JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1996, 47, 2, 329—339

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VASOPRESSIN CONTENT IN THE CEREBROSPINAL FLUID AND FLUID PERFUSING CEREBRAL VENTRICLES IN RATS AFTER THE AFFERENT VAGUS NERVE FIBRES STIMULATION

Experiments were carried out on male rats in urethane anaesthesia. Cerebroventricular system was perfused with McIlwain-Rodniht's solution from lateral ventricles to cerebellomedullary cistern. Both vagus nerves were cut and the central ends of the nerves were electrically stimulated during the collection of the third 30-min portion of perfusing fluid. Vasopressin (AVP) was determined by radioimmunoassay in samples of the cerebrospinal fluid (CSF) (the first portion) and in five succesive samples of the perfusing fluid. AVP concentration in the CSF was several times greater than in the fluid perfusing cerebral ventricles. Alternate electrical stimulation of both vagus nerves did not change considerably the release of AVP into the fluid perfusing the cerebral ventricles in rat, although a certain upward tendency could be observed. It seems that only AVP raised in circulating blood (13) and not in CSF, after vagus nerves stimulation may act on the central nervous structures.

K e y w o r d s: *vasopressin (AVP), cerebrospinal fluid (CSF), vagus nerve, cerebroventricular system*

INTRODUCTION

It is apparent that stimuli causing elevation of plasma vasopressin (AVP) may also elevate cerebrospinal fluid (CSF) AVP concentration, although the strength of the stimulus needed and the magnitude as well as the time course of changes seem to be different (1). The threshold for changes in AVP levels in CSF is generally higher than that for the corresponding changes in blood. Moreover, changes in AVP levels in the circulation are usually more pronounced and precede the changes in the CSF after peripheral stimulation (1, 2).

Chang et al. 1973 (3) as the first demonstrated delayed vasopressor reactions in dogs due to AVP release into the blood after stimulation of the central end of the vagus nerve (the vagus-pituitary reflex). Antidiuretic responses in animals were obtained (4) and enhanced AVP release into the blood was observed (5) in the same experimental conditions and after unilateral vagotomy (6).

The excitation of vagus afferents modified the activity of some neurones within or near the supraoptic nuclei of hypothalamus (7) and markedly intensified the activity of neurosecretory neurones in the supraoptic and paraventricular area in rats (8) and in the supraoptic area in cats (9).

Bilateral vagotomy abolished the inhibition of neurosecretory cells activity in the supraoptic and paraventricular nuclei after the left atrial stretch receptors activation (10). The authors assumed that the blood loss, by action of the left vagal afferents, caused an increased release of AVP into the blood. It is suggested that the A_1 noradrenaline cell group neurones are an essential component in a pathway which relays facilitatory vagal input of cardiopulmonary origin to neurosecretory vasopressin cells (11).

In the report on AVP release into the CSF significant elevation of AVP concentration in the CSF after electrical stimulation of the central end of a servered vagus nerve was found in the rabbit (12).

In recent experiments we have confirmed earlier reports, mentioned above, on AVP release into the blood after the vagus nerves stimulation (13). The aim of the present study is to investigate AVP release into the CSF at the same experimental conditions.

MATERIAL AND METHODS

The experiments were performed on male rats weighing 380 ± 18 g (mean $\pm$ SE), about 5—6 month old, F_1 generation of Wistar females and Buffalo males from the stocks of the Institutes of Oncology in Warsaw and in Gliwice, respectively, bred in our Department. The animals were kept in standart conditions of a 14-hr light: 10-hr darkness cycle and received standard rat pellets and water ad libitum. They were anaesthetized with intraperitoneal injection of urethane 120.0 mg/100g b.w. and chloralose 12.0 mg/100 g b.w.

