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Hippocampal Vasopressin Release Evoked by N-Methyl D-Aspartate (NMDA) Microdialysis

M. ORŁOWSKA-MAJDAK, W. Z. TRACZYK, D. SZYMAŃSKI

Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry, Medical University of Łódź, Poland

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Summary

Hippocampus is a brain structure containing vasopressin (AVP) fibers and specific binding sites for this peptide. There is growing evidence that AVP and its metabolites participate in glutamate-mediated plasticity of the hippocampus. The aim of the present study was to evaluate the influence of NMDA on AVP release in the rabbit hippocampus. Caudate nucleus was chosen as the reference structure. The mentioned brain structures were simultaneously microdialyzed with 0.9 % NaCl solution. AVP was determined in the outflowing fluid by radioimmunoassay. The mean basal AVP content in the fluid outflowing from the hippocampus was significantly greater than that from the caudate nucleus. The addition of K^+ into the fluid perfusing the probes implanted into the hippocampus and caudate nucleus significantly increased AVP release into the extracellular fluid of both brain structures. NMDA applied into the mentioned brain structures increased AVP release only from the hippocampus but not from the caudate nucleus. Our findings indicate a role which NMDA receptors play in AVP release into the extracellular fluid of the hippocampus.

Key words

Hippocampus • Caudate nucleus • Microdialysis • NMDA • K^+ • Vasopressin • Rabbit

Introduction

The brain vasopressinergic endings were shown to innervate, among other limbic system structures, the ventral hippocampus (Buijs 1978). Some evidence has indicated that AVP could function as an excitatory neurotransmitter in the hippocampus (for review see Urban 1998). It has been suggested that AVP and its metabolites may act as memory facilitating principles (de Wied and van Ree 1982). The hippocampus seems to be the site of AVP action on memory processes (Kovacs *et al.* 1982, Ibragimov 1990, Orłowska-Majdak *et al.* 2001).

The excitatory amino acids, glutamate and aspartate, are the primary mediators of excitatory transmission in the central nervous system and play an important role in neuroendocrine regulation (van den Pol *et al.* 1990, Brann and Mahesh 1994). Glutamate receptors can be categorized into two principal classes: ionotropic and metabotropic. Both classes of receptors are involved in the learning and memory processes in the hippocampus (Riedel and Reymann 1996). It is speculated that glutamate acts as the fast excitatory transmitter through its ionotropic receptors and the feedback regulation *via* metabotropic receptors acts to protect neurons from excessive excitation (Kullmann 1999).

Circuits within the hippocampus are remarkably plastic, and this plasticity is mediated in part through

Fax+420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

changes in synaptic strength and revealed by long-term potentiation (LTP) and long-term depression (LTD) (Shapiro 2001). Both classes of glutamate receptors participate in these phenomena (Asztely and Gustafsson 1996, Bortolotto *et al.* 1999). N-methyl-D-aspartate (NMDA) receptors, a subtype of ionotropic glutamate receptors are crucial for inducing these plastic changes, whereas blockade of these receptors reduces plasticity and impairs learning in tasks that require the hippocampus (Shapiro 2001).

Excitatory amino acids were suggested to be an important fast excitatory transmitter system in the paraventricular and supraoptic nuclei responsible for the secretion of vasopressin (AVP) and oxytocin (OXT) (van den Pol *et al.* 1990). NMDA injected peripherally enhanced AVP and OXT concentrations in the rat plasma (Ježová and Michajlovskij 1992) and *in vitro* stimulated AVP release from perifused explants of the hypothalamo-neurohypophyseal system (Swenson *et al.* 1998). Moreover, it was demonstrated that NMDA (Swenson *et al.* 1998) and non-NMDA (Sladek *et al.* 1998) glutamate receptors participate in the osmotic regulation of AVP release.

There is growing evidence that AVP and its metabolites participate in glutamate-mediated plasticity in the hippocampus (for review see Urban 1998). Present experiments were performed to ascertain if NMDA can modify AVP release from the rabbit hippocampus. The caudate nucleus was chosen as the reference structure.

Methods

Animals

Adult male white New Zealand rabbits, weighing over 3 kg, were used in this study. They were housed individually in their cages with food and water *ad libitum* and kept under controlled laboratory conditions in a light regulated room (lights on at 06:00 h and off at 20:00 h). Eight rabbits were microdialyzed intrahippocampally and intracaudally with 0.9 % NaCl or NMDA or K^+ solutions, whereas one rabbit underwent microdialysis with 0.9 % NaCl solution only. All experimental procedures were carried out in accordance with the NIH guide for care and use of laboratory animals.

