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INCREASE IN VASOPRESSIN CONCENTRATION AND CARDIODEPRESSANT ACTIVITY IN THE BLOOD DIALYSATES AFTER NMDA AND HYPERTONIC SALINE ADMINISTRATION[1]

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It has been demonstrated that electric stimulation of the central ends of cut vagus nerves or angiotensin II infusion cause an increase in vasopressin concentration and cardiodepressant activity in the sella turcica venous blood (1). The present study was an attempt to determine if the cardiodepressant factor and vasopressin were simultaneously released from the pituitary into the blood dialysate after osmotic stimulation, and whether excitatory amino acids are involved in this mechanism. The samples of dialysates of venous blood flowing from the sella turcica region and, for comparison, from the femoral vein were collected in anaesthetised rats. The concentration of vasopressin in blood dialysate was determined by radioimmunoassay, and cardiodepressant activity on spontaneously discharging pacemaker tissue of the right auricle of the right heart atrium. Osmotic stimulation or N-methyl-D-aspartic acid infusion caused an increase in cardiodepressant activity and vasopressin concentration in the blood dialysate from the sella turcica and from the femoral vein. A blockade of the excitatory amino acids receptors by specific and non-specific antagonists significantly inhibited the increase in the blood dialysate vasopressin concentration and cardiodepressant activity elicited by an intra-arterial injection of hypertonic saline. These data indicate that excitatory amino acids are involved in the mechanism of increase in blood vasopressin and cardiodepressant factor concentration in response to osmotic stimulation. These results also demonstrate the utility of blood minidialysis for simultaneous monitoring of active substances concentration in the blood.

Key words: *osmotic stimulation, cardiodepressant activity, vasopressin, excitatory amino acids blockers.*

INTRODUCTION

Toxic humoral agents have been regarded for many years as playing a key role in the pathogenesis of shock. Many of these substances appear to be small peptides in the range of 250 to 1,000 daltons (2, 3) and to originate in the ischemic splanchnic region (i.e., pancreas, intestine, liver). The best known and

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best characterised of these cardiotoxic peptides appearing in shock is the myocardial depressant factor (MDF). MDF was initially discovered in 1966 in cat plasma during hemorrhagic shock (4, 5). Cardiodepressant factor (CDF) was also revealed by Hallström *et al.* (6, 7) in blood of animals during post-traumatic shock. Moreover, Iha *et al.* (8) found cardiodepressant activity in the 10,000 to 30,000 mol wt fraction of plasma during sepsis.

The presence of the cardiodepressant factor has been demonstrated in the bovine hypothalamus (9) and in the fluid incubating *in situ* the posterior pituitary lobe in rats (10).

The relationship between plasma osmolality and plasma vasopressin has been described in a number of species. Increases in plasma osmolality after both intravenous and intra-arterial infusion of hypertonic saline above normal results augment in the linear manner in plasma vasopressin (11, 12). It also has been shown that electric stimulation of the central ends of the cut vagus nerves or angiotensin II infusion caused an increase in vasopressin concentration and cardiodepressant activity in the sella turcica venous blood (1).The present study was an attempt to determine: 1) if the cardiodepressant factor and vasopressin were simultaneously released from the pituitary into blood after osmotic stimulation, and 2) whether the excitatory amino acids are involved in the mechanism of the response of blood vasopressin and cardiodepressant factor to osmotic stimulation.

MATERIALS AND METHODS

The experiments were carried out on male rats weighing 380-400 g, the F1 generation of cross-bred of Buffalo strain males and Wistar strain females from the stock of the Institute of Oncology in Warsaw. The animals were anaesthetised by an intraperitoneal injection of a solution containing 6 mg of chloralose (Roth) and 60 mg of urethane (Flucka AG, CH-3470 Bucks) per 100 g of body weight. The experimental animals were divided into 5 groups.

In group 1 (n = 10) six 30 min blood dialysates samples were collected from the femoral vein and from the vicinity of cavernous sinus of the sella turcica.

In group 2 (n = 10) six 30 min blood dialysates samples from the femoral vein and from the vicinity of cavernous sinus of the sella turcica were collected in the same way. On collecting the second dialysate samples, hyperosmotic NaCl solution (1M) in the volume of 0.2 mL was injected into the internal carotid artery.

In the 3rd group (n = 10), N-methyl-D-aspartic acid (NMDA) at the dose of 0.53 mg/kg was injected into the internal carotid artery at the beginning of collecting of the second dialysate sample.

In the 4th experimental group (n = 10), NMDA competitive receptor antagonist D,L-2-amino-5-phosphonopentanoic acid (AP-5) at the dose of 0.53 mg/kg was administered, at the beginning of collecting of the second dialysate sample, 1 min prior to hypertonic saline infusion into the internal carotid artery.

In the 5th experimental group (n=10), NMDA noncompetitive receptor antagonist 6,7-dinitroquinoxaline-2,3 (1h,4H)-dione (DNQX) at the dose of 0.53 mg/kg and 1 mol/L NaCl were administered in the same way at the beginning of collecting the second blood dialysate sample.

Drugs

All reagents were directly dissolved in 0.9% NaCl. The acid pH of the solution was neutralised to pH 7.4 using 5N sodium hydroxide. The volume of each intra-arterial injection was 0.2 mL. The solutions included NMDA (N-methyl-D-aspartic acid) (200 μg) (Sigma, Lot 99 F5805, No 6384-92-5); NMDA receptor antagonists AP-5, D,L-2-amino-5-phosphonopentanoic acid (200 μg) (Sigma Lot 64H4067, No 2379-57-9); a non-NMDA receptor anagonist DNQX, 6,7-Dinitroquinoxaline-2,3 (1H,4H)-dione (200 μg) (Sigma, Lot 108F3810, No 76326-31-3).