Perfusion of the cerebral ventricles

The head of the animal was immobilized in a simple stereotaxic frame used for rats but specially adapted for the experiment. The clamp for the maxilla placed about 3 cm over the base of the apparatus and ear bars inserted into the external auditory meatus about 5.5 cm over the base of the the apparatus fixed the animals' head at the position allowing for the introduction of a cannula into the cerebellomedullary cistern according to the method described earlier (14). During the whole experiment the cannula for cerebellomedullary cistern was fixed to the cannula holder attached to the ear bars as was earlier described (14). The skin and subcutaneous tissue on the head in the midline were locally anesthetized with 2% polocainum and then incised. Cranial bones were exposed and the following points were marked on them: 5 mm anteriorly to the frontal zero plane and 3 mm laterally on either side of the sagittal zero plane. Two holes were drilled in the cranial bones at these points with an electric dental drill. Through these holes two cannulas of stainless steel with external diameter of 0.6 mm were inserted stereotaxically into the lateral ventricles to a depth of 4 mm from the surface of the skull. The cannulas were connected with polyethylene tubes to a vessel containing McIlwain-Rodnight's solution composed according to Daniel and Lederis (15), as follows (millimoles per 1 litre): NaCl — 120.0, KCL — 4.8, KH_2PO_4 — 1.2, $MgSO_4$ — 1.3, $CaCl_2+2H_2O$ — 2.8, $NaHCO_3$ — 2.6 and glucose — 10.0.

The cannula for the cerebellomedullary cistern consisted of two stainless steel tubes: guide tube and inner tube of 0.6 mm O. D. The skin and muscles over the atlantooccipital membrane and the membrane itself were punctured by the guide tube, and the tip of the inner tube was introduced into the cistern.

The sites of introduction of the cannulas were established according to the stereotaxic atlas of König and Klippel (16) and after experiments in a separate group of animals with a 1% trypan blue solution used during the perfusion.

Polyethylene tubing was connected with the guide tube and outflowing fluid flowed down into the ampoule placed on melting ice. Each ampoule contained 0.02 ml of glacial acetic acid and 4 mg of dextran (110,000 MW). The vessels with the inflow and outflow perfusion fluid were kept in such a position above and below the rat's head, respectively, to obtain a perfusion volume of about 0.4 ml per 30 min.

Electrical stimulation of the vagus nerve

The skin on the rat's neck and subcutaneous tissue in the midline were locally anaesthetized with 2% polocainum and then incised. Both vagus nerves were exposed and cut. The central end of each nerve, covered with liquid paraffin was placed on a thin bipolar silver electrode connected to a Grass stimulator model S4K. Both nerves were stimulated bipolarly with electric pulses at frequency of 60 Hz, duration 2 msec and amplitude up to 8 V, intermittently 30 sec on and 30 sec off simultaneously each of two nerves during 30 min i. e. during the collection of the 3rd sample of the perfusing fluid. The amplitude of electric pulses stimulating the vagus nerves was so adjusted as not to stop the respiratory movements.

Experimental procedure

The vagus nerves were exposed and incised. Then the animal was placed in the stereotaxic frame, central ends of the cut vagus nerves were placed on the electrodes and the perfusion of the cerebral ventricles was begun. The first collected portion was the cerebrospinal fluid in volume of about 0.2 ml, the second portion of the fluid perfusing the cerebral ventricles was treated as control, and during the collection of the third portion the vagus nerves were stimulated. Then three subsequent portions were collected if it was possible.

During each experiment the animal's breathing remained under control, especially after general anaesthesia set in (an), as soon as the vagus nerves were cut (nXc), during collection of the IInd sample — control perfusion (p), during the vagus nerves stimulation — as soon as the initial breath-holding was ended (nXs), during the collection of the IVth sample — just as stimulation procedure was finished (sf) and at last just before the ending of the whole perfusion procedure (pe).

Perfusion fluid in each ampoule was centrifuged at 10.000 G and the supernatant was frozen and lyophilized and kept in sealed ampoules until determination of vasopressin by radioimmunoassay.

Radioimmunoassay of vasopressin

Anti-AVP antibodies (serum no. 1228/1987-08-24) were raised in rabbits according to Moore et al (17). Arginine vasopressin used for immunization was synthesized in Institute of Organic Chemistry, Technical University of Lodz. AVP was iodinated with ^{125}I using chloramine-T method (18). Unreacted iodide was removed by mixing the reaction mixture with Amberlite (Ion Exchange Resin, type CG-400, Serva). Further purification was carried out on a column of Sephadex G-25 fine preequilibrated and eluated with 0.05 mol/l acetic acid. Labelled AVP was identified in the third peak (*Fig. 1*). The top or the first descending portion of this peak was used as the tracer in RIA. Arginine vasopressin used for iodination and as standard derived from Serva.

