学位論文　博士(医学)甲

Effects of adrenaline and vasopressin on cerebral microcirculation in the normal state and during global brain ischemia/reperfusion injury in rabbits
(ウサギにおける広範脳虚血と再灌流において、アドレナリンとバソプレシンが脳の微小循環に及ぼす影響)

近藤　大資

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Effects of adrenaline and vasopressin on cerebral microcirculation in the normal state and during global brain ischemia/reperfusion injury in rabbits

Daisuke Kondo[1], Nobumasa Asano[1], Tadahiko Ishiyama[2], Noriyuki Shintani[2], Takashi Matsukawa [1]

1. Department of Anesthesiology, Faculty of Medicine, University of Yamanashi, Chuo, Yamanashi, Japan
2. Surgical Center, University of Yamanashi Hospital, University of Yamanashi, Chuo, Yamanashi, Japan

Corresponding author: Daisuke Kondo

Mailing address: Department of Anesthesiology, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan.

Tel: +81-55-273-1111

FAX: +81-55-273-6755

E-mail: drnosada@gmail.com

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Abstract

Purpose: We aimed to investigate the direct effects of adrenaline and vasopressin on cerebral microvasculature in both normal and ischemia/reperfusion states.

Methods: The closed cranial window method was used to visualize cerebral microcirculation and changes in the pial arteriole diameter. First, various adrenaline and vasopressin concentrations were administered to evaluate the response. Subsequently, the effects of adrenaline and vasopressin on the brain during ischemic/reperfusion injury were investigated. Global brain ischemia/reperfusion was induced by clamping the brachiocephalic, left common carotid, and left subclavian arteries for 15 min. Adrenaline, vasopressin, or artificial cerebrospinal fluid was infused 5 min after initiation of ischemia through 120 min after reperfusion. Pial arteriole diameter and hemodynamic and physiological parameters were recorded before ischemia, during ischemia, and after reperfusion.

Results: In the first experiment, adrenaline did not act directly on the cerebral pial arterioles in the normal state. At 10^{-11} and 10^{-7}mol/L, vasopressin tended to produce pial arteriolar dilation and constriction, respectively; however, these changes were not significant. In the second experiment, the pial arterioles were constricted in the control and vasopressin groups. Topical administration of adrenaline counteracted the vasoconstriction during ischemia/reperfusion injury, with no significant differences in hemodynamic and physiological parameters among the control, adrenaline, and vasopressin groups.

Conclusions: Adrenaline did not act directly on the cerebral pial arterioles, while vasopressin may have dilator and constrictor effects on cerebral pial arterioles in the normal state. During ischemia-reperfusion, adrenaline increased cerebral pial arteriolar diameters, and may thus

counteract cerebral vasoconstriction during the global brain ischemia-reperfusion period.

Key words: pial arterioles, adrenaline, vasopressin, ischemia/reperfusion injury

Introduction

Adrenaline and vasopressin have been recommended for use during cardiopulmonary resuscitation. Adrenaline shows a strong vasoconstrictor action through α-adrenergic stimulation and increases myocardial contractility through β-adrenergic stimulation. In cerebral circulation, adrenaline constricts pial arterioles via α-adrenergic stimulation [1,2], dilating them via a β-adrenergic mechanism [3]. Thus, the direct effects of adrenaline on pial arterioles are controversial [4,5].

Vasopressin is a potent vasoconstrictor that constricts systemic arteries by stimulation of V1 vasopressin receptors [6]; however, the effects of vasopressin on cerebral vessels are controversial. One study reported that vasopressin induced a dual action (low concentration: dilation: high concentration: constriction) in pial arterioles [7]. Another study reported that vasopressin elicited pial arteriolar dilation [8].

During cardiopulmonary resuscitation, the brain falls into ischemia; after successful resuscitation, the brain is reperfused. One study reported that adrenaline was superior to vasopressin during resuscitation [9];however, although adrenaline is essential for cardiopulmonary resuscitation, it has been shown to reduce cerebral perfusion during the procedure[10]. We previously reported that pial arterioles were constricted during the reperfusion period [11]. Adrenaline may exacerbate cerebral hypoperfusion following cerebral ischemia. In cardiopulmonary resuscitation, the degree of brain damage after resuscitation determines a patient's quality of life; therefore, adrenaline is not always preferred from the viewpoint of brain resuscitation. On the other hand, vasopressin improved vital organ blood

flow during resuscitation [12]; furthermore, it increased microvascular cerebral blood flow in comparison with adrenaline after cardiopulmonary resuscitation [13]. Thus, vasopressin may be superior to adrenaline in terms of brain resuscitation.