Dialysate blood sampling

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 cannulae 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 (*Fig. 1*).

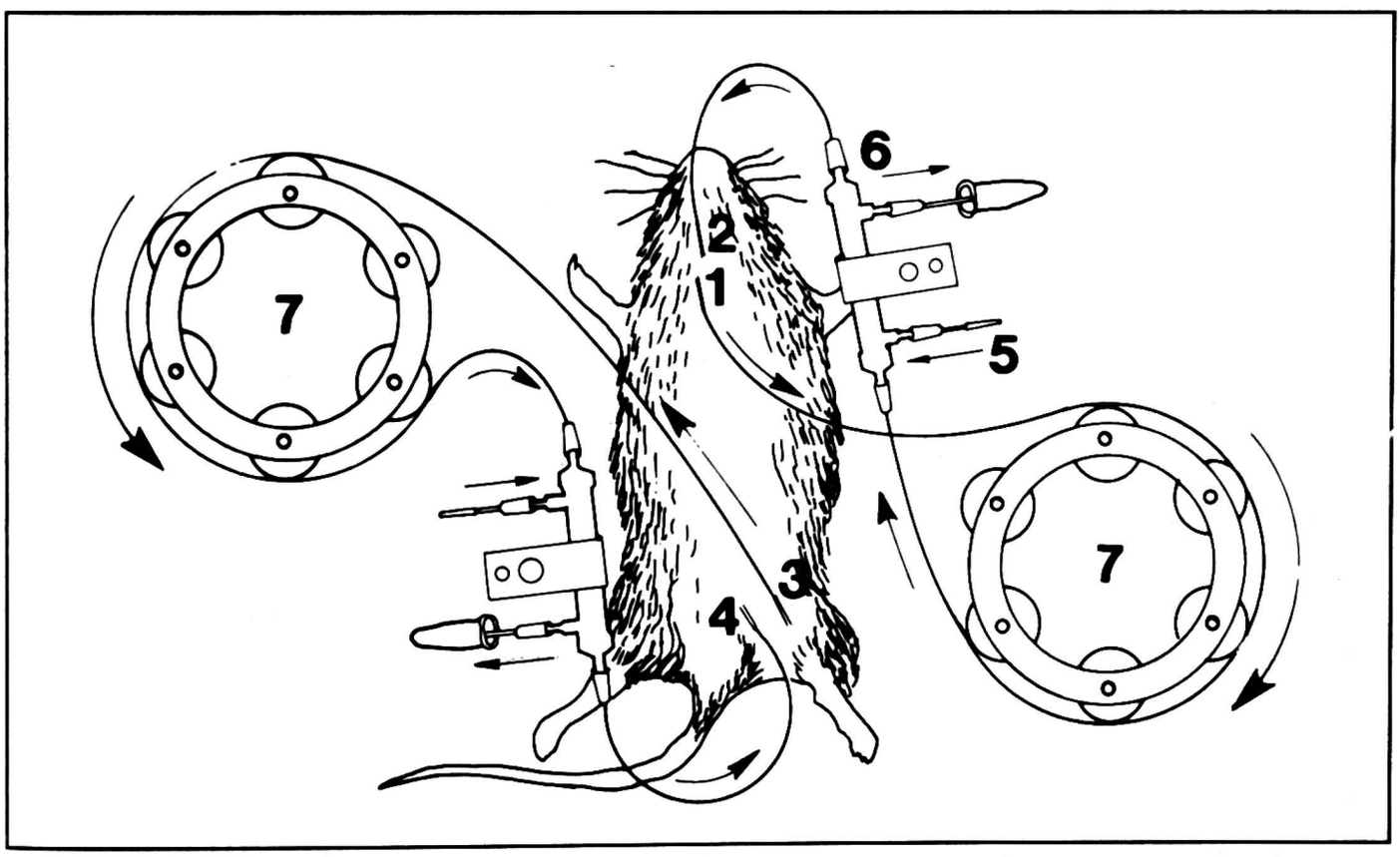

Fig. 1. Dialysis of venous blood outflowing from the cavernous sinus vicinity of the sella turcica and from the femoral vein in rats. 1 — cannula inserted into the sella turcica end of the internal maxillary vein. 2 — cannula inserted into the heart end of the internal maxillary vein. 3 — cannula inserted into the peripheral end of the femoral vein. 4 — cannula inserted into the central end of the femoral vein. 5 — inflow tube for filling minidialyser housing. 6 — outflow tube for collecting dialysing medium from the minidialyser housing. 7 — peristaltic pump.

Blood was drawn from the region of the sella turcica through the polyethylene cannula (and a tube) to a minidialyser by means of a peristaltic pump. It was then returned to the organism 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 a 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 experiment 2 mL of Locke solution with heparin (400 UI/mL) was injected into the femoral vein. The rate of blood flow through the minidialyser was 0.25 mL/min and the pressure at which blood was dialysed was 10 kPa. The whole amount of dialysing fluid was exchanged every 30 min for 3 hrs by draining it directly into a test tube. Six 1 mL dialysate samples were obtained in this way. Before refilling the minidialyser with dialysing fluid its housing was rinsed with dialysing fluid. The dialysates were divided into two parts and lyophilised. One portion was used for the determination of vasopressin (AVP) by radioimmunoassay (13) and the other was assigned for the biological test on a spontaneously discharged pacemaker tissue of the right auricle of the right heart atrium of a two-day-old rat. At the end of the experiment 1% solution of trypan blue was injected into the vicinity of the cavernous sinus of the sella turcica *via* a cannula inserted into the internal maxillary vein. The animals were decapitated and the heads were kept in 10% formalin for several days. The brains were then removed from the skull and the posterior pituitary lobe was verified under a stereomicroscope. Only the animals whose posterior pituitary lobes demonstrated staining were included in our experimental groups. 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.