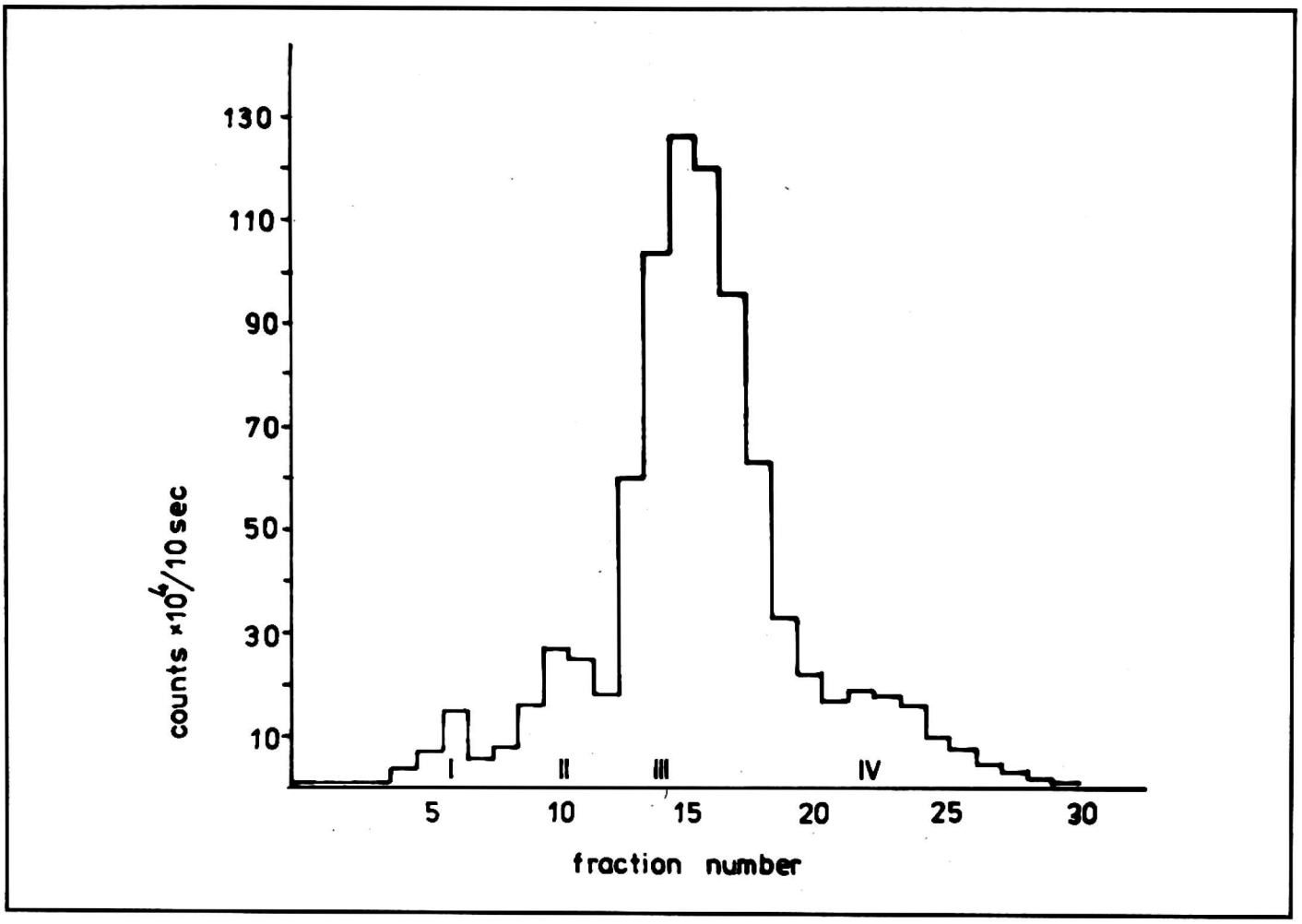

Fig. 1. Elution pattern of ^{125}J AVP on Sephadex G-25 column. Peak I contains ^{125}I BSA (bovine serum albumin used for termination the reaction of iodination), peak II — unreacted free iodide, peak III — ^{125}I AVP and peak IV represents diiodo AVP.

Final dilution of the anti-AVP antibodies was 1:32000. Sensitivity was about 2 pg/tube, the within-assay and between-assay CV were 3.9% and 6.3%, respectively. Cross-reactivity with other peptides expressed as a percentage cross-reactivity relative to AVP is shown in *Table* 1 beneath.

Table 1. Specificity of the anti-AVP antiserum used, expressed as a percentage cross-reactivity relative to AVP.

