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ATRIAL NATRIURETIC PEPTIDE SECRETION FOLLOWING SUBARACHNOID HEMORRHAGE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Atrial natriuretic peptide (ANP) is released excessively in spontaneously hypertensive rats (SHR), and vasodepression is its main effect on the blood vessels. The aim of the study was to investigate the changes in ANP secretion in the cerebral vasospasm following subarachnoid hemorrhage (SAH) in SHRs. The SAH was induced by the injection of 100 µl of unheparinized, autologous blood into the cerebrospinal fluid (CSF), *via* a canule formerly inserted into the cisterna magna (CM). In the sham SAH group the SAH was imitated with 0.9% saline injection. The concentrations of ANP in the blood samples obtained in the acute and chronic stages of vasospasm were radioimmunoassayed with commercial RIA kits (Peninsula RIK 9103). It was found that both SAH and sham SAH induced a significant increase in plasma ANP in the chronic phase of vasospasm. No such changes were observed in the acute phase. This shows that the chronic cerebral vasopasm following SAH considerably enhances the ANP secretion in SHRs, probably through the increased endothelin release. These compensatory and regulatory mechanisms help prevent the development of brain oedema and the progression of vasopasm through secondary vasodilation.

Key words: *atrial natriuretic peptide (ANP), spontaneously hypertensive rats (SHR), subarachnoid hemorrhage (SAH), cerebral vasopasm.*

INTRODUCTION

Atrial natriuretic peptide (ANP) is synthesized, stored and secreted in the granules of the mammalian atrial cardiocytes as well as in the kidneys, lungs, aorta, lymphatic and salivary glands, central nervous system and sympathetic ganglia (1). This group of peptides includes the C-type natriuretic peptide (CNP) and brain natriuretic peptide (BNP), both found in the CNS, mainly in the neurons involved in the regulation of water-electrolyte balance and arterial pressure (2, 3).

Among multiple biological actions of ANP, the effects on cerebral circulation deserve particular attention. They are: strong natriuretic and vasodilating effects, inhibition of endothelin-1 secretion and restriction of the sympathetic activity. In hypoxia, ANP, like many other hormonal and metabolic substances such as CO_2, H^+, K^+, adenosine, histamine, prostaglandins and neuropeptides, plays a significant role in producing vasodilatation (4). This concerns only arterial vessels due to the direct influence of ANP on myocytes (5). The veins, on the contrary, are found to constrict in response to ANP action (6).

Patients with essential hypertension have plasma ANP concentrations twice higher than those in normotensive groups. Similarly high levels of ANP are found in spontaneously hypertensive rats (SHR) (10—12).

The subarachnoid hemorrhage (SAH) may be the cause of cerebral vasospasm leading to considerable cerebral ischemia. The acute phase of vasospasm is induced by a number of vasoconstrictive substances like: F2α-prostaglandin, serotonin, TGF, oxyhemoglobin, and bilirubin released into the cerebrospinal fluid cisterns surrounding the arterial trunks at the base of the brain. The chronic phase of vasospasm occurs several days after SAH and is caused by progressive proliferative changes in the blood vessel walls, resulting in a considerable narrowing of the lumen and, in consequence, deep brain ischemia (7—9).

The aim of the present study was to investigate the dynamics of changes in plasma ANP levels in SHRs both in the acute phase cerebral vasospasm following SAH and in its chronic phase when brain hypoxia develops.

MATERIALS AND METHODS

The study was performed on spontaneously hypertensive male rats (SHR) weighing between 200 and 250 g. The animals were housed in couples in cages under controlled standard microclimate conditions (temerature 20—22°C, humidity of 50—60%) and illumination (light on from 06:00 to 18:00) and had free access to standard food (obtained from „Murigan" Motycz Factory) and tap water. All experiments were performed between 14:00 and 16:00, on animals anesthetized with intraperitoneally injected Ketamine in the dose of 100 $mg \times kg^{-1}$.

The canulation of the cisterna magna (CM) was undertaken according to the original technique reported by Solomon and co-workers (1985) [13] with own modifications.

The surgery was performed using operative microscope and the stereotactic device. After the median incision of the scalp and muscles over the parieto-ocipital region and the neck, the parietal, and occipital bones, the atlas arc and atlantooccipital membrane were exposed. The trephine hole of 0.8 mm diameter was drilled medially over the parieto-occipital suture and the Venocath 18 canule (Abbott) was inserted. The correct position of the canule was maintained by observing: a) the localization of its end in the CM through the atlantooccipital membrane, b) the outflow of CSF through the canule. The canule was attached to the parietal bone using the cyanoacrylic glue.