Characteristics of minidialyser

The minidialysers used in this study have been manufactured according to our design by EURO-SEP-Ltd Warsaw. They have been described in detail previously (14) and are depicted in *Fig. 1* and *Table 1.*

Table 1. Membrane characteristics of the minidialyser used for the blood dialysis.

PARAMETERS	
Molecular cut off (kDa)	20
Active surface (m^2)	0.1
Lumen/wall thickness of capillary (μm)	200/0.8
Number of capillaries	270
Length of capillary (mm)	59
Inner diameter of a capillary (mm)	0.2
Priming volume:blood (mL)	0.8
Priming volume:dialysate	1.6
Membrane material	Cuprophan®
Housing material	Lustran-San®
Polting compound	Polyurethane
Net Weight (g)	8
Maximal working pressure (tor)	500

Determination of Cardiodepressant Activity

Cardiodepressant activity was determined on the pacemaker tissue of the isolated right auricle of the right heart atrium of a two-day-old rat. Two-day-old rats (5.5 g) were decapitated. Each heart was isolated, the auricle of the right heart atrium was dissected under a stereomicroscope and placed on a platinum wire electrode in a 100 μL chamber in Ringer-Locke solution at 20—22°C. Ringer-Locke solution contained in mml/L H_20: NaCl–153; KCl–5.6; CaCl2–3.3; NaHC03–1.7, glucose –5.5 and was oxygen — saturated. The solution additionaly contained atropine sulphate in the amount of 5×10^{-6} mol/L. Ringer-Locke solution was constantly exchanged with an infusion pump at a rate of 0.25 ml/min. After keeping the isolated atrium auricle for 15—30 min in the chamber, it contracted spontaneously at a constant rate. Lyophilised 30 min samples of blood dialysate were dissolved in 0.3 mL redistilled water and injected into the chamber of 100 μL in the volume of 20 μL. The contractions of the atrium auricle were observed under a stereomicroscope and the spontaneous discharge of pacemaker tissue was recorded on the ECG apparatus specially adopted for this purpose.The changes of discharge frequency were analysed by the computer and expressed as the greatest decrease in discharge rate in relation to the control using specially developed software. Discharge rate of the pacemaker tissue was recorded during 20 sec before and 100 sec after administration of the studied sample. Each sample was tested on five preparations.

Statistical analysis

Data are presented as mean ± SE. and were analysed by one-way analysis of variance (ANOVA) followed by Student's unpaired t-test. $P < 0.05$ was considered as significant.

RESULTS

Vasopressin concentrations and cardiodepressant activity were detected in blood dialysate samples from the region of the sella turcica and from the femoral vein.The basal levels of blood vasopressin from the femoral vein (32.92 ± 10.18 pg/mL; $n = 10$) were lower than from the region of the sella turcica (51.09 ± 13.55 pg/mL; $n = 10$) (*Fig. 2*).

Similarly, baseline cardiodepressant activity in blood dialysate samples from the femoral vein were lower ($9.9 \pm 0.08\%$; $n = 10$) than in blood dialysate samples from the region of the sella turcica ($11.8 \pm 0.09\%$; $n = 10$) (*Fig. 2* and *Fig. 3*). Vasopressin content and cardiodepressant activity did not change in the course of dialysis.

In rats that received an intra-arterial injection of hypertonic saline, the dialysate AVP concentration from the sella turcica markedly increased with a peak of 81.4 ± 16 pg/mL ($P < 0.05$; $n = 10$) occurring in the first poststimulus period. Also, in blood dialysate samples from the femoral vein, AVP levels increased significantly during the administration of hyperosmotic NaCl with the peak observed in the first poststimulation period (42.5 ± 8.8 pg/mL, $P < 0.05$; $n = 10$, compared with initial levels) (*Fig. 2*).

