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VASOPRESSIN RELEASE INTO THE 3RD CEREBRAL VENTRICLE AND INTO THE BLOOD AFTER SCIATIC AND TRIGEMINAL NERVE STIMULATION

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The aim of the experiments was an assessment of vasopressinergic (AVP-ergic) neurons response to somatic afferent impulsation in the spinal and in the cranial nerves. Experiments were carried out on male rats in urethane anaesthesia with the perfusion cannula introduced into the 3rd cerebral ventricle and with the catheter in the internal maxillary vein, in the vicinity of the cavernous sinus. Four samples of perfusing fluid, each of about 1.5 ml/30 min., and four samples of blood, 0.8 ml each, were collected. Vasopressin (AVP) was determined by radioimmunoassay in samples of perfusing fluid from the 3rd cerebral ventricle and in blood plasma. The central end of the left sciatic, supraorbital or infraorbital nerves was electrically stimulated with an intensity which increased the respiratory rate by 10—15% during collection of the IIIrd samples.

The AVP concentration in the fluid perfusing the 3rd ventricle did not change during stimulation of the nerves. On the contrary, sciatic and supraorbital nerve stimulation caused a significant increase of AVP concentration in the blood. It can be concluded that sciatic and supraorbital nerve afferentation induced AVP release from the posterior pituitary lobe and did not affect AVP-ergic neurons projecting to the ventricular ependyma and responsible for AVP release into the cerebrospinal fluid.

K e y   w o r d s: *vasopressin, 3rd cerebral ventricle, blood.*

INTRODUCTION

Studies by Theobald and Verney 1934 (1) showed for the first time that afferent impulsation probably influenced vasopressin release into the blood. The antidiuretic response was also demontrated by electrical stimulation of the ulnar nerve in dogs (2). Then afferent impulsation from the skeletal muscles have been reported to affect the firing rate of neurons in or near the supraoptic nuclei (3—5). The nociceptive stimuli evoked the same influence (5, 6). Afferent impulsation evoked by nociceptive stimuli enhanced the AVP release into the blood (7—9).

Although electrical activation of somatic afferents and application of noxious stimuli have been shown to excite neurosecretory cells and prompt the release of AVP from the neurohypophysis, little is known about the supraspinal pathways involved. It seems likely that most spinal afferents relay in the brainstem, probably in the nucleus tractus solitarius (NTS) (10).

The aim of the present investigation was an assessment of vasopressinergic neurons response to somatic afferent impulsation in the spinal nerve — sciatic, and cranial nerve — branches of trigeminal nerve, and an answer to the question if the response depends on the localization of the receptor fields.

MATERIAL AND METHODS

The experiments were performed on male rats, weighing $350-380$ g, about 6 months old, F_1 generation of cross-breeding of August strain males and Wistar strain females from the stock of Institute of Oncology in Warsaw. The animals were kept in standard conditions of a 14-hr light: 10-hr darkness cycle and received standard rat pellets and water ad libitum.

The animals were anaesthetized by an intraperitoneal administration of the urethan in dose of 100 mg/100 g of body weight.

Perfusion of the 3rd cerebral ventricle by the perfusion cannula

The head of the animal was immobilized in a simple stereotaxic instrument. The skin and subcutaneous tissue on the head in the midline were locally anaesthetised with 2% novocaine and then incised. Cranial bones were exposed and a hole was drilled by the use of an electric drill, exactly in sagital zero plane, 7 mm anteriorly to the frontal interaural zero plane. The perfusion cannula was introduced into the 3rd cerebral ventricle through the drilled hole. This site of cannula introduction was established according to stereotaxic atlas of König and Klippel (11) and after experiments in a separate group of animals with a 1% trypan blue solution used during the perfusion. The tip of the cannula was in the 3rd ventricle when the cannula was introduced to a depth of 5.5 — 6.0 mm from the skull surface.

The perfussion cannula consisted of two coaxial stainless-steel tubes, the external — 1 mm and the internal — 0.5 mm in diameter, respectively. The tip of the internal tube protruded 2.5 mm from the external tube. The cannula was connected through a poliethylene tubing with a peristaltic pump Unipan 304. Through the internal tube the affluent perfusion fluid followed into the 3rd cerebral ventricle, returning through the external tube.

