主　論　文

DIFFERING EFFECTS OF VASOPRESSIN ON REGIONAL CEREBRAL BLOOD FLOW OF DOGS FOLLOWING INTRACISTERNAL VS. INTRAARTERIAL ADMINISTRATION

バゾプレッシンの脳槽内投与と椎骨動脈内投与に対するイヌ局所脳血流の変化

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Abstract: We investigated the differential effect of the intracisternal and intraarterial administration of vasopressin on the regional cerebral blood flow (rCBF) in the parietal cortex of dogs. Regional CBF, velocity and blood volume were assayed by laser flowmetry. The intracisternal injection of 1 nmol vasopressin significantly increased the rCBF and velocity, without affecting blood volume. However, the intravertebral arterial injection of 1 nmol vasopressin significantly decreased the rCBF and velocity. This discrepancy can be explained by a difference in the affected vasculature; large blood vessels in the subarachnoid space vs. whole cerebral vascular system. The intracisternal and intraarterial injection of the nitric oxide inhibitor N^G-monomethyl-L-arginine reduced the rCBF from the base line, and significantly suppressed the rCBF elevation induced by vasopressin. The effect of vasopressin may be considered as the summation of the increased flow from the dilated large vessels via the release of nitric oxide from the endothelium, and of the decreased flow from the contracted small vessels.

Introduction

Vasopressin may contribute to the regulation of the regional cerebral blood flow (rCBF) not only via circulating hormone from the neurohypophysis, but also via an active mediator of local origin, since several studies have demonstrated the possible existence of vasopressin in the endothelium or nerve terminals of the blood vessels (1-4). The demonstration of a vasopressinergic pathway in rat brain capillaries (5), the receptors in rat and pig microvessels (6-8) and of vasoactive function in rat intracerebral arterioles (9) supports the importance of vasopressin in regulating the microcirculation.

We previously reported that the intracisternal and intraarterial administration of vasopressin dilated the major cerebral arteries of the dog including the basilar artery, the circle of Willis and its main branches (10-12), while it potently contracted the intracerebral arterioles (9). Several authors have shown that the response to vasopressin differs in arteries from different brain region (13-16). Vasopressin induces vasoconstriction by directly stimulating the receptor on the vascular smooth muscle coupled with an increase in calcium mobilization and in inositol phosphate turnover (8,17). The vasodilation induced by vasopressin seems to be mediated via an endothelium-dependent mechanism, probably the L-arginine nitric oxide synthase pathway (13,18). The synthesis of nitric oxide from L-arginine is competitively inhibited by such agents as

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analogues of L-arginine inducing N^G-monomethyl-L-arginine (L-NMMA) (19,20), nitro-L-arginine methyl ester (20) and L-N^G-nitroarginine (21,22).

Our objective was to investigate the effect of vasopressin on the rCBF in the parietal cortex of dogs using different routes of administration: intracisternal injection, which allows vasopressin to mainly reach the large blood vessels in the subarachnoid space from the extraluminal side, and intraarterial injection, which allows it to spread throughout the cerebral circulation from the intraluminal side. The effect of L-NMMA on rCBF following the extraluminal and intraluminal application of vasopressin was further studied.

Methods

Preparation of animals: All protocols and surgical procedures used in this study followed the Guideline of the Institute for Laboratory Animal Research, Nagoya University School of Medicine. Adult mongrel dogs of either sex (7-18 kg) were anesthetized with sodium pentobarbital (25 mg/kg, i.v.) and intubated. During the experiment, they were maintained with 2.2 % enflurane in 30% oxygen/70% nitrous oxide, paralyzed with pancuronium (0.2 mg/kg/hour, i.v.), and artificially ventilated to maintain arterial blood gases within physiological limits. A femoral artery was cannulated to allow the repeated measurement of arterial blood pressure and for arterial blood sampling for analysis of blood gases. We administered a slow continuous saline infusion to prevent dehydration and avoid an increase in endogenous plasma vasopressin. The right vertebral artery and the cisterna magna were cannulated to allow the administration of test solutions. The body temperature was controlled with a heating/cooling pad.