Surgical procedure

Surgery for the implantation of the guide cannulae was performed. The animals were premedicated with a subcutaneous injection of atropine sulphate (1.0 mg/animal) and anesthetized with intravenous hexobarbital sodium (40.0 mg/kg b.w. Germed). A plexiglass headpiece with guide cannulae for the microdialysis probes was stereotaxically implanted: two cannulae for the caudate nuclei and two others for the hippocampi, according to a rabbit stereotaxic atlas (Sawyer *et al.*1954). An additional fifth cannula inserted into the third cerebral ventricle was used as a reference cannula for four others. The whole procedure was described in detail earlier (Traczyk *et al.*1997). After the surgery, each rabbit received intramuscular injections of 100 000 IU of benzylpenicillin potassium (Polfa-Tarchomin) and 0.5 g streptomycin (Polfa-Tarchomin) daily during the five consecutive days. The guide cannulae were filled with stilettes, so they remained patent for several weeks until implantation of the probes. An aluminium cover was fixed on the headpiece as a protection against damage. The animals with implanted headpieces were kept in their cages under standard laboratory conditions as mentioned above.

Microdialysis probe implantation

One week before implantation of the microdialysis probes, the rabbits were accustomed to spend several hours daily in a special box designed to facilitate probe implantation and microdialysis. The box restricted body rotation but allowed free access to food and water. On the day of implantation the rabbits received an i.v. infusion of a 20 % solution of mannitol (600 mg/kg b.w.). The stilletes were removed from the guide cannulae to hippocampus and caudate nucleus contralaterally. Microdialysis probes (CMA/ Microdialysis, Stockholm, Sweden) 20 mm long, with the membrane of 4 mm in length, molecular cut off approximately 20 000 Daltons (Cat.No. 8309504) were perfused with degassed 0.9 % NaCl solution at a rate of 1 µl/min. 0.9 % NaCl solution was used instead of artificial cerebrospinal fluid because much earlier loss of efficacy of the probes perfused with artificial cerebrospinal fluid than with 0.9 % NaCl solution was observed in our earlier experiments. This was probably caused by crystal formation inside the probe from the perfused artificial cerebrospinal fluid during weeks of experiments (Traczyk *et al.* 1997). The outlet tubings of the probes were cut shorter to the length of 2/3 of the inlet tubing length. Microdialysis probes filled and continuously perfused with 0.9 % NaCl solution were inserted into the guide cannulae under the operation

microscope and fixed in the headpiece by screws. After 10 min of tentative microdialysis the stand with a coil was fixed to the rear side of the headpiece, microdialysis was finished and inlet and outlet tubings of microdialysis probes were wound round the coil. An aluminium cover was put over the headpiece for protection. Figure 1 shows the rabbit's head during microdialysis procedure.

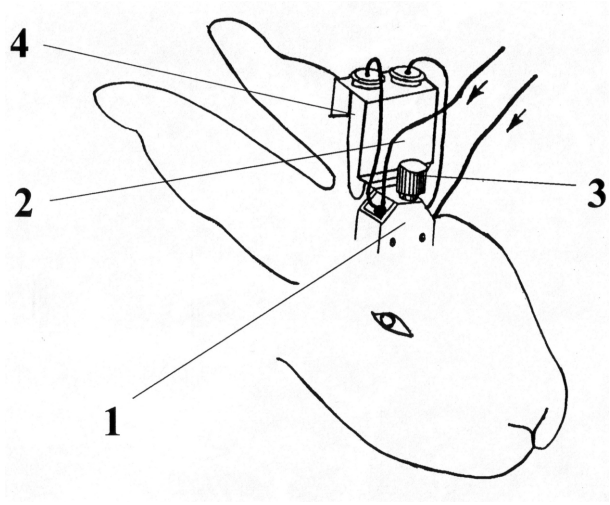

Fig. 1. *Rabbits with implanted headpiece and microdialysis probes during collection of the outflowing dialysate: 1 – plexiglass headpiece, 2 – aluminium stand for tubes, 3 – screw fixing the stand, 4 – tubes for outflow fluid collection.*