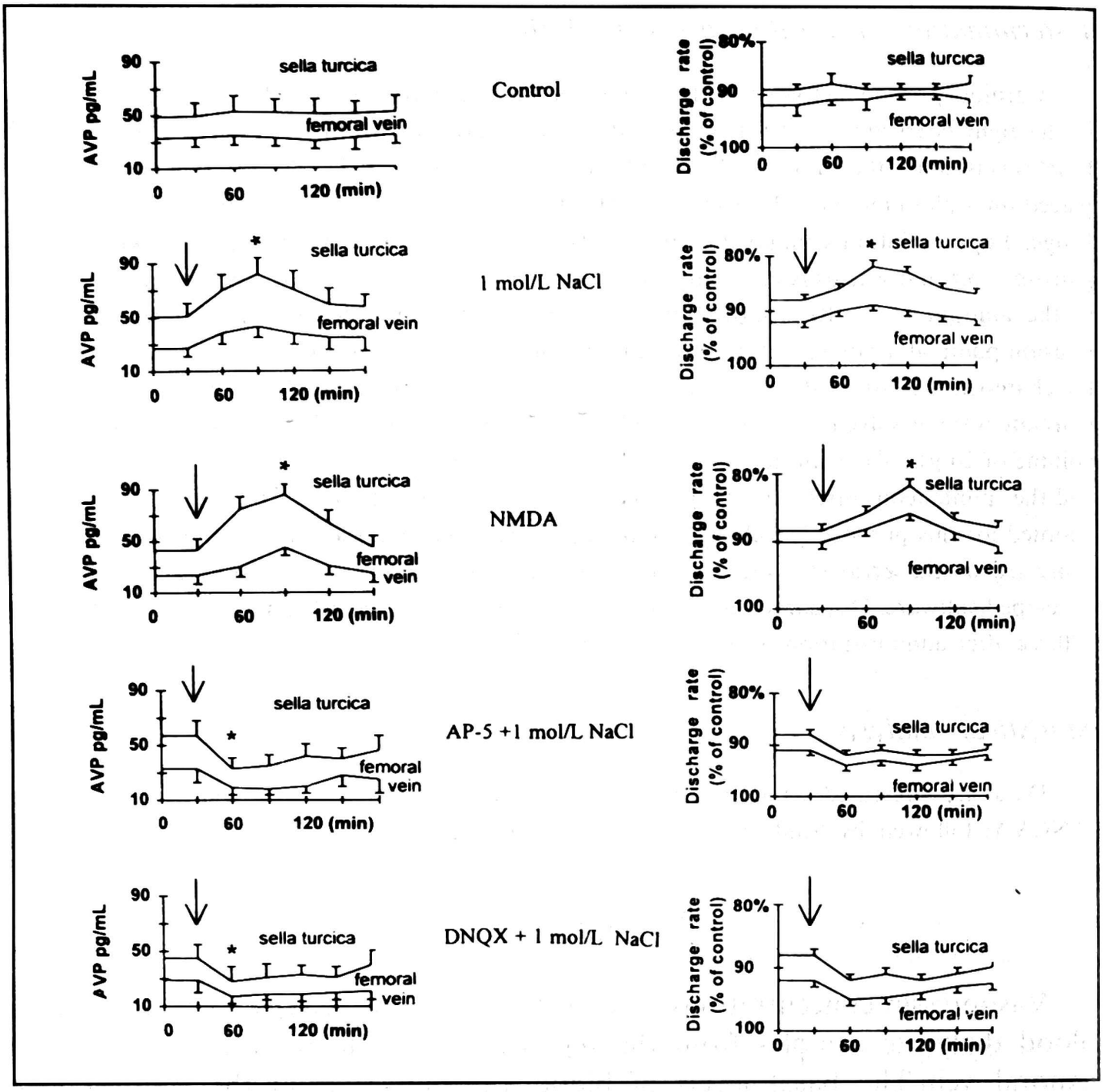

Fig. 2. Vasopressin contents (left hand panel) and cardiodepressant activity (right hand panel) (means ± SE) in consecutive 30-min blood dialysate samples from the region of the sella turcica and from the femoral vein in control and after administration of 1 mol/L NaCl alone , NMDA alone, 1 mol/L NaCl 1 min after pretreatment with AP-5 or DNQX. Arrows indicate time of 20 µL sample administration into the chamber with spontaneously discharging isolated auricle of the right atrium. Each sample was tested on five preparations. Asterisks indicate values significantly different. (Values are expressed as means ± SE, $P < 0.05$, $n = 10$ compared to pre-stimulation values).

This stimulation also produced significant increases in cardiodepressant activity in dialysates from the region of the sella turcica and from the femoral vein by 18.96 ± 2.32% and 13.59 ± 0.87% ($P < 0.05$; $n = 10$), respectively, compared with the initial value) (*Fig. 2* and *Fig. 4*). Infusion of NMDA caused a significant increase in AVP content of both dialysates from the region of the sella turcica (to 85.7 ± 10 pg/mL, $P < 0.05$; $n = 10$ compared with the initial value) and from the femoral vein (to 31 ± 8 pg/mL, $P < 0.05$; $n = 10$ compared