Measurement of regional CBF, velocity and blood volume: Regional CBF, velocity and blood volume were assayed with a laser flowmetry device (ALF 21R, Advance Co., Ltd., Tokyo, Japan) that was placed on the surface of the left parietal cortex. It includes calibration factors that provide digital flow direct readings in ml/min/100g. Velocity and blood volume values can be taken as abstract numbers using this device.

Parietal trepanation was performed and a hole that measured 15-mm in diameter was drilled, leaving the dura mater intact. The probe (Type N) containing an emitter and a detector was gently placed (using the balancer ALF-B) over the dura of the parietal cortex. Care was taken to avoid placing the probe above the pial arteries and veins. The measured values of rCBF, velocity and blood volume over the dura were confirmed to be almost same as those on the cortex, and were stable, without the influence of cerebrospinal fluid leaked from the subarachnoid space.

Protocols: A volume of 0.5 ml physiological saline was used as a control solution, and the injected substances, vasopressin, L-NMMA or L-arginine, were dissolved in the same amount of physiological saline just before use. After measuring the base line value of rCBF, velocity and blood volume, physiological saline or the test solution was injected intracisternally or intraarterially. Synthetic arginine-vasopressin was obtained from Peptide Institute (Osaka, Japan). L-NMMA was purchased from Calbiochem (La Jolla, CA, USA). L-arginine and all other chemicals were of reagent grade.

Intracisternal administration: Each solution was injected into the cisterna magna after gently aspirating the same amount of cerebrospinal fluid so as to maintain the intracranial pressure as constant as possible. In studying the effect of 10 μmol of L-NMMA on the change in rCBF, we administered a dose of L-NMMA (10 μmol) sufficient to inhibit the synthesis of nitric oxide from L-arginine 20 minutes before injecting the vasopressin. The pH of all test solutions except for L–arginine, an alkaline amino acid, was similar to that of the control solution. Previously confirmed that the vasodilatory effect of L-arginine solution neutralized with HCl does not differ from the original effect (12).

Intraarterial administration: Each solution was infused into the right vertebral artery. Since the effect of such administered agents was relatively brief, we intraarterially injected 10 μmol of L-NMMA with vasopressin at the same time.

Statistical analysis: Results were expressed as the mean ± S.E. Changes in response were calculated as percentage of baseline value. Drug effect was calculated by one-way analysis of variance and Fisher's Protected Least Significant Difference (PLSD) multiple range test. Statistical significance was accepted at a level of $p < 0.05$.

Fig. 1

Percentage change in response of rCBF to vasopressin administered into the cisterna magna (**A**) and into the vertebral artery (**B**), and the response to L-arginine administered into the cisterna magna (**C**). Values are means ± S.E. Number of animals appears in parentheses. *: $p<0.05$ (vs. saline)

Results

The intracisternal injection of saline solution used as a control produced no significant change in rCBF, velocity or blood volume from base line (17.4±0.9 ml/min/100g, 0.66±0.05, 750±27, respectively). Intracisternally injected vasopressin significantly increased rCBF and velocity without affecting blood volume in a dose dependent manner. A dose of 1 nmol of vasopressin induced a maximal rise in rCBF and velocity at 129.5±7.9 % and 123.3±9.3 % of the base line (Fig. 1A, 2A). This significant increase in CBF and velocity persisted for 30 min.

Though the intravertebral arterial injection of 0.1 nmol of vasopressin did not show any significant effect, the administration of 1 nmol vasopressin significantly decreased rCBF and velocity (Fig. 1B, 2B).

The intracisternal injection of 10 μmol of L-NMMA given alone reduced the rCBF from the base line (Fig. 3A). Pretreatment with 10 μmol of L-NMMA significantly suppressed the elevation in rCBF induced by the intracisternal injection of 1 nmol of vasopressin.

The intravertebral arterial injection of 10 μmol of L-NMMA significantly reduced the rCBF to 92.2 ±0.9 % of the base line (Fig. 3B). The simultaneous administration of L-NMMA and 1 nmol of vasopressin also significantly reduced the rCBF to 87.2±1.5 % of the base line.