Substance tested	% cross-reactivity
Arginine vasopressin (1)	100
Lysine vasopressin (2)	2.7
Oxytocin (3)	0.016
LH-RH (4)	<0.002
TRH (5)	<0.002
Leu-enkephalin (6)	<0.002
Angiotensin II (2)	<0.002
Substance P (8)	<0.002
(pyrGlu^{6}Tyr^{8})SP$_{6-11}$ (9)	<0.002
(Tyr^{8})SP$_{6-11}$ (10)	<0.002

(1) and (3) synthesized in Institute of Organic Chemistry, Technical University of Lodz, Poland
(2) Sigma, Lot 123F-0600
(4) synthesized in Department of Organic Chemistry, Institute of Chemistry, Pedagogical College of Opole, Poland

(5) synthesized in Department of Bioorganic Chemistry, University of Gdańsk, Poland
(6) synthesized in Laboratory of Peptides, Department of Chemistry, University of Warsaw, Poland
(7) Ciba
(8) synthesized in Institut für Wirkstofforschung in Berlin, Germany
(9) and (10) synthesized in Department of General Chemistry, Institute of Physiology and Biochemistry. Medical University of Lodz, Poland.

RESULTS

Changes of frequency of breathing during the experimental procedure

Cutting of vagus nerves caused a statistically significant decrease of frequency of breathing from 71±7 to 44±2 per minute (mean±SE) (*Fig. 2*). The mean values of frequency of breathing maintained during the perfusion procedure at more or less the same level. Stimulation of vagus nerves had no significant effect on mean frequency of breathing. However, we observed great alterations of frequency of breathing, specially during vagus nerves stimulation, in a few animals, for example: from 48/min before to 24/min during stimulation in rat No 16311 and from 32/min before to 44/min during stimulation in rat No 16605. Temporary breath holding was observed in almost all animals which underwent stimulation of vagus nerves.