The subarachnoid hemorrhage (SAH) was induced by the injection of 100 μl of unheparinized arterial blood into the CM *via* the formerly inserted canule. The blood was obtained from the axillar artery prepared under the microscope control with the Neoflon 0.6 mm canule. The

imitation of SAH in the sham SAH group was performed with 100 µl of 0.9% saline injected into CM.

The protocol: 7 days prior to the artificial SAH or its imitation with saline, the canulation of CM was performed. This period helped to avoid the influence of the surgery and the manipulation in CM.

Directly after the canulation of CM, 3 ml samples of blood were collected from the orbital plexus to measure the initial control concentration of ANP (control group-CON).

The animals were divided into two groups: 1) experiment — SAH group, and 2) sham SAH group (0.9% saline). In both of them the blood samples were collected from the orbital plexus or axillary artery 20 minutes and two days following SAH or its imitation. The blood was collected into tubes containing EDTA (1 $mg \times ml^{-1}$) and Aprotinin (500 $KIU \times ml^{-1}$), cooled to 0°C, centrifuged (1600 rpm, 15 min) and stored at −25°C until assayed within two weeks.

The radioimmunoassay of ANP concentration was performed using commercial RIA Peninsula kits (RIK 9103 — Atrial Natriuretic Factor, ANF, Rat). The peptides were isolated from the serum on the SEP-COLUMNS (RIK-SEPCOL 1) using A and B buffers.

The statistical analysis of differences was performed using Student's t-test for unpaired variables. A p value of < 0.05 was considered to be significant.

RESULTS

The initial, mean plasma concentration of ANP (control group-CON) in SHRs, assayed prior to the experiment, was 114.2 $pg \times ml^{-1} \pm 43.1$

Twenty minutes following SAH or saline injection the ANP concentrations showed no significant changes and amounted to 134.6 $pg \times ml^{-1} \pm 164.9$ for experimental-SAH group and 109.0 $pg \times ml^{-1} \pm 68.8$ for sham SAH group (*Fig. 1 and 2*).