The intracisternal injection of 1 and 100 μmol of L-arginine dose-dependently increased the rCBF and velocity, but not the blood volume (Fig. 1C). The elevation in rCBF produced by 100 μmol of L-arginine persisted for 60 min with a maximal 123.3 ±6.3 % of base line.

The intracisternal and intraarterial injections of the test solutions showed no significant change in physiological parameters such as heart rate, blood pressure, arterial blood gas analysis data and body temperture.

Fig. 2

Percentage change in flow, velocity and blood volume following the intracisternal administration of 1 nmole of vasopressin into the cisterna magna (**A**) and into the vertebral artery (**B**). Values are means ± S.E. *: $p<0.05$ (vs. saline)

Discussion

This study showed that vasopressin increased the rCBF of the canine parietal cortex when applied from the intracisternal space, but did not increase the rCBF when it was injected into the vertebral artery. This discrepancy can be explained by the differing effects of vasopressin on the cerebral vascular system. In previous studies, we demonstrated using angiography that the intracisternal administration of vasopressin dilated the major cerebral arteries such as the vertebral artery, the basilar artery and the circle of Willis and its main branches, all of which are present in the

Fig. 3

Percentage change in rCBF response to 10 μmol of L-NMMA and simultaneously administered 10 μmol of L-NMMA and vasopressin into the cisterna magna (**A**) and vertebral artery (**B**). Values are means ± S.E. Number of animals appears in parentheses. *: $p<0.05$ (vs. vasopressin 1 nmol)

subarachnoid space (10-12). A vasodilator response to vasopressin that was mediated via the release of nitric oxide from endothelium in the arteries present in the subarachnoid space probably induced the significant increase in rCBF in the parietal cortex. Although intracisternal administration of vasopressin may influence small perforating arteries in the subarachnoid space which are regarded to be constricted by application of high concentration of vasopressin, our observation that velocity rather than blood volume significantly increased the rCBF supports the idea that large vessels are dominantly affecting rCBF. The increase in rCBF produced by vasopressin resembles that produced by L-arginine.

The intravertebral arterial injection of vasopressin as well as the intracisternal injection dilated the major cerebral arteries on angiography (10). However, it failed to increase the rCBF in the parietal cortex. The vertebral artery appears to be the major artery that supplies blood to the brain of dogs, based on angiographical studies which show that the cerebral arteries including the anterior circulation could be opacified via the vertebral artery in this species (11). Our recent study using the rat intracerebral arterioles (diameter 50 μm) in vitro demonstrated a different response to increasing concentrations of vasopressin (9): we observed a small relaxation at lower concentrations (10^{-12} - 10^{-11} M) of vasopressin and a major constriction at higher concentrations (10^{-10} - 10^{-8} M). It is therefore suggested that the response of rCBF to the intraarterial injection of vasopressin depends on its concentration and/or regional differences in responsiveness to this agent. The regulation of rCBF by 1 nmol of vasopressin can be considered as the sum of the increased flow from the dilated large vessels which is produced by the release of nitric oxide from the endothelium, and of the decreased flow from the contracted small vessels produced by a direct effect on smooth muscle.

We found that the intracisternal injection of L-NMMA induced a long-lasting vasoconstriction, and produced a slight decrease in rCBF. Conversely, L-arginine significantly increased rCBF. Pretreatment with L-NMMA significantly suppressed the elevation in rCBF induced by vasopressin. These results suggest that the nitric oxide released from endothelium has a role in maintaining the basal CBF; the vasodilator effect of vasopressin is a consequence of the nitric oxide release. The suppressive effect of L-NMMA on the action of vasopressin is reversed

by high concentrations of L-arginine, a substrate for the formation of nitric oxide (12). L-NMMA probably suppresses the synthesis of nitric oxide in the endothelium and nerve terminals when applied intracisternally, since immunohistochemical studies have demonstrated that dense staining for nitric oxide synthase is observed in the nerve terminals that innervate the blood vessels of humans and rats (23,24). L-NMMA blocks the transient vasodilator responses following the transmural electrical stimulation of nonadrenergic noncholinergic nerves (20,22). However, vasopressin can not evoke the release of nitric oxide from nerve terminals, since the vasodilation induced by vasopressin is completely abolished following removal of the endothelium (20).