The 3rd cerebral ventricle was perfused with McIlwain-Rodnight's solution composed according to Daniel and Lederis (12) as follows (millimoles per 1 litre): NaCl — 120.0, KCl — 4.8, KH_2PO_4 — 1.2, $MgSO_4$ — 1.3, $CaCl_2 + 2 H_2O$ — 2.8, $NaHCO_3$ — 2.6 and glucose — 10.0. Four 30-min, 1.5 ml samples of perfusing fluid were collected into glass ampoules placed on melting ice. Each ampoule contained 0.02 ml of glacial acetic acid and 6 mg of dextran (110,000 MW). Perfusion fluid in each ampoule was centrifuged at $10°C$ and the supernatant was frozen and lyophilized, and kept in sealed ampoules until determination of vasopressin by radioimmunoassay.

Blood sampling

Polyethylene tubing was inserted into the internal maxillary vein in the vicinity of the cavernous sinus. 1% trypan blue solution injected through this vein at the end of the experiment immediately stained the posterior pituitary lobe. 0.5 ml of 0.9% NaCl with heparin (400 UI/ml) was

injected through the internal maxillary vein at the beginning of the experiment. Then during the last 10 min of the collection of each sample of fluid perfusing the 3rd cerebral ventricle 0.8 ml of venous blood was slowly drawn out. The blood was immediately centrifuged, plasma was frozen and the cells were resuspended in 0.9 % NaCl with heparin and returned via the internal maxillary vein before the next sample was collected.

Extraction of vasopressin from blood plasma

0.4 ml of cold aceton was added to 0.4 ml of plasma. The mixture was stirred 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 $35-40^{\circ}C$, and stored at $-20^{\circ}C$ until assayed.

For the estimation of recovery of known quantities of the added hormone through the extraction procedure, unlabelled vasopressin was added to the plasma to give a concentration of $2.2-35.7$ pg AVP/tube, extraction was performed and vasopressin was determined. The recovery was estimated to approximate 56%. Values given for plasma AVP in this paper have not been corrected for losses during extraction.

Radioimmunoassay

Anti-AVP antibodies were raised in rabbits according to Moore at all. (13). Arginine vasopressin used for immunization, iodination and as standard was synthesized in Institute of Organic Chemistry, Technical University of Lodz. AVP was iodinated with ^{125}I using the chloramine-T method (14). The sensitivity was about 2 pg/tube, the within-assay CV was 3.9% and the between-assay CV 6.3%. Characteristics of anti-AVP antiserum obtained from rabbits and used in the radioimmunoassay were described previously (15).

Electrical stimulation of the nerves

The left sciatic, supraorbital or infraorbital nerve was exposed at the beginning of collection of the 3rd perfusing fluid sample. The nerve was cut and the central end was placed on a thin bipolar silver electrode connected to a Grass stimulator model S4K. Each nerve was stimulated bipolarly with electric pulses at frequency of 100 Hz, duration 3 msec and amplitude 8 V, intermittently: 1.5 sec on and 1.5 sec off during 30 min, i.e. while the collection of the 3rd sample of perfusing fluid and the 3rd sample of blood. Such electrical stimulation of the nerves accelerated the respiratory rate in rats by $10-15\%$.

Statistical evaluation of the results

Group means $\pm$ SE were calculated for data points. 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