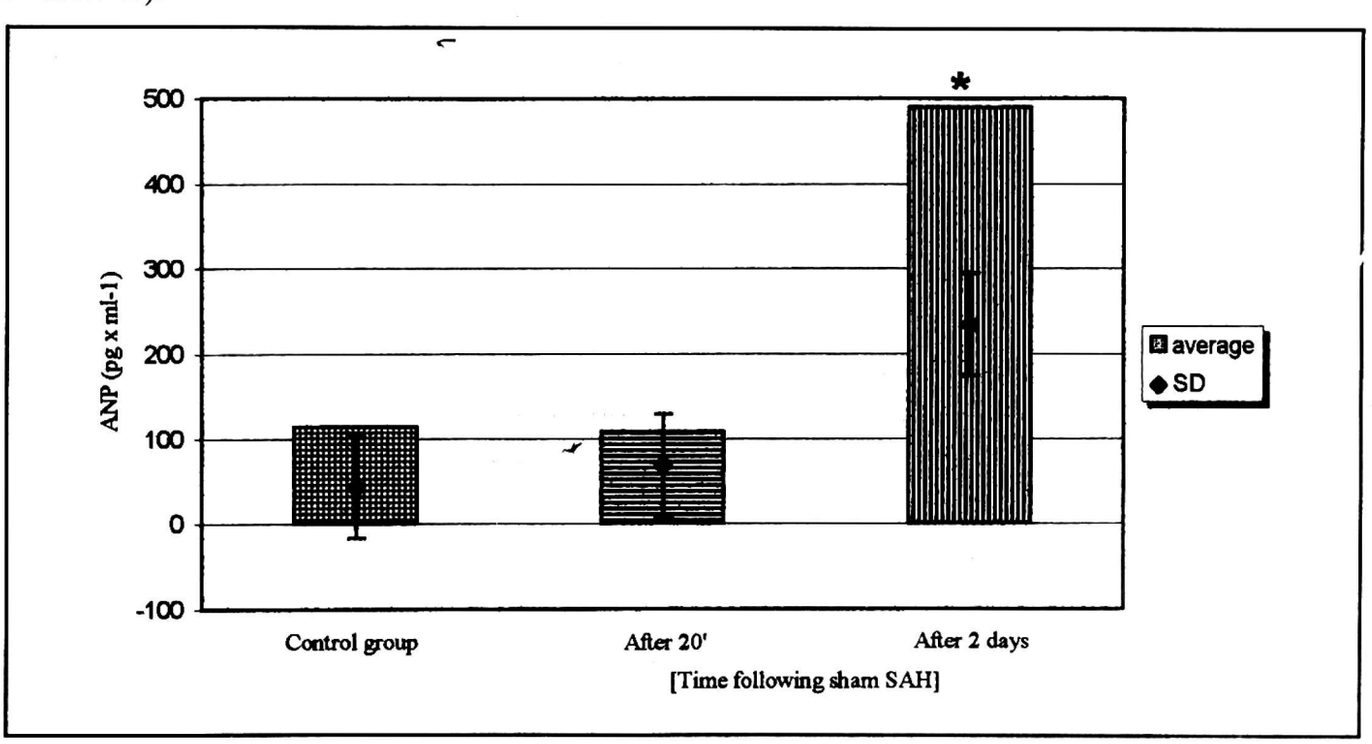

Fig. 1. ANP levels in plasma of the SHRs after SAH. The SAH was induced by the injection of 100 µl unheparinized arterial blood into the CM *via* the formerly inserted canule. Data. are presented as mean ± SD. Statistical analysis was performed using Students t-test. A $p < 0.05$ was considered as significant. Significant differences were only in the rats of 2 days after SAH * in comparision to the control roup (CON) ($p < 0.01$).