Microdialysis procedure

All dialysis experiments were begun at least 24 h after probes implantation, i.e. when disturbed function of blood-brain barrier had probably recovered (Westergren *et al.* 1995). The probes inserted into the hippocampus and the caudate nucleus were simultaneously perfused with degassed 0.9 % NaCl at a rate of 1 μl/min three times or at least twice weekly. Samples of the outflowing fluid were collected in two polyethylene tubes containing 18 μl of 1 N acetic acid solution and 90 μl of dextrane 110 000 m.w. solution. Two samples of the outflowing fluid were collected daily from the each structure. The first sample was collected during the first 180 min of dialysis procedure (1-180 min) and the second sample was collected during the next 180 min of dialysis (181-360 min). All samples were immediately frozen and lyophilized.

Experimental protocol

In one rabbit the hippocampus and the caudate nucleus were repeatedly dialyzed with 0.9 % NaCl solution to determine the release of vasopressin in the basic conditions. The microdialysis procedure lasted two months. Moreover, in eight rabbits the initial three microdialysis procedures were performed in the basic conditions using 0.9 % NaCl (Table 1). These 0.9 % NaCl samples obtained in nine rabbits were used to calculate the mean basic AVP concentration in the dialysate from both structures. The fourth procedure of microdialysis was performed in eight rabbits using 56 mM K^+ solution in 0.9 % NaCl (0.9 % NaCl + K) during the whole procedure to stimulate vasopressin release from the AVP-ergic endings. Then microdialysis of the brain structures was repeated three times with 0.9 % NaCl solution to lead to the basal level of intracerebral vasopressin release. From this moment, N-methyl-D-aspartic acid (NMDA, Feinbiochemica, Lot 500183) was added to the dialyzing medium during the first 30 min of the collection of the second (181-360 min) sample of the dialysate.

To compare the effects caused by different doses of NMDA, increasing concentrations of NMDA were used (2, 4, 8, 16 and 32 mmol/l). Successive concentrations of NMDA were applied at intervals of at least 10-12 days. In the meantime, microdialyses of the brain structures were done using 0.9 % NaCl solutions. Taking into consideration the rate and the time of the infusion, and the value of 24 % as typical recovery determined for aspartate by CMA/Microdialysis – the manufacturer of the probes – the amounts of NMDA reaching the brain structures could have values of about 2, 4, 8, 16 and 32 μg. At the end of experiments 0.9 % NaCl + K solution was used as the dialyzing medium again in each rabbit, if possible, to compare the excitability of AVP-ergic endings at the beginning and at the end of the experimental procedure. Samples of dialysate from the hippocampus and from the caudate nucleus were collected to determine vasopressin content.

Upon completion of the experiments, the position of the probes in the nervous structures was marked by perfusion of 30 μl of a 10 % Ianus Green solution through both probes. Later the animals were sacrificed with a letal dose of urethane (Fluka), their heads were perfused through both carotid arteries with 200 ml of isotonic saline with heparin followed by 1-1.5 l of a formalin solution, cut off and immersed in a 10 % formalin solution for at least one week.

Table 1. Protocol of animal treatment.

Days	
1	
4	microdialysis with 0.9 % NaCl
7	
10	microdialysis with 56 mmol/l K^+
13	
16	microdialysis with 0.9 % NaCl
19	
22	control microdialysis with 0.9 % NaCl (0-180 min) microdialysis with 2 mmol/l NMDA (181-360 min)
25	
28	microdialysis with 0.9 % NaCl
31	
34	control microdialysis with 0.9 % NaCl (0-180 min) microdialysis with 4 mmol/l NMDA (181-360 min)
37	
40	microdialysis with 0.9 % NaCl
43	
46	control microdialysis with 0.9 % NaCl (0-180 min) microdialysis with 8 mmol/l NMDA (181-360 min)
49	
52	microdialysis with 0.9 % NaCl
55	
58	control microdialysis with 0.9 % NaCl (0-180 min) microdialysis with 16 mmol/l NMDA (181-360 min)
61	
64	microdialysis with 0.9 % NaCl
67	
70	control microdialysis with 0.9 % NaCl (0-180 min) microdialysis with 32 mmol/l NMDA (181-360 min)
73	
76	microdialysis with 0.9 % NaCl
79	
82	microdialysis with 56 mmol/l K^+
85	
88	microdialysis with 0.9 % NaCl
91	

The interval between succeeding doses of NMDA was occasionally greater than 12 days and at that time microdialysis was repeated more than three times.