AVP concentration in control samples of fluid perfusing the 3rd cerebral ventricle, i.e. in sample I and II in all three groups of animals was 5.5 ± 0.9 pg/10 min ($n = 29$) and 6.2 ± 0.7 pg/10 min ($n = 29$), respectively. Stimulation of the sciatic, supraorbital and infraorbital nerves did not have a significant
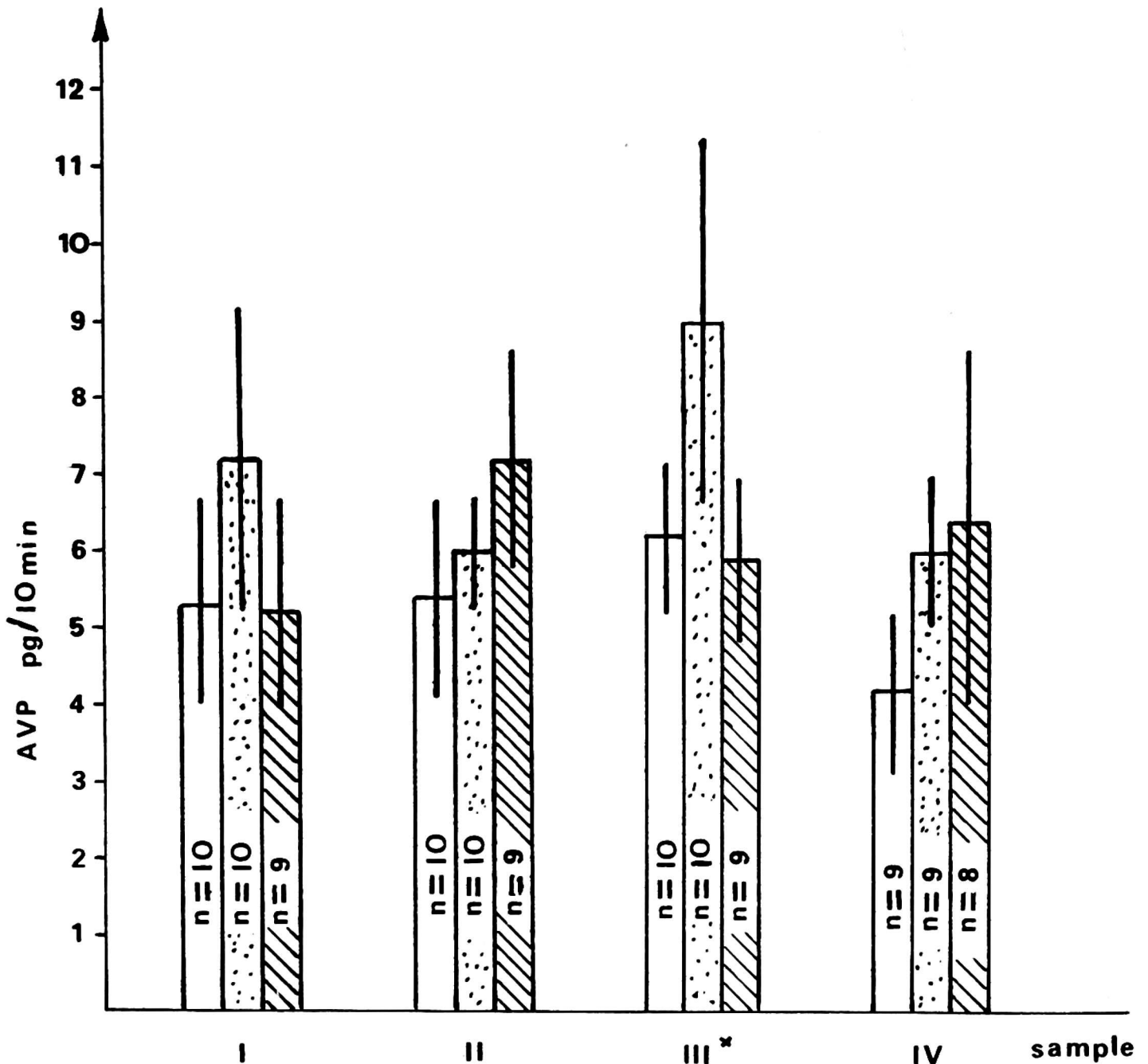
Fig. 1. Vasopressin release into the fluid perfusing the 3rd cerebral ventricle in rat before (I—II samples), during (III samples) and after (IV samples) the electrical stimulation of the sciatic nerve ☐, supraorbital nerve ▨ and infraorbital nerve ▨ (mean ± SE, $n = 8$—10 animals per group). Nerves were stimulated during the collection of the IIIrd sample (III*) of perfusing fluid.

influence on the AVP release into the fluid perfusing the 3rd ventricle. Only a slight, insignificant increase is shown after the sciatic and supraorbital nerve stimulation (*Fig. 1*).