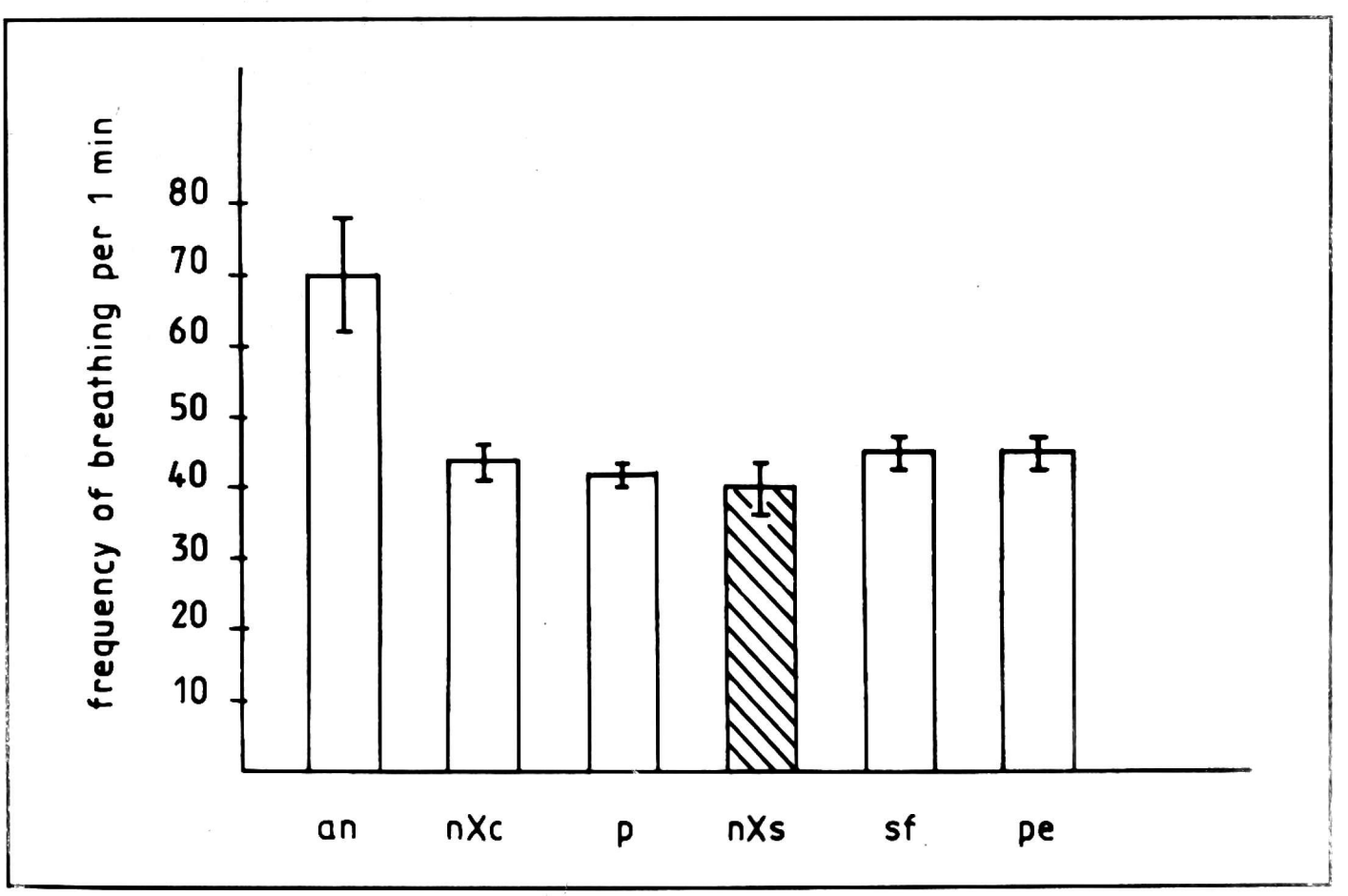

Fig. 2. Frequency of breathing in rats: after anaesthesia set in (an), as soon as cutting the vagus nerves (nXc), during control perfusion (p), during the vagus nerves stimulation (nXs), just as stimulation was finished (sf), just before the ending of the perfusion (pe).

Vasopressin concentration in the CSF and in the fluid perfusing the cerebral ventricles

Vasopressin concentration in the CSF was several times (4.7) greater than the concentration in the fluid perfusing the cerebral ventricles (*Fig. 3*). The difference was statistically significant ($p < 0.05$).