Histological verification of the position of microdialysis probe tips

The block containing specified pieces of the hippocampus or caudate nucleus and neighboring structures was excised from the rabbit's brain, washed in tap water for 24 h and then dehydrated in ethanol, clarified in methyl salicylate and embedded in paraffin wax. The time of processing was longer than in routine histology. Serial coronal sections at 12 μm thickness were cut off from paraffin block on a rotary microtome. Subsequent procedure was the same as previously described (Traczyk *et al.*1997), except that the last immersing procedure was performed in xylene with eosine.

Radioimmunoassay of AVP

Each sample of the dialysate was dissolved in 250 μl of distilled water and AVP was determined in duplicate. Anti-AVP antibodies were raised in rabbits according to Moore *et al.* (1977). Characteristics of the antiserum obtained were described previously (Orłowska-Majdak and Traczyk 1996). Additionally cross-reactivity with NMDA was determined, and it amounted to <0.002 %. AVP (Serva, Lot 02424) was iodinated with ^{125}I using chloramine-T method (Greenwood *et al.*1963). The sensitivity was about 2 pg/tube, the within-assay CV amounted to 3.9 % and between-assay CV was 6.3 %.

Statistics

The data were expressed as % of control per sample. The control for AVP release into the hippocampus and into the caudate nucleus following 0.9 % NaCl + K dialysis was the value of AVP content in the dialysate from each structure during the previous day microdialysis with 0.9 % NaCl solution. The control for AVP release into the both structures following NMDA dialysis was the value of AVP content in the dialysate collected this day from each structure as the first sample, that is during microdialysis of both structures with 0.9 % NaCl solution. Significant differences between control values and 0.9 % NaCl + K or NMDA evoked values of AVP in dialysate were calculated using Student's "t" test for paired variables. Mean basic AVP concentration in the fluid perfusing the hippocampus was compared with the value obtained in the fluid perfusing the caudate nucleus using the Cochran-Cox test. In all tests, $p<0.05$ was taken as the level of significance and the data were expressed as mean ± S.E.M.

Results

Position of microdialysis probe tips and the periprobe tissue reaction

Histological verification of the probe tips showed a great spatial distribution of the dye dialyzed through the brain structures. Microdialysis probe tips were most frequently localized in the medial part of the brain structures, both in the hippocampus and the caudate nucleus (Traczyk *et al.* 1997). Hypercellularity around the track left by the microdialysis probe was shown in all rabbits, both in those dialyzed during two months and in these dialyzed during 3 and 5 months (Fig. 2). The cells were not distinguishable by eosin staining, but they could be fibroblasts, astrocytes and granulocytes (de Lange *et al.* 1995).