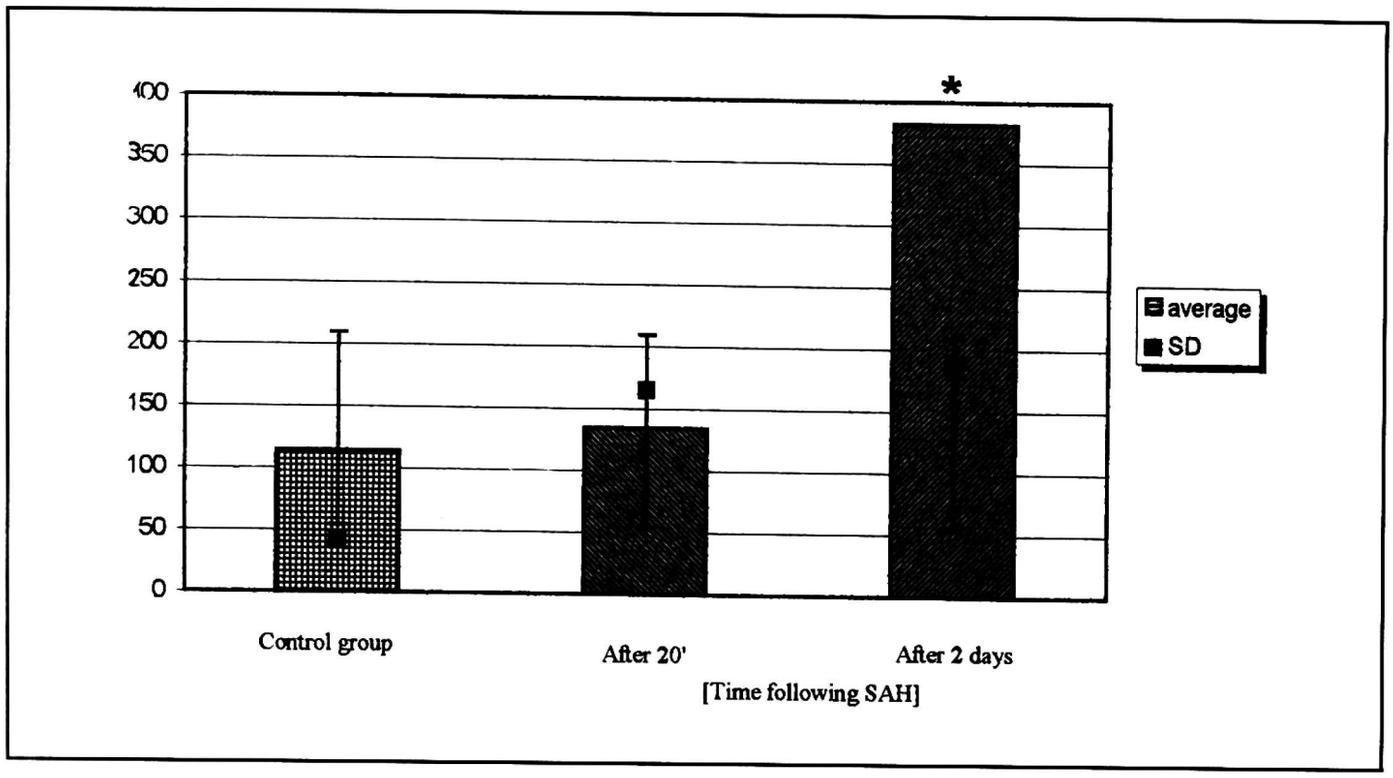

Fig. 2. ANP levels in plasma of the SHRs after sham SAH with 100 µl 0.9% NaCl was injected into the CM *via* the formerly inserted canule. Data are presented as mean ± SD. Statistical analysis was performed using Student t-test. A $p < 0.05$ was considered as significant. Significant differences werer only in the rats of 2 days after sham SAH * in comparison to the control group (CON) ($p < 0.001$).