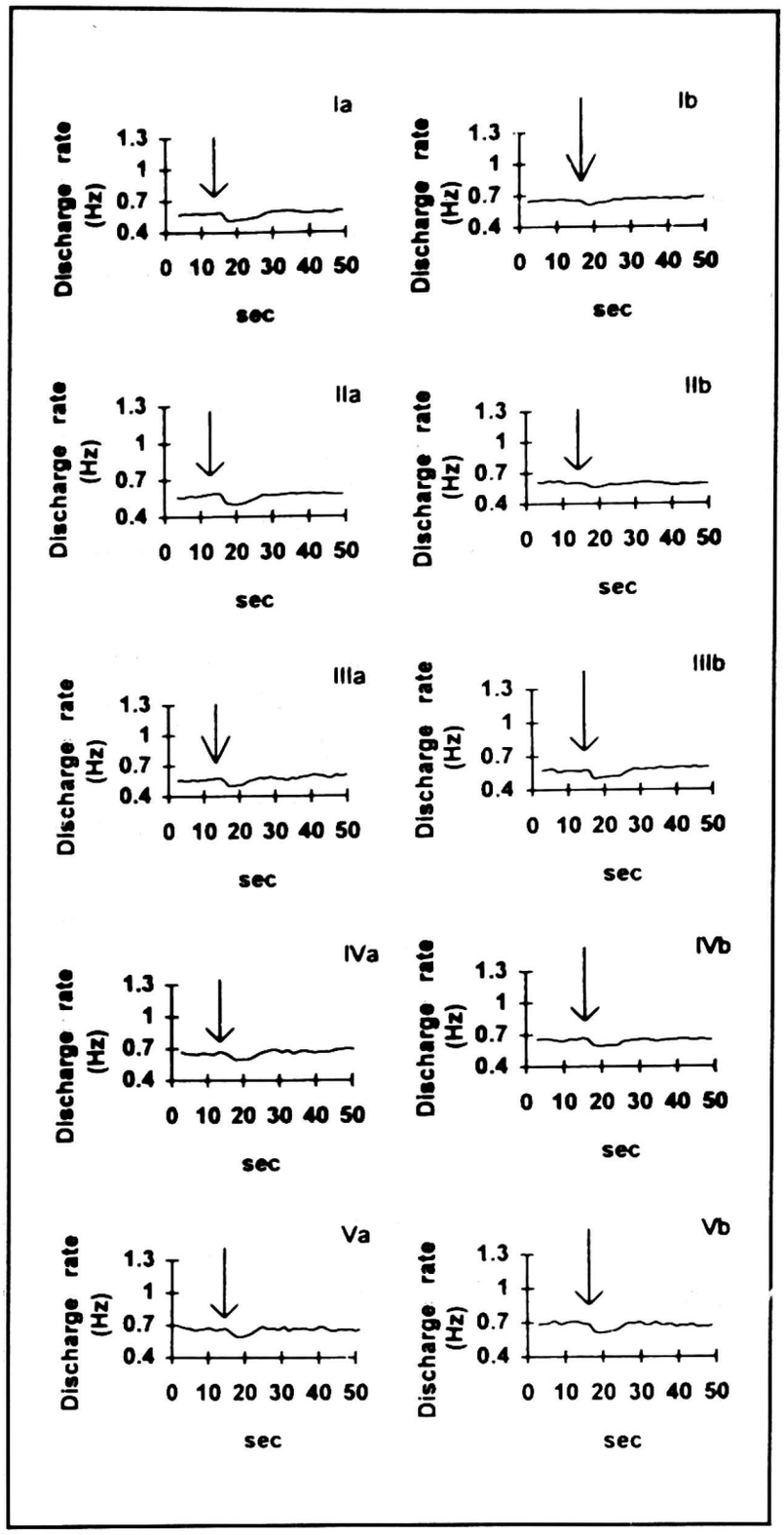

Fig. 3. Original records illustrating changes in spontaneous discharge rate of an isolated right auricle after administration of the first blood dialysate sample (control) from the sella turcica (Ia—Va) and femoral vein (Ib—Vb). Arrows indicate the time of 20 μL sample administration into the chamber with spontaneously discharging isolated auricle of the right atrium.

with the inital value) (*Fig. 2*). The increase in cardiodepressant activity in dialysates from the region of the sella turcica by $19.2 \pm 1.6\%$ ($P < 0.05$; $n = 10$ compared with the initial value) and from the femoral vein by $14.1 \pm 1\%$ ($P < 0.05$ compared with the initial value) after i.a. infusion with NMDA was synchronous with AVP (*Fig. 2* and *Fig. 5*).

To determine whether excitatory amino acids are involved in the mechanism of the increase in the AVP levels and cardiodepressant activity after i.a. injection of EAA antagonist AP-5 or DNQX we investigated the effect of injection of hypertonic saline on the inrease in the plasma AVP concentration and cardiodepressant activity.