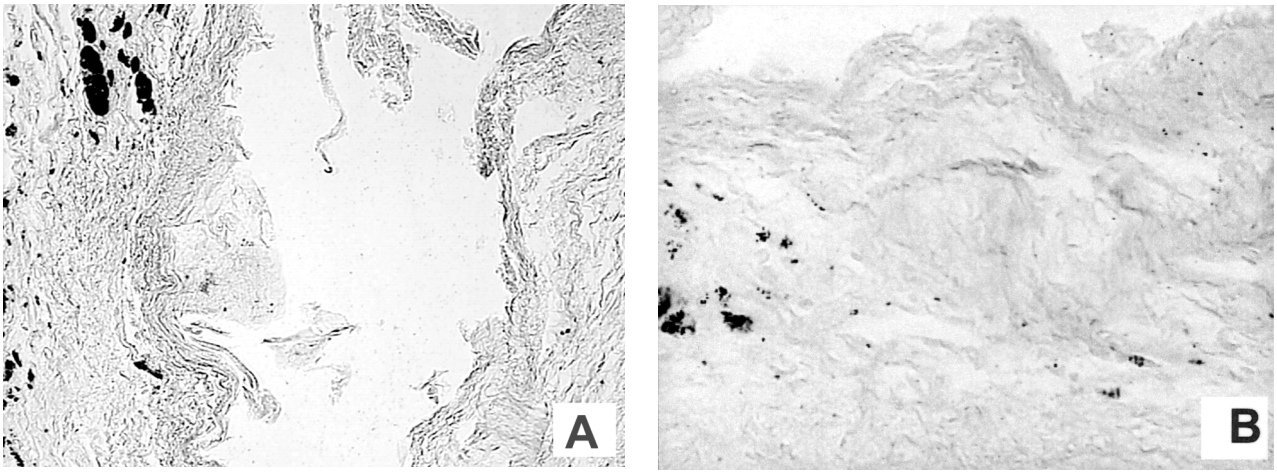

Fig. 2. A. *A photomicrograph of the brain section indicating the region of dialyzed hippocampus in rabbit no 1449, whose brain structures were repeatedly dialyzed during about 3 months, and all five concentrations of NMDA were applied into the brain structures. Microdialysis probe has been removed from the hippocampus after the experiment.* *Periprobe reaction is visible round the hole made by the tip of the probe. Dark spots especially visible on the right present the dye – Ianus Green, which have penetrated into brain tissue far from the hole. Magnification x 10.* ***B.*** *The same as Fig. 2.A but magnification x 20. The edge of the hole made by the probe is shown on the upper side of the photomicrograph. Periprobe reaction is shown beneath. The dye spots are visible on the right.*

Release of vasopressin into the fluid dialyzing brain structures

AVP was consistently detectable in almost all dialysates from the hippocampus and caudate nucleus, however, basal values during 0.9 % NaCl dialysis observed in nine rabbits varied from the limit of detection to the values approximately 100 times higher in both samples. The mean basal AVP concentration amounted to 3.1±0.6 pg in the first sample and 5.7±1.3 pg in the second sample of the fluid dialyzing the caudate nucleus. In the fluid dialyzing the hippocampus the basal values of AVP concentration were greater and amounted to 5.5±1.6 pg in the first sample and to 11.8±4.2 pg in the second. Differences between the mean AVP values in the first and second samples of dialysate from both structures in the rabbits were not significant. Total (first sample + second sample) mean basal AVP concentrations in the dialysate from the hippocampus in all rabbits amounted to 8.6±2.3 pg/sample, and in the dialysate from the caudate nucleus to 4.4±0.7 pg/sample, the difference being highly significant ($p<0.001$) (Fig. 3).

During K and NMDA treatment, the release of AVP was also irregular, for example, from the value at the limit of detection 0.4 pg/sample to 14.2 pg/sample in rabbit no. 1393 (Fig. 4). To analyze the K^+ effect, the AVP concentrations per sample after treatment with 0.9 % NaCl + K at the beginning and at the end of experiments were summed and compared with the AVP concentrations in dialysates obtained during microdialysis of brain structures with 0.9 % NaCl solution. As shown in Figure 5, AVP content in the dialysates from the hippocampus increased significantly to 245±62 % of control value after 0.9 % NaCl + K treatment ($p<0.05$). Greater increase in AVP release was observed into the dialysates from the caudate nucleus, i.e. to the value of 336±95 % of the control release ($p<0.02$).

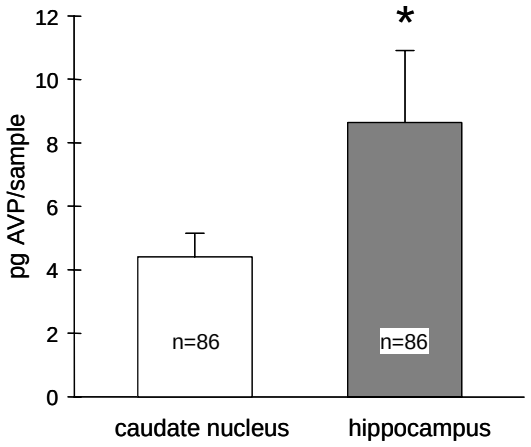

Fig. 3. *Mean content of AVP in pg/sample in the fluid dialyzing the hippocampus and the caudate nucleus in control conditions, when the brain structures were dialyzed with 0.9% NaCl only (mean ± S.E.M.). Significantly different from values found in the caudate nucleus: * $p<0.001$.*