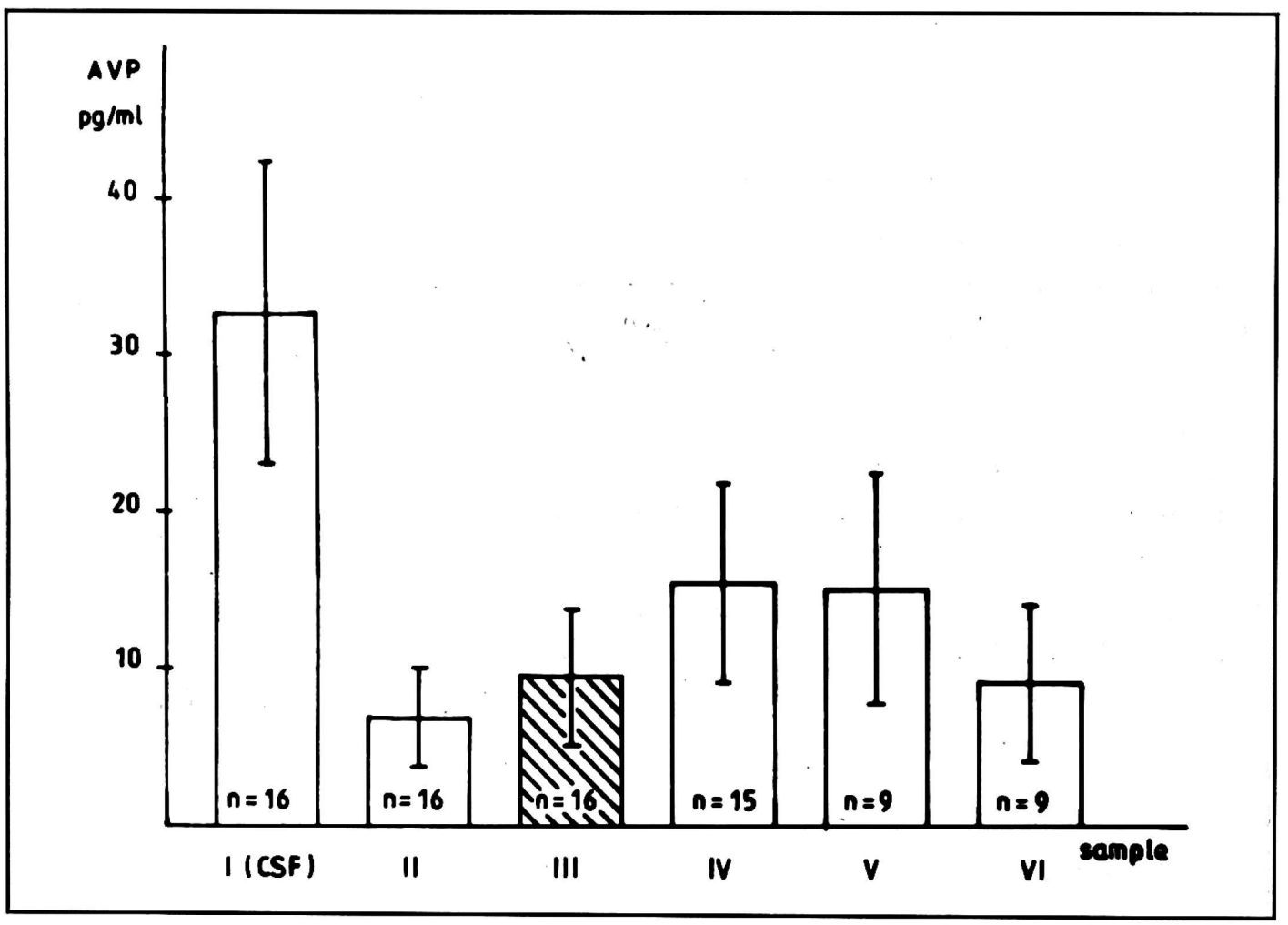

Fig. 3. Vasopressin concentration (pg/ml) in the cerebrospinal fluid (Ist sample) and in the fluid perfusing cerebral ventricles (IInd to VIth sample): before (Ist to IInd sample), during (IIIrd sample) and after (IVth to VIth sample) the vagus nerves stimulation (mean $\pm$ SE, $n = 9$ to 16).

The effect of vagus nerves stimulation on vasopressin concentration in the fluid perfusing the cerebral ventricles

In the course of the vagus nerves stimulation mean vasopressin concentration was somewhat greater (sample III vs. sample II) and still rose (sample IV and V) even when the stimulation was off, but the differences were not statistically significant ($p > 0.05$) (*Fig. 3*).

DISCUSSION

Vasopressin present in the cerebrospinal fluid (CSF) may, in principal, originate from the blood through the blood-brain barrier, reach the CSF *via* the circumventricular organs, or be the result of direct release or diffusion from

brain extracellular space (19). Basal CSF level of AVP was measured by Dogterom et. al. 1977 and it amounted to 11.5 pg/ml (20). The AVP concentration in CSF may be affected by both age and sex (21). The lowest concentration (4.9 pmol/l, i.e. about 5.3 pg/ml) was found in young freely moving rats, and the highest (11.8 pmol/l i.e. about 12.8 pg/ml) in aged male freely moving rats (21). Slightly higher concentrations of AVP in control CSF samples obtained from conscious rats were found by Ivanyi et al. (22) and they amounted to about 20—25 pg/ml. In our earlier experiments concentration of AVP was 27 pg/ml (23) or 68.1 pg/ml (24) in rats under urethane anaesthesia.

In present experiments mean AVP concentration in CSF is 32.9 pg/ml. The values observed by us are slightly greater than values obtained by others, mentioned above (20, 21). It has to be noted that our experiments were done on 5—6 mounth old animals so we expected higher concentration of AVP in CSF. The animals better tolerate long-lasting experiment and the perfusion of the cerebral ventricles can be also performed properly. The rise in AVP concentration might be also caused by vagotomy done at the beginning of experiment. In conscious dogs after 10 days unilateral vagotomy (left) the concentration of vasopressin in the blood was higher than in control animals (6). This might be also caused by anaesthesia applied to animals and by noxious impulsation connected with the surgical procedure (introducing of steel cannulas into the lateral ventricles and into the cerebellomedullary cistern). Mentioned factors caused a twofold (anaesthesia) or tenfold (surgical procedure) increase of CSF AVP in dogs (25). It seems that urethane anaesthesia applied in our experiments raises vasopressin and oxytocin release (26) probably as a result of increased plasma osmolality (27) but reduces the responsivenes of AVP- and OT-neurones to acute salt loading (28).