In this study, we first investigated the direct effects of adrenaline and vasopressin on cerebral arteriolar tone in the normal state using the cranial window technique in rabbits, hypothesizing that both adrenaline and vasopressin produced pial arteriolar constriction. We evaluated the direct effects of adrenaline and vasopressin on cerebral pial arterial diameter changes during the ischemia-reperfusion period, and hypothesized that both adrenaline and vasopressin induced equivalent pial arteriolar vasoconstriction during the reperfusion period. The primary outcome was pial arteriolar diameter changes; the secondary outcomes were changes in hemodynamic and blood gas analyses parameters.

Materials and Methods

The experimental protocol was approved by the Committee on Animal Research at the University of Yamanashi, Japan. Experiments were performed on Japanese White rabbits weighing between 2.8 and 3.6 kg. After gaining intravenous access via an ear vein, the animals were anesthetized with pentobarbital sodium (20 mg/kg), and maintained with a continuous infusion (5 mg/kg/h). The animals were tracheostomized and mechanically ventilated with 60 % oxygen. End-tidal CO_2 ($EtCO_2$) was monitored; tidal volume and respiratory rate were adjusted to maintain arterial carbon dioxide partial pressure ($PaCO_2$) between 35 and 45 mmHg. A catheter was inserted into the femoral artery to measure mean arterial blood pressure (MAP) and for blood sampling. Rectal temperature was monitored constantly and maintained at 39 ℃ ± 1 ℃ with a heating blanket.

Cranial window installation

After undergoing a thoracotomy, the rabbits were placed in the sphinx posture and a closed cranial window was implanted over the parietal cortex. The diameter of the cranial window was 8 mm, and was made in the parietal bone. The dura and arachnoid membranes were cut, and a ring with thin glass was then positioned over the hole, secured with bone wax and dental acrylic. The space under the window was filled with artificial cerebrospinal fluid (aCSF), and three polyethylene catheters were inserted; one catheter was attached to a reservoir bottle containing aCSF, which was continuously bubbled with 5% CO_2 in air. The aCSF was suffused at 0.1 mL/min. Two other catheters served as an inlet and an outlet for the aCSF and study drug solutions; the level of the outlet was maintained at approximately 5–6 cm above the

window to maintain normal intracranial pressure. The fluid volume in the window was between 0.5 and 0.7 mL. The composition of the aCSF was as follows: Na^+ :151 mEq/L; K^+ :3.5 mEq/L; Ca^{2+} :2.5 mEq/L; Mg^{2+} :1.3 mEq/L; HCO_3^- :25 mEq/L; urea: 40 mg/dL; and glucose: 65 mg/dL. We measured the diameters of pial arterioles diameters using a digital video analyzer (VH-E500, Keyence, Osaka, Japan):the measured diameters of the pial arterioles were between 15 and 90 μm.

Experiment 1 (Normal state)

After the baseline measurements of hemodynamic parameters and pial arteriole diameters, the cranial window was superfused with four increasing concentrations of adrenaline (10^{-9}, 10^{-7}, 10^{-5}, and 10^{-4} mol/L dissolved in the aCSF; n = 5) or vasopressin (10^{-11}, 10^{-9}, 10^{-7}, and 10^{-6} mol/L dissolved in the aCSF; n = 5). The perfusion rate was initially set at 0.5 mL/min for 2 min, decreasing to 0.1 mL/min for 5 min. The pial arteriole diameter, MAP, heart rate (HR), and rectal temperature were measured 5 min after application of each concentration. The window was then flushed with aCSF for 30 min before the next concentration was tested.

Experiment 2 (Ischemia/reperfusion injury)

Before the experiments, the brachiocephalic artery, left common carotid artery, and left subclavian artery were exposed. Global brain ischemia was produced by a 15 min clamp of the brachiocephalic artery, left common carotid artery, and left subclavian artery. Either aCSF (control group, n = 7), adrenaline 10^{-5} mol/L (adrenaline group, n = 7), or vasopressin 10^{-7} mol/L (vasopressin group n = 7) were infused from 5 min before ischemia (baseline) to 120 min after unclamping. Cerebral pial diameter and hemodynamic parameter measurements were made at

the following time points: 5 min before ischemia, 10 min after clamping, and at 5, 10, 20, 40, 60, 80, 100, and 120 min after unclamping; arterial blood samples were collected from the femoral artery 5 min before ischemia, 10 min after clamping, and at 5, 10, 20, 40, 60, 80, 100, and 120 min after unclamping.