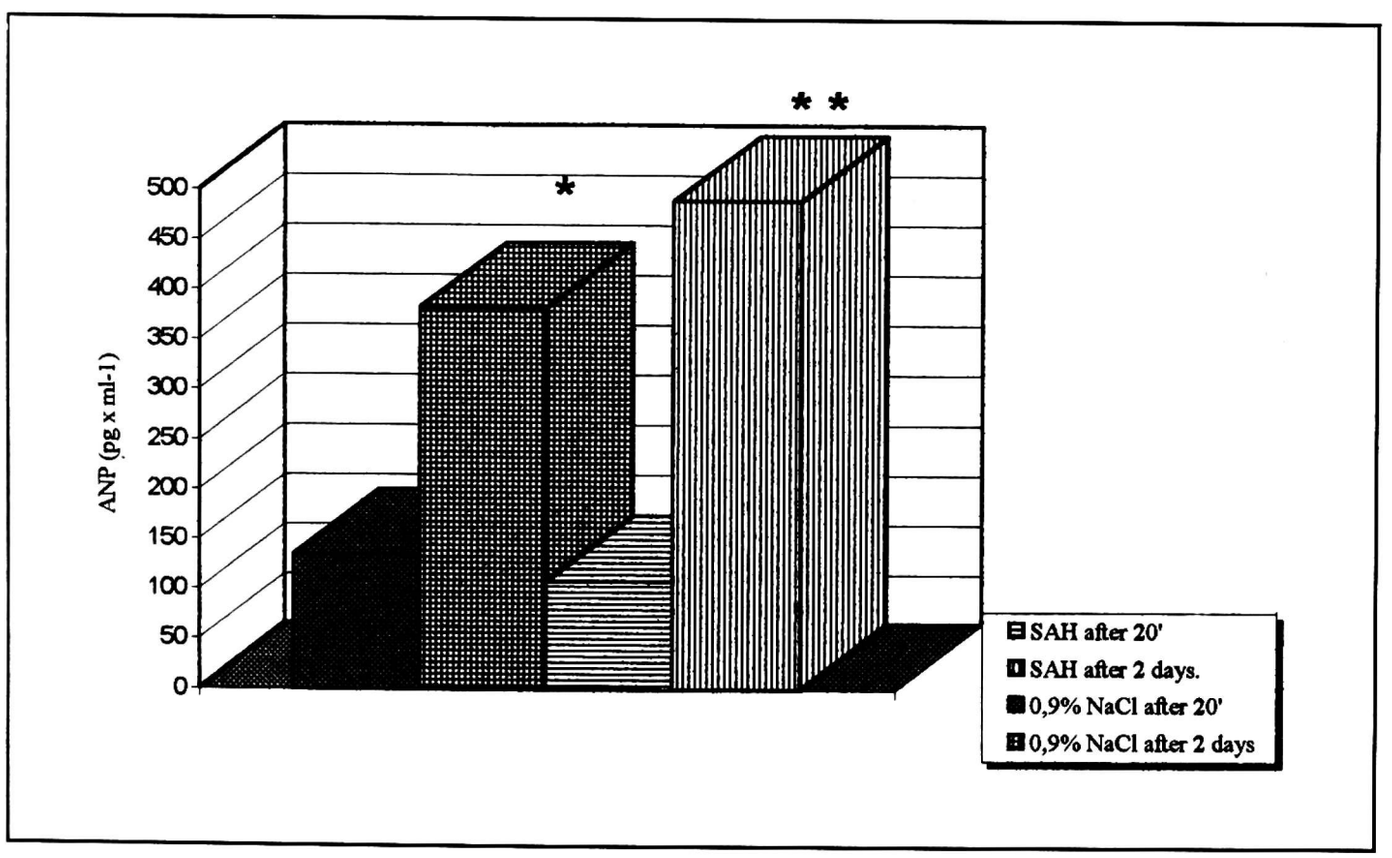

Fig. 3. Plasma ANP levels in SHRs 20' and 2 days after SAH and sham SAH. The SAH was induced by the injection of 100 µl unheparinized arterial blood into the CM, and sham SAH with 100 µl 0.9% NaCl, *via* the formerly inserted canule. The differences in the SAH group after 2 days * in comparison to 20' were significant ($p < 0.05$). Similarly, differences in the sham SAH group ** were significant ($p < 0.001$).

Marked increase of ANP was observed in the samples collected on the second day following SAH or saline injection. The respective values for SAH and sham SAH animals were 382.3 $pg \times ml^{-1} \pm 187.3$ and 489.5 $pg \times ml^{-1} \pm$ ± 234.5.

The comparison of ANP levels measured 20 minutes and 2 days after SAH or saline injection showed over a threefold increase in the latter periods and the differences were significant (in SAH $p < 0.05$ and sham SAH $p < 0.001$) (*Fig. 3*).

The comparison of mean plasma ANP levels 2 days after SAH or its imitation with saline to the initial control values prior to the experiment (CON group) also showed statistically significant differences in both groups ($p < 0.01$ and $p < 0.001$) (*Fig. 4*).