All the five mentioned doses of NMDA (see *Experimental protocol*) were applied in one rabbit only, the remaining rabbits received 4, 3 or 2 successive doses of NMDA. The reason for shortening the duration of the experiment was the blocked flow through the microdialysis probe, or the damage of probe tubings or the probe itself during several weeks of experiments. The increased locomotor activity observed in rabbits a few days after NMDA treatment was the reason of the deviations from the assumed experimental protocol (Table 1). Therefore, the intervals between successive doses of NMDA were sometimes greater than 12 days and the experimental protocols were somewhat different for each rabbit. The analysis of AVP release in relation to the applied doses of NMDA was impossible due to the great scatter of experimental data. Therefore, it was decided to pool all data of AVP concentrations in the dialysates from each brain structure after NMDA treatment (after all concentrations of NMDA had been applied) and to compare them with control AVP release into this brain structure. It was found that NMDA in the hippocampus significantly enhanced AVP release to 365±127 % of the control value (Fig. 5). In the caudate nucleus, AVP release into the dialysate increased after NMDA treatment to the value of 307±131 % of the control value, but this increase was not significant (Fig. 5).

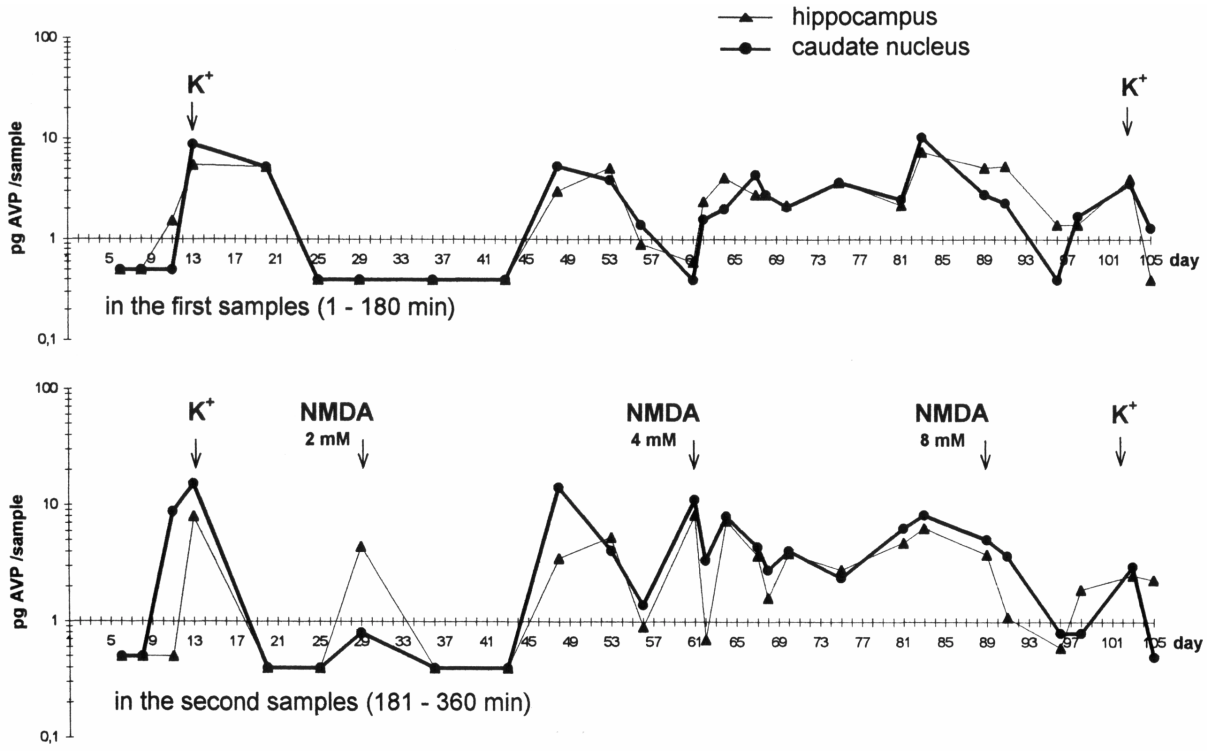

Fig. 4. *Vasopressin content (pg/sample) in the dialysate outflowing from the hippocampus and from the caudate nucleus in the rabbit No. 1393. The upper graph represents AVP contents in the first samples of dialysate (0-180 min) and the bottom graph shows AVP contents in the second samples (181-360 min). At the beginning and at the end of experimental procedure 56 mM K^+ solution as dialysis medium was used. NMDA (2, 4 and 8 mmol/l) was dialyzed into both structures during the first 30 min of the second sample collection. Subsequent microdialysis was done with 0.9 % NaCl.*

Locomotor activity

Enhanced locomotor activity was observed both in rabbits receiving 0.9 % NaCl + K and NMDA. Most animals exhibited rhythmic jaw movements imitating chewing, moreover they smacked, licked and salivated. Sometimes the rabbits vigorously shook their heads or tried to squirm and jump in their boxes. The frequency and time of appearance of such behavior varied from animal to animal. There was a tendency to increased locomotor movements with increasing NMDA concentrations.