Statistical analysis

Values are represented as the mean ± SD. Power analysis indicated that a sample size of seven rabbits per group was sufficient to detect a 15% change in pial arteriolar diameter from the control values with a power of 0.8 and $\alpha < 0.05$. Intragroup differences were compared using analysis of variance and Dunnett's post-hoc comparisons; intergroup differences were compared with analysis of variance and the Newman-Keuls post-hoc test. A *P* value less than 0.05 was considered statistically significant.

Results

Topical administration of adrenaline and vasopressin in the normal state

In the topical experiments using adrenaline and vasopressin, MAP, HR, arterial pH, $PaCO_2$, PaO_2, base excess (BE), and plasma Na^+, K^+, and glucose levels did not change significantly during the experimental period (Tables 1and 2).

While topical application of adrenaline had no effect on pial arterioles (Figure. 1a), vasopressin tended to produce pial arteriolar dilation at 10^{-11} mol/L ($P = 0.1567$), and, pial arteriolar constriction at 10^{-7} mol/L ($P = 0.1604$); however these changes were not statistically significant (Figure. 1b).

Ischemia and reperfusion

In the control, adrenaline, and vasopressin groups, HR, $PaCO_2$, PaO_2, and plasma Na^+, and K^+ levels did not change significantly during the experimental period. In all groups, MAP, arterial pH, and BE decreased, while the glucose level increased after unclamping (Tables 3, 4, and 5).

Pial arteriolar diameter temporarily increased, decreasing after unclamping in the control group ($P < 0.01$, Figure 2a). Compared with the control and vasopressin groups, the adrenaline group indicated dilation of the pial arterioles during the reperfusion period (Figure 2b). In the vasopressin group, pial arteriolar diameter significantly decreased after unclamping ($P < 0.01$, Figure 2c); the control and vasopressin groups showed similar changes in arteriolar

diameter (Figures 2a and 2c).

Discussion

This study showed that adrenaline had no direct effects on the pial arterioles. While low concentrations of vasopressin tended to produce pial arteriolar dilation, at high concentrations tended to induce pial arteriolar constriction. During the ischemia-reperfusion period, pial arterioles were slightly dilated and then constricted in the control group; adrenaline counteracted cerebral vasoconstriction during the global brain ischemia-reperfusion period. Conversely, vasopressin did not show any effect on pial arterioles during the global brain ischemia-reperfusion period.

Adrenergic receptors such as α1-, α2-, β1-, and β2-receptors are localized on pial vessels [14]. Activation of α-adrenergic receptors causes pial arteriolar constriction; thus, constriction of the pial arterioles occurs via α1-adrenergic receptor- [2] and α2-adrenergic receptor-mediated mechanisms [1]. On the other hand, pial arterioles are dilated via β1-adrenergic receptor- [3] and β2-adrenergic receptor-mediated mechanism [3]. Although blockade of the β1-adrenergic receptor has been recently reported to produce pial arteriolar dilation, suggesting that β1-adrenergic receptor activation results in pial arteriolar constriction. The number of β2-adrenergic receptors is approximately twice as high as that of β1-adrenergic receptors [15,16]. Activation of β-adrenergic receptors could produce pial arteriolar dilation. Adrenaline acts on α1-, α2-, β1-, and β2-adrenergic receptors. The effect of adrenaline on pial arterioles is determined by the balance between its effects on these receptors. One study reported that adrenaline constricted cerebral pial arterioles in cats [4]; another study using dogs demonstrated that adrenaline did not affect cerebral pial arterioles in dogs [5]. Our findings concur with the latter study, and demonstrating that adrenaline did not dilate, nor constrict the

pial arterioles; thus our study suggests that adrenaline could act on α1-, α2-, β1-, and β2-receptors to an equivalent extent in rabbits of a normal state.

Various studies have suggested that vasopressin induces vasodilation [17] or vasoconstriction [18]. Vasopressin induced the production of nitric oxide from vascular endothelial cells through V1- and V2-receptors [19, 20]. Through this mechanism, vasopressin dilates the cerebral pial arterioles [20]; conversely, vasopressin can also constrict the cerebral vessels through a V1-receptor mediated mechanism [20]. Kumazawa et al. [7] reported that pial arterioles were dilated at lower doses (10^{-11} mol/L) of vasopressin, whereas they were constricted at higher doses (10^{-9}, 10^{-7}, and 10^{-5} mol/L). Takayasu et al. [20] showed that vasopressin induced a triphasic response (vasodilation, vasoconstriction and vasodilation) in these arteries. Our results demonstrated that vasopressin at 10^{-11} mol/L tended to produce pial arteriolar dilation, while pial arteriolar constriction was induced at 10^{-7} mol/L; however, these changes were not statistically significant. Our results are roughly compatible with those of previous studies; thus, it is possible that vasopressin has both dilating and constricting effects on normal cerebral pial arterioles.

