EFFECT OF TOOTH PULP STIMULATION ON OXYTOCIN AND VASOPRESSIN RELEASE INTO THE CEREBROSPINAL FLUID AND FLUID PERFUSING THE CEREBRAL VENTRICLES IN RATS

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Objective. Stress- and pain-related stimuli cause a release of vasopressin (AVP) and oxytocin (OT) into the cerebrospinal fluid (CSF) and extracellular fluid of the brain in various animal species. The aim of the study was to investigate the effect of stimulation of the nociceptive afferent terminals in the tooth pulp on the release of AVP and OT into CSF in rats under chloralose anesthesia.

Methods. Cerebrospinal fluid was collected from the cerebellomedullary cistern and then 30-minute perfusions of the lateral cerebral ventricles with artificial cerebrospinal fluid (aCSF) were carried out. The perfusate was collected from the cerebellomedullary cistern at rest (control), during electric stimulation of the tooth pulp which induced nociceptive trigemino-hypoglossal reflex, and after stimulation. In the collected CSF and aCSF perfusates, AVP-like immunoreactivity (AVP-LI) and OT-like immunoreactivity (OT-LI) were determined by radioimmunoassay (RIA).

Results. The concentrations of AVP-LI and OT-LI in CSF were found to reach 21 pg/ml and 67 pg/ml, respectively. Electric tooth pulp stimulation exerted no effect on AVP and OT release into the fluid perfusing the cerebral ventricles during stimulation.

Conclusion. It was found that noxious stimulus from the tooth pulp is not a factor affecting significantly AVP and OT release into CSF.

Key words: Vasopressin-like immunoreactivity – Oxytocin-like immunoreactivity – Radioimmunoassay – Cerebroventricular system – Pain

Vasopressin (AVP) and oxytocin (OT) are synthesized mainly in the magnocellular neurons of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei (Buijs 1978). The AVP and OT nerve fibers from hypothalamic paraventricular and supraoptic nuclei were found to project to the other brain nuclei and spinal cord regions, including the periaqueductal gray (PAG), raphes magnus (NRM), raphes dorsalis nucleus and dorsal horn of the spinal cord, which are involved in antinociception (BUIJS 1978; ANTUNES and ZIMMERMAN 1978; DE VRIES and BUIJS 1983; SWANSON et al. 1980; SAWCHENKO and SWANSON 1982; JENKENS et al. 1984). The data concerning the effect of noxious stimuli on AVP and OT release into cerebrospinal fluid (CSF) are scarce. Noxious stimuli were shown to increase synthesis of AVP and OT in the hypothalamus (HAMAMURA et al. 1984) and its release into the CSF in rats (DOGTEROM et al. 1977) and dogs (BROWN and PERKOWSKI 1988).

YANG et al. (2006 a,b,c,d) have found, that pain stimulation elevated AVP concentration in the liquid perfusing the NRM, caudate nucleus and PVN, but OT concentrations in perfusates from these structures were not elevated. Moreover, the antinociceptive effect of AVP was limited to the brain nuclei, not to the spinal cord and peripheral organs in the rat.

We previously found that CSF is a route of transmitting SP-mediated information between the brain

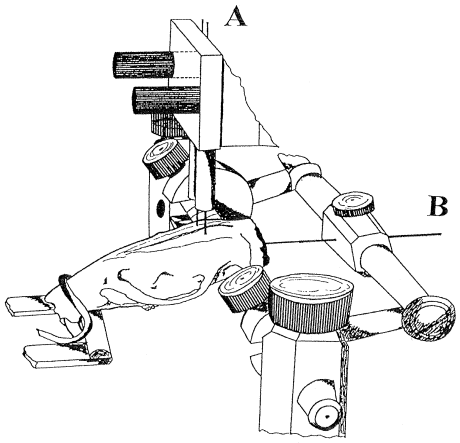

Fig. 1. Position of a rat skull in a stereotaxic instrument adapted for perfusion of cerebral ventricles. A- inflow cannulae for lateral ventricles, B – outlow cannula for cerebellomedullar cistern.