Injection of L-NMMA into the vertebral artery suppressed the basal rCBF. The simultaneous administration of L-NMMA and vasopressin further reduced the rCBF, probably because the vasoconstrictive action of vasopressin lingered after the inhibition of the nitric oxide synthesis by L-NMMA.

In conclusion, vasopressin exhibited a differing effect on rCBF of the parietal cortex of dogs following its intracisternal vs. intraarterial administration. This finding reflects the regional differences in the vasoactive response to vasopressin in the cerebral blood vessels. The increase in rCBF may be derived mainly from the vasodilation produced by stimulating the L-arginine nitric oxide pathway in the endothelium, while the decrease in rCBF may occur mainly via the vasoconstriction produced by stimulating the smooth muscle directly.

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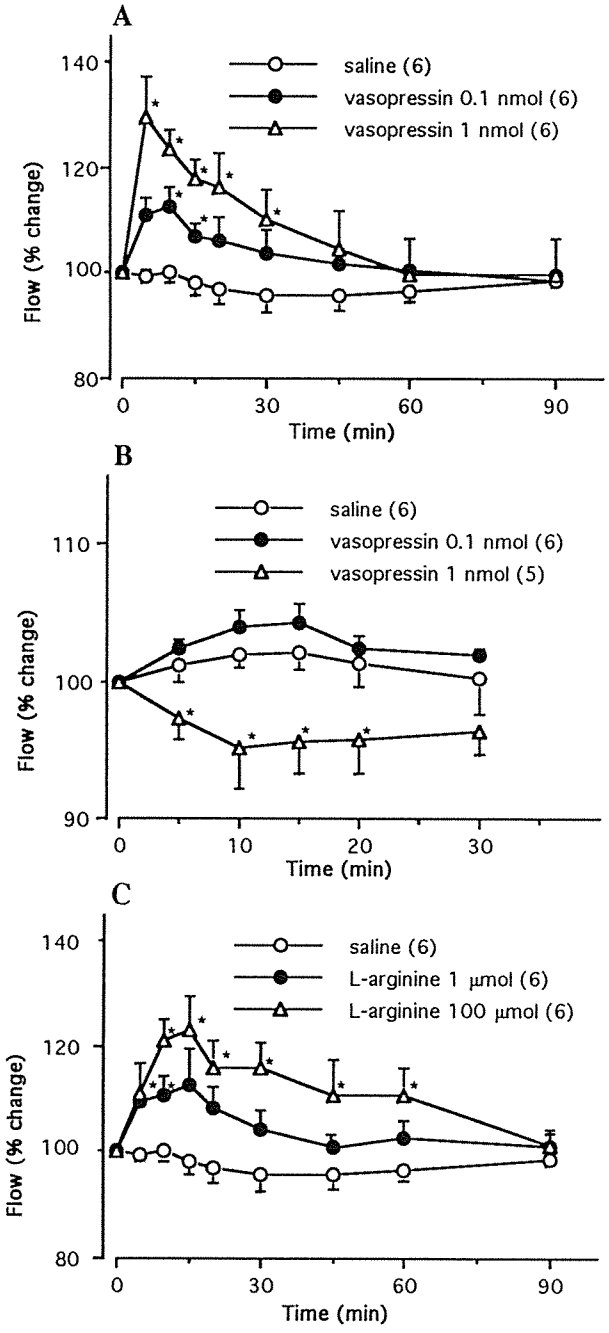
A
saline (6)
vasopressin 0.1 nmol (6)
vasopressin 1 nmol (6)
Flow (% change)
Time (min)
B
saline (6)
vasopressin 0.1 nmol (6)
vasopressin 1 nmol (5)
Flow (% change)
Time (min)
C
saline (6)
L-arginine 1 μmol (6)
L-arginine 100 μmol (6)
Flow (% change)
Time (min)