I and II control samples of blood contained 39.0 ± 5.0 pg AVP/ml ($n = 30$) and 40.6 ± 5.6 pg AVP/ml ($n = 29$) of plasma, respectively. Electrical stimulation of the sciatic nerve caused a significant increase of AVP concentration in the IVth sample of blood, i.e. with a 30 min latency period. Electrical stimulation of the supraorbital nerve intensified the AVP release into the IIIrd sample of blood, i.e. instantly during the stimulation. This elevation was statistically significant in relation to control samples. The AVP concentration in the blood changed neither during nor after the infraorbital nerve stimulation (*Fig. 2*).
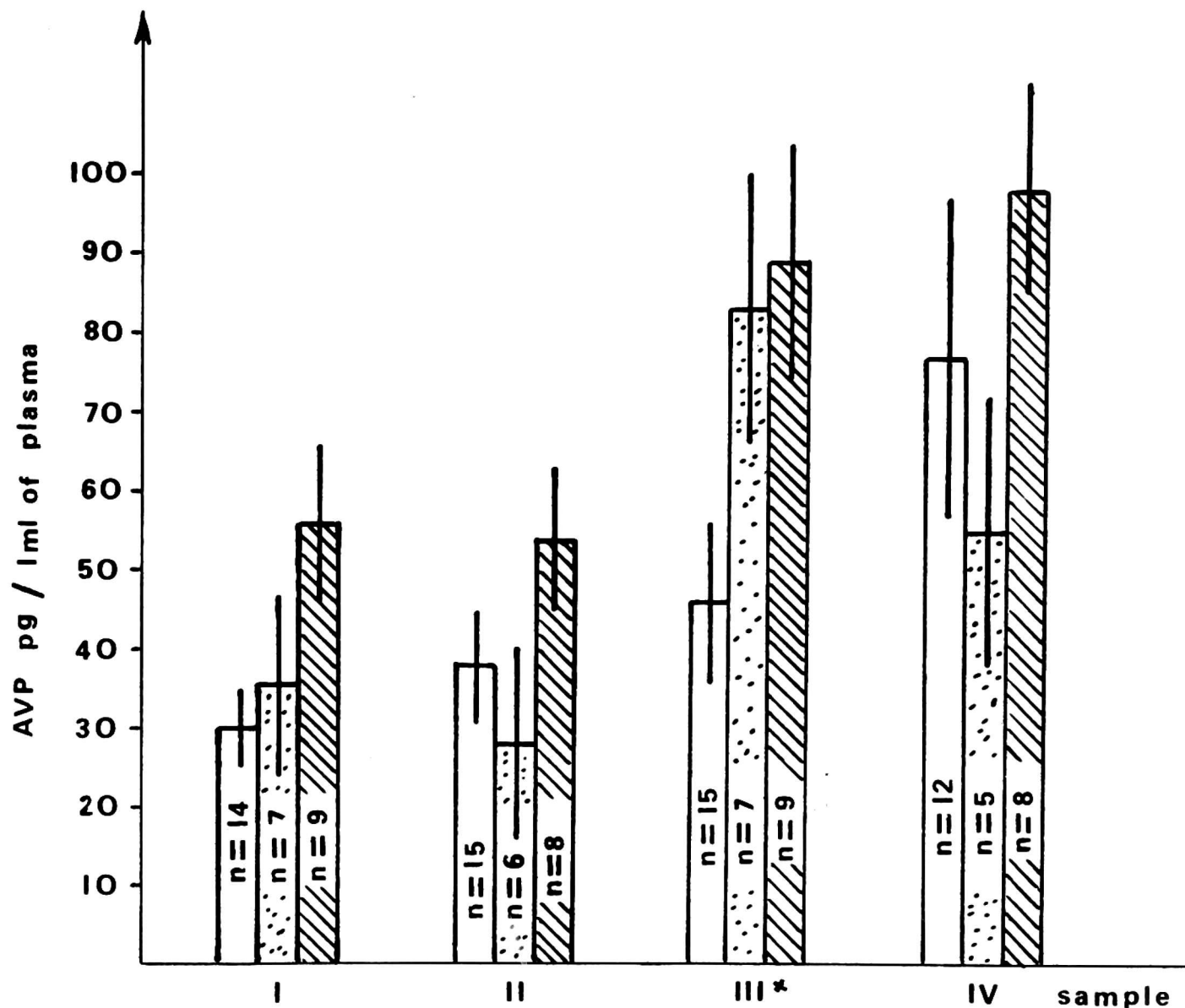
Fig. 2. Vasopressin concentration in the blood from the internal maxillary vein (mean ± SE, $n = 5 - 15$ animals per group) before (I—II samples), during (III samples) and after (IV samples) the electrical stimulation of the nerves. During the collection of the IIIrd sample of blood (III*) sciatic nerve ☐, supraorbital nerve ▨ or infraorbital nerve ▨ was stimulated.

DISCUSION

The "push-pull" type cannula used for the study of neurosecretory function of the hypothalamus neurons was first applied in dogs subject to chronic experiments (16). The use of the "push-pull" cannula for the perfusion of the 3rd cerebral ventricle in conscious rats allowed to determine the vasopressin release into the perfusion fluid at rest (17). Our results are about 4-fold higher than those of the mentioned authors, but comparable with values obtained in an earlier study, where the whole cerebroventricular system between the lateral ventricles and the cerebellomedular cystern was perfused (18). Vasopressin concentration in the fluid perfusing the 3rd cerebral ventricle depends mainly on the position of the cannula in relation to structures surrounding the 3rd ventricle and upon the construction of the cannula itself. In the perfusion

cannula used in our laboratory the distance between the internal tube letting the perfusion fluid in (push) and the external tube leading the fluid out (pull) was 2.5 mm. The amount of vasopressin released into the 3rd ventricle per time unit was also dependent on other factors, such as the kind of anaesthetic applied, noxious impulsation connected with the technique of introducing the perfusing cannula into the 3rd cerebral ventricle and of inserting the tube into the vein (19).