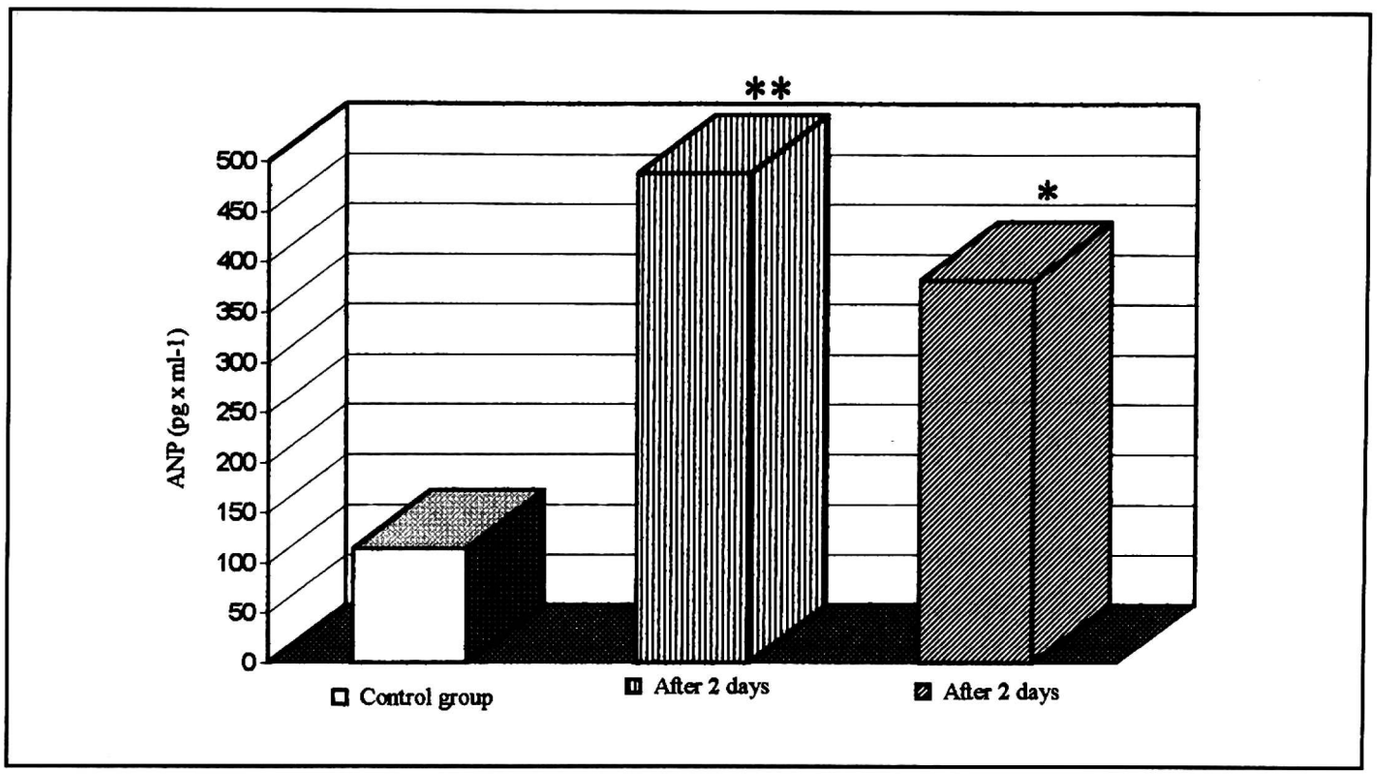

Fig. 4. The comparison of mean plasma ANP levels 2 days after SAH * and its imitation with saline (sham SAH) ** to initial control values prior to the experiment (control group CON) showed statistical significant differences in both groups (respectively $p < 0.01$ and $p < 0.001$).

DISCUSSION

The study has shown a considerable increase in plasma ANP concentration in the chronic phase of cerebral vasospasm following SAH in SHRs. A similar increase was observed in rats injected 0.9% saline solution into CM as an imitation of SAH. No significant changes in ANP concentration were found in the acute phase of vasospasm either in the SAH or sham SAH group.

Among a many complex causes of essential hypertension, the role of ET-1 — a strongly vasoconstrictive peptide-deserves attention (14—16). Several of pathological factors injuring vascular endothelium cause a considerable increase of ET-1 concentration (17—19). The general and local influence of

ET-1 on the smooth muscle cells *via* ETA receptors may play a role in the development of chronic vasospasm following SAH (20). Several attempts at reducing the ischemic effect of vasopasm by selective blocking of ETA receptors were reported (21).

Particular attention should be paid to the studies of Mitaka and co-workers (22), who compared the levels of ANP and ET-1 in the blood serum and found a highly significant correlation between these adversely angioactive peptides. It was found that ET-1 is a potent factor stimulating, *in vivo* and *in vitro*, the secretion of ANP following both extrinsic ET-1 injection and elevation of endogenic ET-1 caused by the intrinsic pathogenic factors. It augments the stimulation of the adrenergic system and increases the prostanoid secretion, which may influence the vasospasm developing after SAH.

It is possible that the considerable increase of the ANP concentration observed in our study in the chronic phase of vasosopasm is the response to the factors leading to intensification of the spasm, including the ET-1. It was found that the highest amounts of ET-1 were secreted in the chronic phase of vasospasm (23, 24). The ANP produced and concentrated in excess is probably one of the important control mechanisms of cerebral hemodynamics.

SAH causes an increase in brain water and sodium contents. It was found that the ANP administration into the cerebral lateral ventricle resulted in their return to normal levels within 90 minutes following SAH, so ANP seems to attenuate brain oedema (25). This is yet another indication of the presence of defense mechanism manifested by the intensified secretion of ANP in the chronic vasospasm following SAH (26, 27).

The cause of the increase of ANP concentration in the sham SAH group imitated by saline injection is unclear, but it is very characteristic that the increased secretion of ANP following either SAH or sham SAH requires similar time to develop.

ANP secreted excessively as a secondary reaction in essential hypertension modifies the distribution and metabolism of intracellularly accumulated catecholamines (28), influences the increased norepinephrine uptake in adrenal medulla and in the central nervous system (29), and inhibits the norepinephrine synthesis and its spontaneous release (30). These findings indicate that the increased levels of ANP may regulate the secretion of catecholamines, especially when it is necessary to change the peripheral vascular resistance and the blood volume, like in hypertension or vasopasm following SAH.

It should also be emphasized that the strong natriuretic action of ANP may possibly play a role in the treatment of brain oedema in the chronic phase of cerebral vasospasm (25).

The significant increase of ANP production and secretion in the chronic stage of vasospasm following SAH may be the result of compensatory and defense reaction which, through the natriuretic effect and the action

antagonistic to ET-1, angiotensin, aldosterone and vasopressin as well as through the modulation of catecholamine metabolism, reduces the effects of cerebral ischemia and brain oedema.

CONCLUSION

The increased ANP production and secretion may be the result of the compensatory and defense mechanisms activated in response to the developing hypoxia and brain oedema in the SHRs in the cerebral vasopasm following SAH.

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