Fig.1

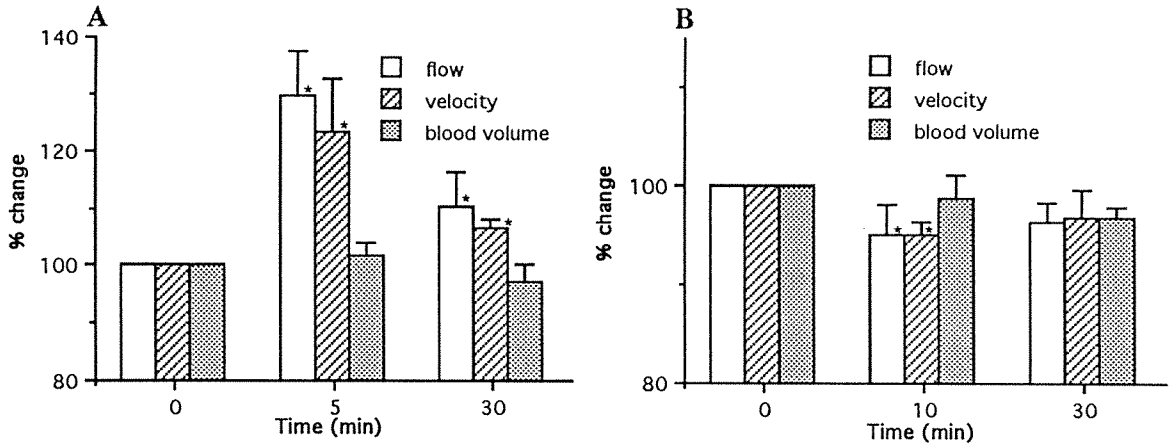
A
140
120
100
80
% change
flow
velocity
blood volume
0
5
30
Time (min)
B
100
80
% change
flow
velocity
blood volume
0
10
30
Time (min)

Fig.2

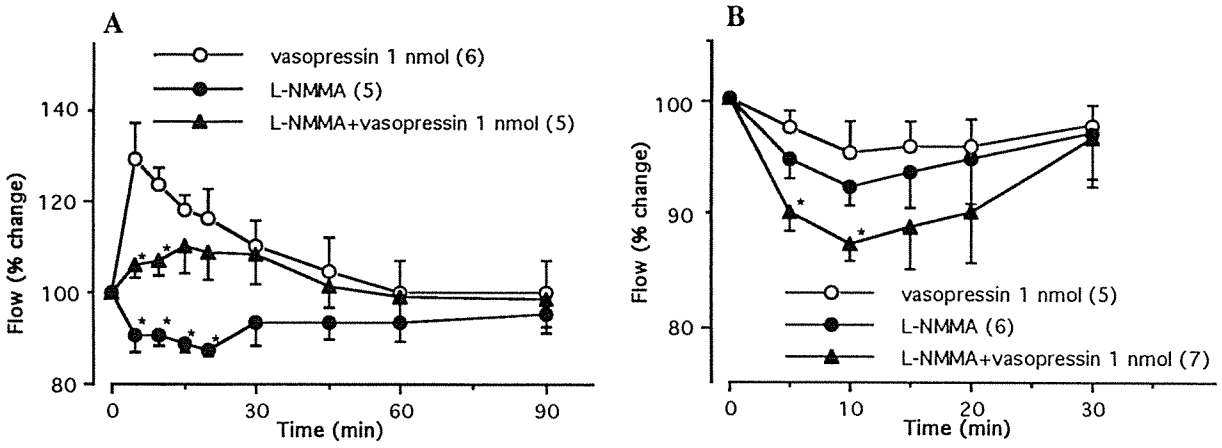
A
vasopressin 1 nmol (6)
L-NMMA (5)
L-NMMA+vasopressin 1 nmol (5)
Flow (% change)
140
120
100
80
0
30
60
90
Time (min)
B
100
90
80
Flow (% change)
vasopressin 1 nmol (5)
L-NMMA (6)
L-NMMA+vasopressin 1 nmol (7)
0
10
20
30
Time (min)

Fig. 3