous sinus following NMDA infusion.

Administration of excitatory amino acids (EAA) antagonists inhibits almost completely synaptic responses in the magnocellular and parvocellular PVN (Wuarin and Dudek 1991). Parker and Crowley (1993) indi-

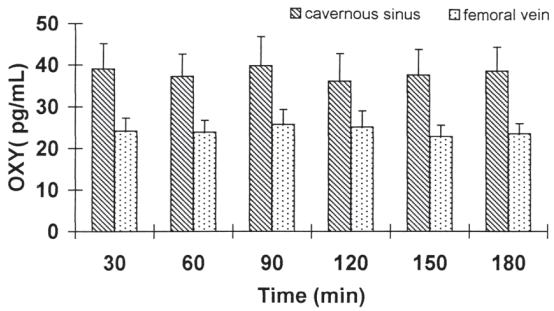

Fig. 1 Oxytocin concentration in consecutive 30-min blood dialysate samples from the vicinity of the cavernous sinus and from the femoral vein (means ± SE).

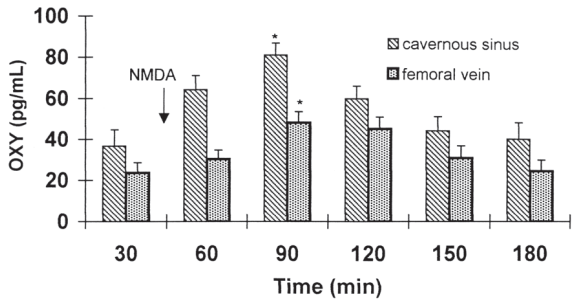

Fig. 2 Oxytocin concentration in consecutive 30-min blood dialysate samples from the vicinity of the cavernous sinus and from the femoral vein after NMDA administration (mean ± SE; p< 0.05; n=10 compared to pre-stimulation values).

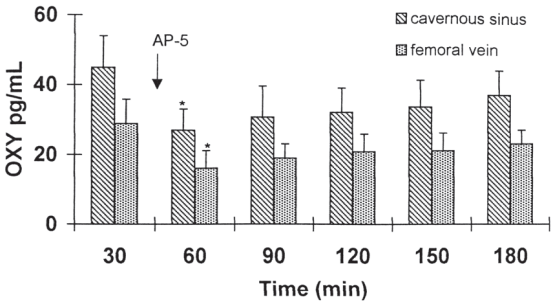

Fig. 3 Oxytocin concentration in consecutive 30-min blood dialysate samples from the vicinity of the cavernous sinus and from the femoral vein after AP-5 administration, 1 min prior to NMDA infusion. Arrow indicates time sample infusion in the internal carotid artery. Asterisk indicates value significantly different (mean ± SE; p< 0.05 ; n=10 compared to pre-stimulation value).

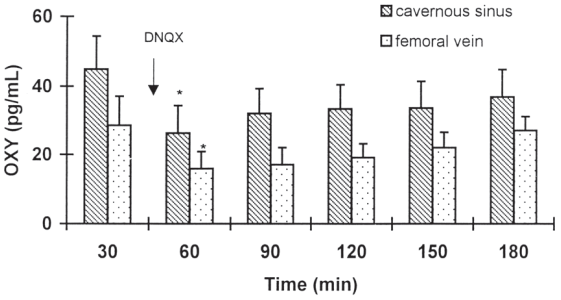

Fig. 4 Oxytocin concentration in consecutive 30-min blood dialysate samples from the vicinity of the cavernous sinus and from the femoral vein after DNQX administration, 1 min prior NMDA infusion. Arrow indicates time sample infusion in the internal carotid artery. Asterisk indicates value significantly different (mean ± SE; p< 0.05 ; n=10 compared to pre-stimulation value).

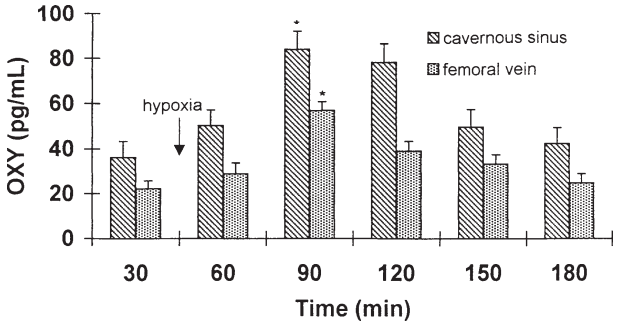

Fig. 5 Oxytocin concentration in 30-min blood dialysate samples from the vicinity of the cavernous sinus and from the femoral vein collected consecutively before, during and after acute hypoxia. Arrow indicates time hypoxia. Asterisk indicates value significantly different (mean ± SE; p< 0.05 ; n=10 compared to pre-stimulation value).

cated that selective non-NMDA receptor antagonist CNQX attenuated oxytocin release. Similarly, a non-competitive NMDA receptor antagonist, MK-801 significantly decreased plasma concentrations of oxytocin and vasopressin after hypertonic saline infusion (Onaka and Yagi 2001). Moreover, NMDA-stimulated AVP but not OXY release was prevented by blockade of non-NMDA-R, but AMPA (aminopropinic acid) – stimulated AVP/OXY release was not prevented by NMDA-R blockade (Morsette et al. 2001).

In the present study, AP-5, a selective antagonist for NMDA receptors and DNQX, which is a non-specific EAA antagonist completely inhibited the increase in the blood dialysate OXY concentration after NMDA administration. It has also been shown that hypoxia increases cerebrospinal fluid flow more than twofold

(Faraci et al. 1994) and activates neurosecretory oxytocin cells (Smith et al. 1999). Chen and Du (1999) indicated that acute hypoxic stress induced a release of OXY in median eminence proportional to its intensity and duration and that such release may be modulated in part by hypoxia-activated high circulating glucocorticoids. However, Stegner et al. (1984) indicated that hypoxia did not significantly affect OXY release in fetal and maternal sheep. Hypoxic stress activates also the secretion of other hormones such as corticosterone (ACTH) via cAMP (Zhi and Ji-Zeng 1996), prolactin (Zhang and Du 2000), atrial natriuretic peptide (Klinger et al. 2001), endothelin, norepinephrine, erythroproietin (Ziegler 2003) and excitatory neurotransmitters (Hylland and Nillson 1999), vasopressin (Stark et al. 1985; Goraca 2000).

In the present experiments, respiration under increased dead space conditions led to a decrease of pH, reduction of pO_2 and increase of pCO_2 in venous blood. In these conditions of reduced oxygen pressure, increased OXY release occurred. In the present study, the dialysis of venous blood outflowing from the region of the cavernous sinus of the sella turcica (hypothalamus, pituitary) was performed, which allowed to determine the level of peptides without collecting blood samples. In addition, average oxytocin content in 30 min dialysate sample outflowing from thefemoral vein was lower than in the blood withdrawn directly from the vicinity of the cavernous sinus of the sella turcica. This is related to the fact that dialysis in blood provides only an average over the collection time, since any sharp changes in peripheral release are blunted. Moreover, dialysis mebrane limits diffusion of neurohormones between the dialysing fluid and the blood (Lipinska 2000). However, using the technique of minidialysis allows to avoid problems with taking blood samples.

In conclusion, basal and NMDA or acute hypoxia stimulated oxytocin concentration were higher in the blood dialysates withdrawn directly from the vicinity of the cavernous sinus than in the blood dialysates from the femoral vein. The results of this study indicate an involvement of the NMDA receptor and acute hypoxia in the hypothalamic regulation of OXY release and demonstrate the utility of blood minidialysis for simultaneous monitoring of central and peripheral peptide release.

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