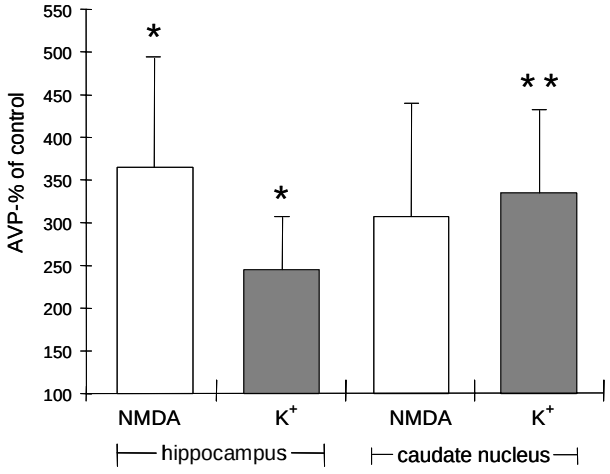

Fig. 5. *AVP in the fluid outflowing from the hippocampus and from the caudate nucleus after 0.9%NaCl + K and NMDA treatment. Data are expressed in percentage of control values. Significantly different from control values:* $p<0.05$ ** $p<0.02$.*

Discussion

Vasopressin was present in appreciable quantities both in the dialysates from the hippocampus and from the caudate nucleus of the rabbit. There was significantly more AVP in the dialysate from the hippocampus than from the caudate nucleus in rabbits under basal conditions. The existence of AVP and its quantity per mg of protein was previously shown in both structures of the rat brain. The caudate nucleus contained nearly twice as much AVP as the hippocampus (Hawthorn *et al.* 1984). On the contrary, AVP was found in the hippocampus but not in the caudate nucleus in human brains (Jenkins *et al.* 1983). Our findings that the amounts of AVP in dialysate from the hippocampus are greater than from the caudate nucleus in the rabbit may be the result of higher resting local AVP release in the hippocampus than in the caudate nucleus in this species.

The central release of AVP was studied in anesthetized (Demotes-Mainard *et al.* 1986) and conscious rats (Langdraf *et al.* 1988) using push-pull perfusions. Resting and stimulus-evoked release of AVP was reported in the hippocampus (Landgraf *et al.* 1988) but not in the caudate nucleus (Demotes-Mainard *et al.* 1986). It is very difficult to compare the AVP release from the hippocampus in the present and in Landgraf's experiments because of many differences in experimental procedures and animals used: rats vs. rabbits, push-pull vs. microdialysis technique, different time and rate of the perfusion. Taking all these into consideration and additionally the duration of passage of AVP through the dialysis membrane (determined *in vitro* earlier to be about 10 % – Orłowska-Majdak *et al.* 2001), the peptide release was similar or slightly greater in the present study compared to that of Landgraf *et al.* (1988). In additional experiments, when the time of perfusion was much shorter than in present experiments, far less AVP was found in the dialysates (unpublished data).

The present experiments were long-lasting, microdialysis was continued for several months and AVP was detectable throughout. Some changes in the morphology of the brain tissue surrounding the microdialysis membrane during a chronic procedure were also shown by others (de Lange *et al.* 1995). There was a reaction to the repeated perfusion procedures but not to the presence of the probe itself (de Lange *et al.* 1995). We therefore carried out an appropriate histological examination of the brain tissue after the microdialysis procedure was performed in each rabbit and a tissue reaction such as hypercellularity was actually observed. But the great concentration of cells shown round the site of probe implantation in the present experiments probably did not disturb molecules of brain AVP to diffuse into the fluid perfusing microdialysis probe. Changes in the permeability of the blood-brain barrier (BBB) during brain microdialysis, especially at the beginning, were possible (for review see Benveniste 1989). Evan's blue extravasation was observed around the dialysis probe 1, 3 and 7 days but not 21 days after the probe insertion (Johansson *et al.* 1995).