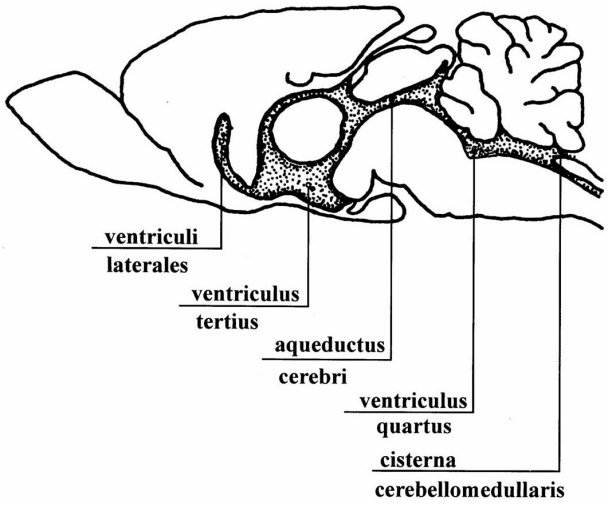

Fig. 2. Brain structures perfused with aCSF

centers. Thus, electric stimulation of tooth pulp caused enhanced substance P (SP) release, whereas stimulation of PAG inhibits SP release to artificial cerebrospinal fluid (aCSF) perfusing the cerebroventricular system in rats (Zubrzycka and Janecka 2001, 2002). We have also shown that intracerebroventricular perfusion of a rat brain with AVP or OT produced a central analgesic effect. AVP exerted its antinociceptive effect by activating vasopressin V_1, opioid and serotonin receptors (Zubrzycka and Janecka 2005). The antinociceptive effect of OT was mediated by the OT receptor and by μ- and ?-opioid receptors (Zubrzycka et al.2005).

The results of these studies allowed to draw a conclusion that AVP and OT are likely to penetrate through the cerebroventricular lining to CSF and exert a modulatory effect on the tongue motor center situated near III and IV cerebral ventricles.

In the present study we wanted to determine in the same experimental model, to what extent noxious stimuli from the tooth pulp can stimulate the release of AVP and OT to aCSF perfusing the cerebral ventricles in rats.

Materials and Methods

Experimental animals and anaesthesia. The experimental protocol was approved by the Local Ethical Committee for Animal Research and it complies with the European Community guidelines for the use of experimental animals. Male Long-Evans rats weighing 350-370 g were kept under standard conditions: temperature 22 °C, 12 h light-dark cycle, and allowed tap water and rodent chow *ad libitum*. The rats were anaesthetised with a single i.p. injection of chloralose (150 mg/kg). For each experiment 10 animals were used.

Chemicals. Artificial cerebrospinal fluid (aCSF) was prepared according to Daniel and Lederis (1967) and contained: 120 mM:NaCl, 2.6 mM $NaHCO_3$, 4.8 mM KCl, 2.8 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1.2 mM $KHPO_4$ and 10 mM glucose. AVP and OT for radioimmunoassay standard curves were purchased from Peninsula Laboratories (San Carlos, USA).

Perfusion of cerebral ventricles in rats. The rat's head was immobilized by introduction of ear bars into the external auditory meati and fixing the maxilla with jaw clamps in a stereotaxic instrument specially adapted for perfusion of the cerebral ventricles (Fig. 1.) (Zubrzycka et al.1997). The skin of the animal's head, anaesthetized with 2 % polocaine solution, was incised in the midline and the skull bones were exposed. On the basis of modified co-ordinates given by De Groott's stereotaxic atlas (1963), the sites for drilling holes in the skull bones were determined: to the lateral ventricles - 9 mm anterior to the frontal interaural zero plane and 3 mm lateral to the sagittal zero plane. The system of cerebral ventricles was perfused by inserting stainless steel cannulas into both lateral ventricles and to the cerebellomedullary cistern. (Fig. 2.). The container with perfusion fluid was positioned 20 cm above the animal's head. aCSF solution was used for perfusion. The outflow cannula inserted into the cerebellomedullary cistern was connected to a polyethylene tube ca 100 cm long which provided the outflow for the perfu-

sion fluid. The flow rate at the end of the tubing in the course of perfusion was 0.5-0.7 ml/10 min.