Afferent fibres were stimulated with electric pulses of 100 Hz frequency, which is a threshold frequency in evoked pain sensation during tooth pulp stimulation in men (20). Frequences lower than 100 Hz produce other sensory but not noxious sensations (20). The same frequences was applied for the sciatic nerve stimulation by Day and Sibbald (1990) (21). It may be thus assumed that among afferent fibres stimulated in our experiment pain fibres constitute the majority.

Vasopressin concentration in the perfusing fluid did not significantly change during the stimulation of the sciatic nerve and both branches of the trigeminal nerve. It is consistent with the results of experiments in which alternate stimulation of both sciatic nerves did not significantly influence the vasopressin release into the fluid incubating the posterior pituitary lobe in situ (22). The method of posterior pituitary lobe incubation in situ may serve as an experimental model of neurohormones release into the cerebrospinal fluid in the subarachnoid cisterns: the interpeduncular cistern and the chiasma cistern.

Vasopressin release in the control blood of the examined rats exceed 20-fold the values known from the literature data (23). It is probably due to the fact that in our experiments vasopressin was determined in blood flowing directly from the area of the sella turcica, as the cannula taking blood was inserted into the internal maxillary vein and its ending was situated in the vicinity of the cavernous sinus. Blood obtained this way contained more vasopressin than peripheral blood (24). Moreover, another factor influencing the vasopressin content in blood may be urethan applied as an anaesthetic. Urethan increases the neurohormones release from the posterior pituitary acting at the level of neurosecretory endings (25), and also influences the volume of body fluids and, through the volume receptors, additionally intensifies ·the neurohormones release (26).

The stimulation of the central end of the cut sciatic and supraorbital nerves evoked a substantial increase of the vasopressin release into the blood. The effect of the supraorbital nerve stimulation manifested itself immediately, while in case of the sciatic nerve stimulation — with certain latency. Differences in the latency of neurohumoral response are probably connected with the properties of the nervous centers processing sensation from the area innervated by the sciatic and trigeminal nerves.

Different results obtained after the stimulation of the supraorbital and infraorbital nerves may be connected with a different processing of impulses in

nonspecific sensory pathways and centers. Early scalp responses evoked by stimulation of the supraorbital and infraorbital nerves in the man do not differ substantially (27). It evidences of the similarity of impulse conduction in specific sensory pathways and centers. The response of vasopressinergic neurons may be evoked by nonspecific pathways and centers which are probably distinct for impulsation produced by stimuli of various biological importance.

Most spinal afferents relay in the brainstem in the nucleus tractus solitarius (NTS) (10). NTS also provides the first central relay for most primary sensory axons of the cranial nerves — VII, IX, X, as well as a few which travel in the trigeminal nerve. Only trigeminal fibres of the ophtalmic and mandibular branches, but not maxillary, enter the NTS (28). It can be supposed that afferent impulses enter the NTS only in case of electrical stimulation of the supraorbital, but not infraorbital nerve. All afferent impulses to the NTS are excitatory (29). It was demonstrated earlier that stimulation of the NTS excites neurosecretory AVP cells by a relay through the A1 cell group (21, 30). It can be thus supposed that different results obtained after the stimulation of the supraorbital and infraorbital nerves originate from NTS excitation during the supraorbital nerve stimulation and from the lack of the NTS excitation during the infraorbital nerve stimulation.

Functional dissimilarity of both nerves is also evidenced by a distinct effect of AVP on trigemino-hypoglossal reflex evoked by electrical stimulation of the supraorbital and infraorbital nerves. AVP perfunded through the cerebral ventricles did not change the strength of tongue jerks (ETJ) evoked by supraorbital nerve stimulation and increased the strength of ETJ during the infraorbital nerve stimulation (31).

The results indicate that afferent stimuli of noxious nature cause the release of AVP mainly to the systemic blood. The nervous centers could be affected by AVP circulating in the systemic blood rather than by the increased of AVP concentration in the cerebrospinal fluid.

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