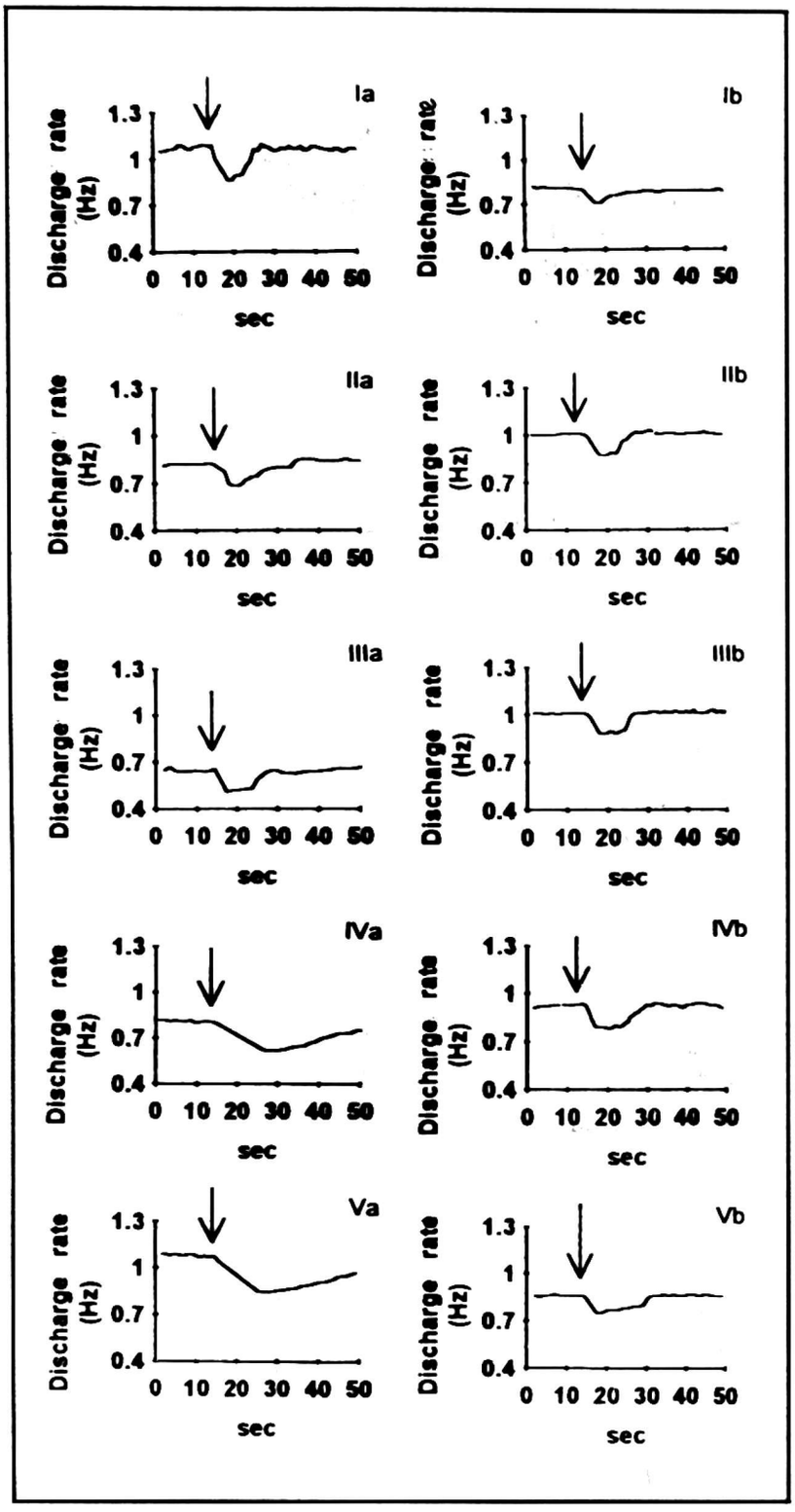

Fig. 4. Original records illustrating changes in spontaneous discharge rate of an isolated right auricle after administration of the third blood dialysate sample from the sella turcica (Ia—Va) and from the femoral vein (Ib—Vb). 1 mol/L NaCl alone was administered at the beginning of 30—60 min dialysis. Arrows indicate the time of 20 μL sample administration into the chamber with spontaneously discharging isolated auricle of the right atrium.

The blockade of NMDA receptors by a competitive receptor antagonist AP-5 and by a noncompetitive NMDA antagonist DNQX significantly reduced vasopressin concentration in blood dialysates from the region of the sella turcica and from the femoral vein in response to hypertonic saline to 28 ± 12.3 pg/mL and 17.3 ± 4.6 pg/mL, respectively, for competentive

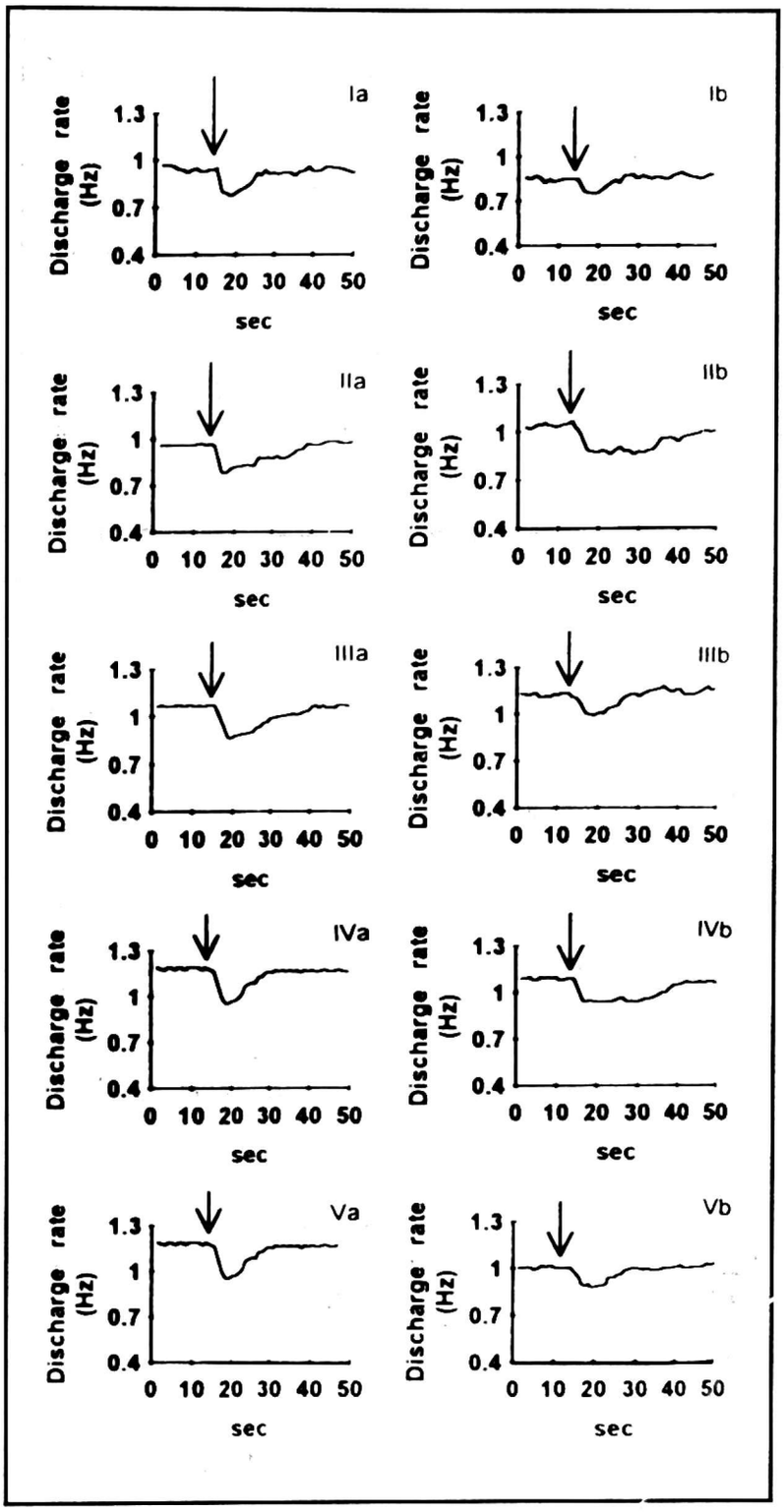

Fig. 5. Original records illustrating changes in spontaneous discharge rate of an isolated right auricle after administration of the third blood dialysate sample from the sella turcica (Ia—Va) and from the femoral vein (Ib—Vb). 0.53 mg/kg NMDA was administered into the internal carotid artery at the beginning of the 30—60 min dialysis. Arrows indicate the time of 20 μL sample administration into the chamber with spontaneously discharging isolated auricle of the right atrium.

blockade ($P<0.05$, $n=10$), and to 33 ± 8 pg/mL and 18.7 ± 4.2 pg/mL, respectively, after noncompetentive blockade ($P<0.05$, $n=10$) (*Fig. 2*).