In rats (Caffe *et al.* 1987) and in mice (Metzger *et al.* 1993), AVP-ergic endings in the hippocampus originate from the amygdaloid nucleus, although new sensitive techniques have recently allowed to identify AVP neurons inside the rat hippocampus itself

(Hallbeck *et al.* 1999). The origin of the AVP or AVP-ergic endings, if there are any in the caudate nucleus of the rabbit, is not known. To demonstrate that AVP-containing endings are able to release their peptide upon depolarization, high potassium or veratridine as depolarizing stimulus were used. In the present experiments, 56 mmol/l potassium solution (0.9 % NaCl + K) was applied into both structures as the depolarizing stimulus. Both in the hippocampus and in the caudate nucleus potassium solution evoked AVP release, greater in the caudate nucleus than in the hippocampus. It is thus tempting to speculate that AVP-ergic endings must be present in the rabbit caudate nucleus.

Apart from its well-known role in the regulation of locomotion, the caudate nucleus may participate in water regulation in monkey (Simonnet *et al.* 1979), dog (Szczepańska-Sadowska *et al.* 1979) and cat (Rosenberg *et al.* 1988). The participation of AVP in this function of caudate nucleus was revealed (Simonnet *et al.* 1979 and Rosenberg *et al.* 1988) and the role of V_1 receptors located in the caudate nucleus was proved (Rosenberg *et al.* 1988). In the rat, the distribution of AVP binding sites both in the caudate nucleus and in the hippocampus changes markedly during postnatal development: they appear early in development but disappear by adulthood (Petracca *et al.* 1986). Moreover, the caudate nucleus may act in the process of learning and it was shown that AVP also participate in this function of caudate nucleus (Hamburger-Bar *et al.* 1984, Orłowska-Majdak *et al.* 2001). Such effects of AVP in the caudate nucleus are possible through its influence on the caudate nucleus dopamine system (Versteeg *et al.* 1979, Van Heuven-Nolsen and Versteeg 1985). This caudate nucleus dopamine system is much more sensitive to some pharmacological agents compared with other brain dopamine systems (Saunders *et al.* 1994).

0.9 % NaCl + K treatment enhanced AVP release in the hippocampus and caudate nucleus of the rabbit in the present experiments. The effect of high K^+ concentration might be in part mediated by a local hyperosmolarity (56 mmol/l K^+ corresponds to 112 mosm/l) of the brain extracellular fluid. Herrera *et al.* (1993) described additionally to depolarizing widespread effects of such treatment in the hippocampus of rats, namely proto-oncogene, c-fos upregulation and enhanced glutamate release. He suggested that c-fos expression in the hippocampus could be regulated by the release of endogenous glutamate in response to KCl treatment, and that c-fos expression is NMDA receptor-mediated (Herrera *et al.* 1993). Moreover, *in vitro* studies indicated that high potassium concentration induced long-term (LTP) potentiation of synaptic transmission in the rat hippocampus, blocked by NMDA-receptor antagonist (Fleck *et al.*1992). Increased release of AVP from AVP-ergic endings treated with high potassium solution may be a result of the above complex effect of potassium.

NMDA significantly enhanced the release of AVP from the hippocampus and non-significantly from the caudate nucleus. The role of the excitatory amino acids and NMDA receptors in the response of plasma AVP to osmotic stimulation was shown by *in vivo* (Gorąca 1998) and *in vitro* studies in rats (Swenson *et al.* 1998). The NMDA receptor is essential for the regulation of AVP gene transcription in response to osmotic stimulation in the supraoptic nucleus of rats (Amaya *et al.* 1999). The influence of the excitatory amino acids and NMDA receptors on hippocampal AVP neurons or AVP endings function is unknown. One may speculate that it is an excitatory influence because nearly all the neurons studied in the ventral hippocampus were briefly excited by glutamate (Urban 1998). Microiontophoretically administered AVP facilitated the response of these neurons to glutamate and the effect was sustained for up to 60 min after the peptide administration (Urban 1998). Thus, the memory enhancing effect of AVP released in the hippocampus could be due to the enhancing effect of this peptide on the excitability of hippocampal neurons in response to glutamate.

Acknowledgements

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Reprint requests

Monika Orłowska-Majdak, Department of Experimental and Clinical Physiology, Institute of Physiology and Biochemistry, Medical University of Łódź, Mazowiecka 6/8, 92-215 Łódź, Poland, e-mail: morlowska@zdn.am.lodz.pl