The cerebral ventricles were perfused with aCSF solution (see above). Four 30-min, 1.5 ml samples of perfusing fluid were collected into glass tubes placed on melting ice. Each tube contained 0.02 ml of glacial acetic and 6 mg of dextran (110,000 MW). Perfusion fluid in each tube was centrifuged at 10 °C and the supernatant was frozen and lyophilized, and kept in sealed tubes until determination of vasopressin by radioimmunoassay.

At the end of experiment the cerebral ventricles were perfused with 1 % trypan blue solution till the stain appeared in the outflow tubing leading out of the cerebellomedullary cistern.

Tooth pulp stimulation. After placing the animal's head in a stereotaxic instrument, the tips of both lower incisors were cut off with a dental separator and stainless steel wire electrodes were inserted into the pulp and fixed with dental cement. The pulp bipolar stimulation was delivered 6 times per minute, with a train of four electrical impulses, of 200 Hz frequency, 3 ms single impulse duration with 2 ms intervals and 4-6 V amplitude, using a programmed stimulator. Trains of 4 impulses were delivered to the pulp at 10 s intervals.

The amplitudes of electrical impulses stimulating the incisor pulp were adjusted individually for each animal. At the beginning of each experiment the intensity of stimulus inducing maximal tongue jerks was determined. Then, the amplitude of impulses was reduced to obtain the amplitude of tongue jerks equal to the half of the maximal value. The amplitude of stimulating impulses adjusted this way, as well as other parameters, remained unchanged till the end of the experiment. The tip of the animal's tongue was attached with a silk thread to an isotonic rotating tensometric transducer. The amplitude of tongue jerks was recorded on a paper using a Line Recorder TZ-4620 (Laboratorni Pristroje Praha, Czech Republic).

Radioimmunoassay. The AVP and OT content in CSF and perfusates was determined by double-antibody specific radioimmunoassay as described previously (Ciosek et al. 1993). Anti-AVP and anti-OT antibodies were raised in rabbits by Dr. Monika Orlowska-Majdak (Department of Experimental Physiology, Medical University of Lodz). Commercial AVP and OT were used for standard curve preparation, as well as for iodination with ^{125}I, using the chloramine-T method (Grenwood et al. 1963). Final dilution of the anti-AVP antibodies was 1:24000 and for anti-OT was 1:80000. Sensitivity was about 2 pg/tube, the within-assay and between-assay cv were 3.9 % and 6.5 %, respectively.

Statistical analysis. The data are expressed as means ± SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by a post-hoc multiple comparison Student Newman-Keuls test**.** $P<0.01$ was considered statistically significant**.**

Results

AVP-like immunoreactivity content in CSF and in the fluid perfusing cerebral ventricles. The experimental animals were divided into four groups of 10 animals each. In Group I CSF was collected from the cerebellomedullary cistern for 30 min. In Group II 30-min perfusates of aCSF were collected and regarded as control. In Group III 30-min perfusates of aCSF were collected during incisor pulp stimulation. Finally, in Group IV 30-min post-stimulation perfusates of aCSF were collected. The concentration of AVP-like immunoreactivity (AVP-LI) in CSF or aCSF in groups I-IV was 21 ± 3.28 pg/ml/30min; 7.6 ± 1.76 pg/ml/30min; 8.2 ± 2.06 pg/ml/30min and 9.07 ± 2.51 pg/ml/30min., respectively. The obtained results show that electric stimulation of the tooth pulp (Group III) did not cause any significant change of AVP content in the perfusate. In the perfusate collected for 30 min after electric stimulation, no significant increase (9.07 ± 2.51 pg/ml/30 min) of AVP content was observed. Fig. 3 shows AVP content in CSF (Group I) and in aCSF (Groups II-IV) collected from the cerebellomedullary cistern. Each bar represents a mean ± SEM ($p<0.01$).