The blood dialysate samples from the region of the sella turcica and from the femoral vein after AP-5 or DNQX infusion caused a decrease in discharge rate of the auricle of about $7.5\pm0.9\%$ and $5.1\pm1\%$, respectively, compared with the initial value ($P>0.05$, $n=10$) (*Fig. 2*, *Fig. 6*).

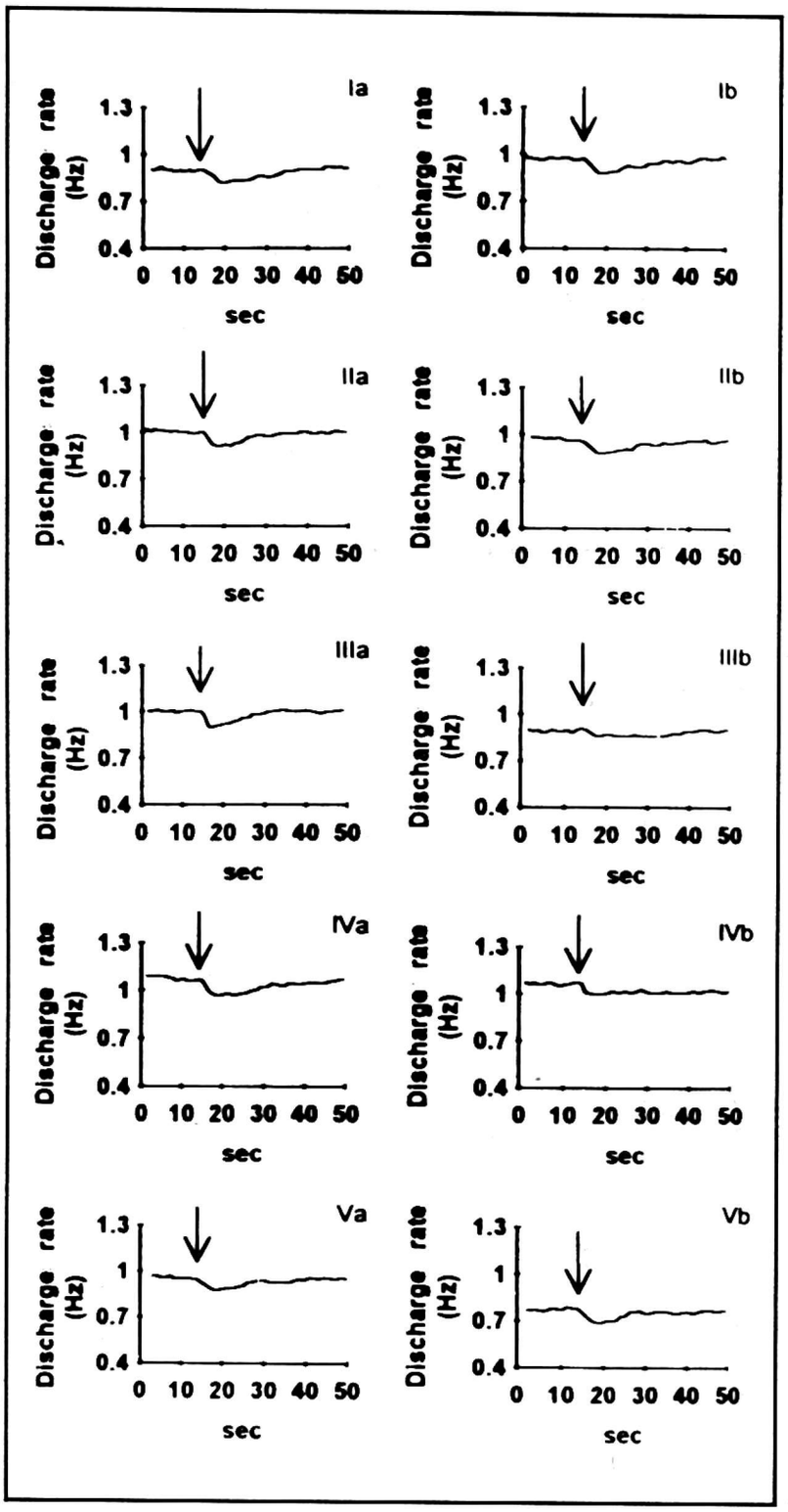

Fig. 6. Original records illustrating changes in spontaneous discharge rate of an isolated right auricle after administration of the second blood dialysate sample from the sella turcica (Ia—Va) and from the femoral vein (Ib—Vb). 1 mol/L NaCl 1 min after pretreatment with AP-5 was administered at the beginning of the 30—60 min dialysis. Arrows indicate the time of 20 μL sample administration into the chamber with spontaneously discharging isolated auricle of the right atrium.

DISCUSSION

In the previous paper results concerning the release of cardiodepressant factor and vasopressin into blood outflowing from the region of the sella turcica have been presented (1). This factor has a small molecular weight (ca 1,000 d) and exerts a negative chronotropic effect on the pacemaker tissue of the right auricle of the right heart atrium in two-day-old rats (10). Electric stimulation of afferent fibres in vagus nerves or angiotensin II infusion significantly increased cardiodepressant factor release from the posterior pituitary lobe into the blood (15).

In the present experiments the technique of *in vivo* minidialysis has been used to demonstrate that the neurohypophyseal hormones can be released into the blood dialysate outflowing from the region of the sella turcica and from the femoral vein under basal conditions and in response to hyperosmotic stimulation.