It is well known that vagus nerve provides afferents from such receptor areas like gastrointestinal, cardio-vascular and respiratory. The most is known about the effect of cardio-vascular receptor area on the vasopressinergic system. During the stimulation of carotid sinus chemoreceptors the vasopressinergic neurones intensified their activity in dogs and cats (10). On the contrary inputs from left atrial stretch receptors or carotid baroreceptors evoked reduction in the vasopressinergic neurones activity (10). The influence of baro- or chemoreceptors on neurosecretory neurones was stronger than that originating from atrial receptors (10). Moreover there are proofs on fundamental difference between the role of the left and right vagal afferents: only left vagus nerve carries impulses from the cardiac area, substantial for vaspressin release in dogs (29). The difference was not found by others (11), because stimulation of the right vagus was marginally but not significantly less effective than stimulation of the left one on AVP-neurones activity. It seems that cervical vagi carry impulses inhibiting and stimulating vasopressin release. Abdominal vagi carry information gastrointestinal origin. Stimulation of the

abdominal vagus had no effect on any AVP-neurone tested in rats (11). The results are in opposition to results obtained in rabbits because plasma vasopressin concentration increased from 35 to 127 pg/ml after electrical stimulation of the abdominal vagus (30).

Alternate electrical stimulation of both vagus nerves in our experiment did not change considerably the release of AVP into the fluid perfusing the cerebral ventricles in rat, although a certain upward tendency could be observed during stimulation and within the first hour after cessation of stimulation.

A similar result of lack of influence, and even devoid of the upward tendency, was observed earlier when in several rats during perfusion of cerebral ventricles, only one vagus nerve was stimulated with electrical impulses of slightly lower frequency and shorter 1-sec bursts (24). The content of AVP in obtained CSF samples and samples of the fluid perfusing the cerebral ventricles was determined by radioimmunoassay in the Netherland's Institute for Brain Research by kidness of Dr Ruud Buijs. The content of AVP in CSF was found to equal 68.1 pg/ml, while in the fluid perfusing the cerebral ventricles (in control sample) it amounted to 13.9 pg/ml, and in the next sample (during vagus nerve stimulation) to 8.2 pg/ml (24). Unfortunately, no subsequent samples of the perfusing fluid were collected and it is not known whether the release of AVP did not rise after the cessation of stimulation as it is evident on the basis of the actually presented results (sample IV and V).

Such delay, but concerning the release of oxytocin (OT) into the CSF was observed by others (31). The peak of OT release into the CSF occurred only 15 minutes after the cessation of stimulation of paraventricular nucleus (PVN). This prolongation may be due partly to the time of penetration into CSF from the release site, but also to the time it takes to reach the sampling site (32).

In our earlier experiments we observed a delay in answer to the stimulus, but only in the release of AVP, and not of OT, into the blood and fluid incubating the posterior pituitary lobe in situ (33—35). Delayed (20 min) increase in plasma AVP concentration after immobilization stress was observed in rats by Ivanyi et al (22). The reason of this delay must be different than that presented by Robinson and Coombes (32).

Electrical stimulation of both vagus nerves caused a statistically significant increase in AVP release to peripheral blood (about 5-fold) in relation to control) and to blood flowing from the area of sella turcica (about 14-fold in relation to control) in rat in our earlier experiments (13). In both cases the highest increase in AVP release was observed during 30 min after the cessation of stimulation, thus also with a certain delay.

It seems that impulsation raised in vagus nerves by electrical stimulation induces, first of all, the release of great amounts of AVP into the blood, and not to the CSF. Vasopressin from the circulation blood can be easily uptaken by CNS structures devoid of blood-brain barriers (36) and in markedly less

amounts by structures possessing such a barrier (37). Therefore, in these conditions it can act peripherally, and its central effect is not excluded, but only through peripheral blood.

Acknowledgements: We thank very much Mrs Beata Biała for help with the experiments. Moreover we wish to thank Professor Ryszard Paruszewski (Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Warsaw) for supplying us with the Angiotensin II and Professor Andrzej Szmigielski (Department of Pharmacodynamics, Faculty of Pharmacy, Medical University of Lodz) for Lysine vasopressin. We thank Professor Stefania Drabarek for providing Leu-enkephalin, Dr Wiktor Koziołkiewicz for (pyr Glu^{6}Tyr^8) SP_{6-11} and (Tyr^8) SP_{6-11}, Professor Gotfryd Kupryszewski for TRH, Professor Peter Oehme for Substance P and Professor Barbara Rzeszotarska for LH-RL. The study was supported by Medical University of Lodz, research grant no 502-11-169.

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Received: April 19, 1995
Accepted: December 27, 1995

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