OT-like immunoreactivity content in CSF and in the fluid perfusing cerebral ventricles: The concentration of OT-like immunoreactivity (OT-LI) in CSF or aCSF in Groups I-IV was 67 ± 7.33 pg/ml/30min; 16.5 ± 3.76 pg/ml/30min; 18.3 ± 5.05 pg/ml/30min and 19.08 ± 4.8 pg/ml/30min., respectively. The obtained results show that electric stimulation of the tooth pulp (Group III) did not cause a statistically significant change of OT content in the perfusate. In the perfusate collected for 30 min after electric stimulation, no statistically significant increase (19.08 ± 4.8 pg/ml/30 min) of OT content was observed. Fig. 4 presents bars illustrating OT content in CSF (Group I) and in aCSF (Groups II-IV) collected from the cerebellomedullary cistern. Each bar represents a mean ± SEM ($p<0.01$).

Discussion

It has been demonstrated that AVP and OT may reach the CSF via the circumventricular organs or be direct-

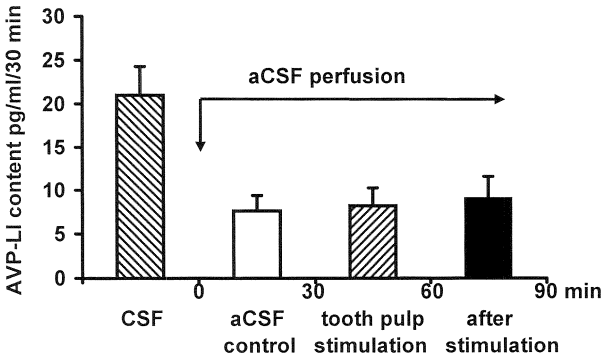

Fig. 3. Vasopressin-like immunoreactivity (AVP-LI) content (pg/ml/30 min) in: CSF, aCSF perfusate without stimulation, aCSF perfusate during tooth pulp stimulation, and aCSF perfusate after stimulation (mean±SEM, n=10)

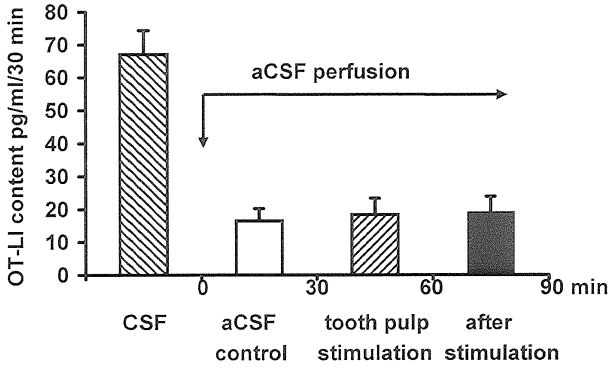

Fig. 4. Oxytocin-like immunoreactivity (OT-LI) content (pg/ml/30 min) in: CSF, aCSF perfusate without stimulation, aCSF perfusate during tooth pulp stimulation, and aCSF perfusate after stimulation (mean±SEM, n=10)

ly released to CSF from brain extracellular space (Sörensen 1986; Engelman et al. 1999; Proeschold et al. 2000; Ludwig et al. 2002; Sewards and Sewards 2003).