Vasopressin and oxytocin are well-characterised nonapeptides synthesised in magnocellular neurons of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN), which project to the neurohypophysis, where the nonapeptides are stored and released into blood (16). Vasopressin and oxytocin release from the posterior pituitary lobe into the blood is stimulated by increases in plasma osmolality (17). Osmotic stimulation causes depolarization of neurons in the supraoptic and paraventricular nuclei and increases AVP mRNA (18,19). It is now accepted that hyperosmotic NaCl microdialysed in the hypothalamic magnocellular nuclei is a potent stimulus for intranuclear and systemic AVP i OT release (17, 20—22) . The release in response to either direct or systemic NaCl stimulation appears to be dependent on synaptic input (17), since administration of tetrodoxin into the SON blocked the intranuclear response to intraperitoneal osmotic stimulation (23).

In our study after intra-arterial osmotic stimulation there was an increase in vasopressin levels blood dialysate from the region of the sella turcica and femoral vein. The plasma peptide response was accompanied by a simultaneous increase in cardiodepressant activity. In fact, osmotic stimulation causes an increase in vasopressin as well as cardiodepressant factor synthesis and secretion.

In our previous experiments it was indicated that vasopressin content in the blood collected from the vicinity of the sella turcica was higher than in the peripheral blood and it was higher than the value known from the literature data (16). In the present experiments average vasopressin content in 30 min dialysate sample outflowing from the region of the sella turcica was lower than in the blood withdrawn directly from the vicinity of the cavernous sinus of the sella turcica (24). It 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 membrane limits diffusion of neurohormones between the dialysing fluid and the blood.

Some authors have observed that intravenous infusion of vasopressin decreases the heart contraction rate (25, 26). Bradycardia induced by vasopressin in rats is caused by reflex decrease of the sympathetic tone to the heart or by the increase in vagal tone. It is supported by the fact that centrally administered vasopressin causes an increase in arterial blood pressure and tachycardia (27).

Other authors performing experiments on the isolated rat heart have concluded that vasopressin contracts coronary vessels which causes heart

muscle hypoxia and a decrease in heart contraction rate (28). Hof (29), however, has indicated that the reduction of coronary blood flow under the influence of vasopressin does not cause a decrease in the heart contraction rate.

The research carried out by Lefer and Inge (30) on isolated papillary muscle demonstrated that vasopressin at the concentrations from 10^{-5} to 10^{-8} mol/L does not exhibit cardiodepressant activity. Also Brizzee *et al.* (31) in their experiments performed on Langendorff isolated rat heart preparation indicated that vasopressin has no chronotropic effect. Similary, in our previous experiments vasopressin had no influence on the frequency of isolated auricle contraction rate (32). Our and other studies reveal that cardiodepressant activity of plasma is not caused by vasopressin.

Neuropeptides present in the posterior pituitary lobe, such as Substance P, leu-enkephalin, met-enkephalin, angiotensin II, atrial natriuretic factor, vasopressin, oxytocin and delta sleep-inducing peptide at the concentrations from 2.1×10^{-7} to 10^{-3} mol/L had no effect on contraction frequency of the isolated auricle of the right heart atrium of a two-day-old rat (32).

In the present study the cardiodepressant activity of plasma was not brought about by acetylcholine, because atropine sulphate (5×10^{-6} mol/L) was added to Ringer-Locke solution perfusing the auricle.

Hyperosmotic NaCl releases excitatory amino acids in various brain areas (33). Endogenous excitatory amino acids (EAA) such as glutamate are the dominant excitatory neurotransmitters in the central nervous system (34,35). Direct activation of cell bodies in the PVN by L-glutamate (36) or N-methyl-D-aspartic acid (37) caused an increased release of vasopressin and oxytocin from the posterior pituitary. Pharmacological studies reported that excitatory amino acids stimulated vasopressin release from the hypothalamic explants (38, 39). Administration of EAA antagonists inhibits almost complectly synaptic responses in the magnocellular and parvocellular PVN (40). Parker and Crowley (41) indicated that selective non-NMDA receptor antagonist CNQX attennuated oxytocin release. It was also shown that basal and osmotically stimulated vasopressin release was decreased by a blockade of synaptic input with MgSO4 and by kynurenic acid which is a non-specific antagonist of excitatory amino acid receptors. Both prevented the hypertonicity-induced increase in vasopressin mRNA content (19).

In the present study, DNQX non-specific EAA antagonist and AP-5, which is a selective antagonist for NMDA receptors significantly inhibited the increase in the blood dialysate AVP concentration and cardiodepressant activity elicited by intra-arterial injection of hypertonic (1mol/L) saline. These findings suggest that excitatory amino acids are endogenously involved in the mechanism of the response of plasma AVP and cardiodepressant factor to osmotic stimulation.

Various biologically active substances are also released into blood during circulatory shock combined with a decrease in arterial blood pressure (3). One of these substances is MDF. The data presented in this report indicate that cardiodepressant substance (s) released from the posterior pituitary lobe can be clearly distinguished from the MDF. The source of MDF is the ischemic pancreas, where a dramatic decrease in blood flow (42) takes place, whereas cardiodepressant factor originates from the posterior pituitary lobe. MDF is a peptide or glycopeptide of molecular weight 500 - 1000 d (4,5) and acts inotropically negatively on the isolated papillary muscle. Cardiodepressant factor is also probably a peptide (14) and passes through the dialysing membrane (20 kDa cutoff) of the minidialyser.

In the present study we indicated that stimulation of the hypothalamus with hyperosmotic NaCl solution caused simultaneously an increase in the cardiodepressant activity and vasopressin concentration in blood dialysate from the vicinity of the pituitary and from the femoral vein. Cardiodepressant activity and vasopressin concentration were significantly decreased by the specific antagonist of excitatory amino acids (AP-5) as well as the non-specific antagonist of excitatory amino acids (DNQX). The results of the present study indicate an involvement of the NMDA receptor in the hypothalamic regulation of AVP and CDF release. The results also demonstrate the utility of blood minidialysis for simultaneous monitoring of central and peripheral peptide release.

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