Basal CSF levels of AVP and OT were measured by Dogterom et al., (1977, 1978) and they amounted to 11.5 pg/ml and 73 pg/ml, respectively. The CSF levels of AVP and OT are estimated to range from 22 pg/ml to 59 pg/ml (Mens et al. 1982), or from 5 to 20 pmol/l (Jolkkonen et al.1986). According to other authors, rat CSF contains from 8 to 20 fmol/ml AVP and from 10 to 70 fM/ml OT (Robinson 1983). The level of AVP in rat CSF obtained by Ivanyi et al., (1991) was from 20 to 25 pg/ml, by Lipinska and Buijs (1988), 27 pg/ml, and 68 pg/ml according to Orlowska and Buijs (1984).

In our experiments, the average content of AVP in CSF was 21 pg/ml, and that of OT was 67 pg/ml, whereas in the fluid perfusing the cerebral ventricles these values reached 7.6 pg/ml for AVP and 16.5 pg/ml for OT. The present results demonstrated that electric tooth pulp stimulation had no effect on AVP and OT content in the fluid perfusing the cerebral ventricles, collected from the cerebello-medullary cistern and are in agreement with the data obtained by Orlowska et al. (1994) during the stimulation of supraorbital, infraorbital and sciatic nerve nociceptive fibers in rat which indicated that impulsation induced by electric stimulation caused a release of large quantities of AVP to the blood rather than to CSF.

Stimulation may cause a release of large quantities of AVP and OT from dendrites into the extracellular fluid without increasing the concentration of these peptides in the CSF. This does not necessarily indicate that AVP and OT released into the extracellular fluid cannot reach the ventricles. It rather suggests that many neuropeptide molecules get lost on their (possibly long-lasting) way to the ventricles, as they are bound by receptors and are degraded by enzymes. The blood-brain barrier (BBB) within the circumventricular organs is not so tight, but, as it seems, still sufficient to prevent the diffusion of AVP and OT from plasma to CSF (Faraci 1989). Receptors for these neurohormons, responding to noxious stimuli from the tooth pulp, are probably located within the blood-brain barrier. High AVP and OT levels in CSF collected from the cerebello-medullary cistern in our experiments indicate that these neurohormons are continually released into the cerebral ventricles. The AVP- and OT-ergic cell bodies and nerve terminals present in the ependymal lining of the cerebral ventricles and the spinal cord are probably the sources of AVP and OT. The presence of mRNAs for AVP and type 1 AVP receptor has also been demonstrated in the choroid plexuses of the lateral cerebral ventricles, which are the main sources of CSF (Zemo and McCabe 2001).

Yang et al. (2006d) demonstrated that pain stimulation elevated the AVP concentration in the perfusing liquid in the rat brain nuclei, but did not affect its concentration in the spinal cord and blood; only intraventricular and intravenous injection of AVP increased the pain threshold, whereas that of anti-AVP serum decreased the pain threshold. This might indicate that AVP, through the brain nuclei, participates in antinociception.

The phenomena of the OT system up-regulation under strong stimulation were described by Brown and

Perkowski (1998), who reported that there was more OT in CSF of dogs with neck and back pain caused by spinal cord compression that in normal dogs. Yang (1994), described acute chronic pain of lower back altering OT content in human CSF.

We presume that noxious tooth pulp stimulation can induce PVH and SON to release AVP and OT, which can be delivered to other brain nuclei including PAG, RMN and raphes dorsalis nucleus through AVP and OT nerve fibers, exerting their effect on sensory and motor centers of the trigemino-hypoglossal reflex so that AVP and OT take part in analgesia in the orofacial area.

The effect of stress and nociceptive factors on AVP and OT release into CSF is very controversial. There are reports where such an effect was observed (Dogterom et al. 1977, Brown and Perkowski 1998), whereas other investigators failed to confirm it (Orlowska et al. 1994, Orlowska and Traczyk 1996). Determination of endogenous peptide levels in CSF can provide information concerning changes in the metabolism of these peptides or activity of the neurons, from which they are released, which can be useful in studies of etiology and pathogenesis of different diseases, as well as in their diagnostics.

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