We used adrenaline at 10^{-5} mol/L for the ischemia-reperfusion study. The plasma adrenaline concentration during cardiopulmonary resuscitation has been reported to range between 5×10^{-7} [21] and 1.2×10^{-6} mol/L [22] or between 0.124 [23] and 1 μg/ml [24]. As the molecular weight of adrenaline is 183.2 g/mol, adrenaline at 10^{-5} mol/L is equivalent to 1.832 μg/mL, a relatively high concentration; however, in the cranial window technique, the drug solution injected into the window is slightly diluted by the cerebrospinal fluid of the animal. The adrenaline concentration of 10^{-5} mol/L should be clinically relevant. On the other hand, 10^{-}

^{7} mol/L vasopressin was used in the ischemia-reperfusion study. Plasma vasopressin concentration during cardiopulmonary resuscitation has been reported to range between 70,000[25] and 110,000 pg/mL [26]. The molecular weight of vasopressin is 1084.2316 g/mol; thus vasopressin concentrations of 70,000 and 110,000 pg/mL are approximately equal to 6.5×10^{-8} and 10^{-7} mol/L, respectively. A vasopressin concentration of 10^{-7} mol/L should therefore also be clinically relevant.

In a previous global brain ischemia-reperfusion study, we had reported that pial arterioles temporarily dilated, subsequently constricting throughout the reperfusion period [11, 27]. Delayed hypoperfusion after brain ischemia has been reported to contribute to development of cerebral edema [28], concurrently, preventing cerebral vasoconstriction in the cerebral vasculature after ischemic stroke in female rats has been shown to improve long-term neurological outcomes [29]. We assumed that cerebral vasodilation after brain ischemia may remove acidic metabolites from ischemic brain tissue. Additionally, cerebral vasodilation may provide sufficient oxygen and glucose to preserve normal neuronal function; thus, attenuation of cerebral vasoconstriction after brain ischemia is important. The current study demonstrated that adrenaline increased pial arteriolar diameter during the reperfusion period, and may counteract cerebral vasoconstriction during the global brain ischemia-reperfusion period, thus, adrenaline could be useful during a period of brain ischemia and reperfusion.

The mechanism underlying pial arteriolar vasodilation during the global brain ischemia-reperfusion period is unclear. Adrenaline dilates cerebral vessels via β2-receptor activation; thus, the β2-receptor may be significantly activated significantly during the ischemia-reperfusion period. Cerebrovascular endothelin-1 hyper-reactivity was associated with delayed cerebral hypoperfusion after forebrain ischemia [30]. Adrenaline might act on

endothelin-1 to attenuate cerebral vasoconstriction during the period of reperfusion, although no reports are available on this issue. Further studies are necessary.

Vasopressin has been reported to constrict cerebral pial arterioles during the ischemia-reperfusion period [7]. We observed that pial arterioles were constricted during the reperfusion period; however, pial arteriolar changes were similar between the control and vasopressin groups during the ischemia-reperfusion periods, suggesting that vasopressin did not exert its cerebrovascular-constricting effects during ischemia-reperfusion period. This result might coincide with Kumazawa's study, which showed that the vasoconstrictor effect of vasopressin was reduced after cerebral ischemia [7]. Ristagno et al. reported that cerebral cortical microcirculatory blood flow was preserved with vasopressin after the restoration of spontaneous circulation from the cardiac arrest [13]; this effect lasted up to 6 min after the restoration of circulation [13]. Cerebral microcirculatory blood flow after resuscitation was equivalent to that before cardiac arrest [13]. In agreement with their findings, pial arteriolar diameters at 5 and 10 min after reperfusion were comparable to those before brain ischemia in the vasopressin group in the present study; thus, vasopressin might not deteriorate cerebral circulation during the brain ischemia-reperfusion period.

Ristagno et al. demonstrated that adrenaline markedly cerebral cortical microcirculatory blood flow up to 6 min after the restoration of circulation [13]; by contrast, adrenaline did not constrict pial arterioles at 5 and 10 min after reperfusion in the present study. There are several differences between Ristagno's study and our own; first, while brain ischemia was induced with cardiac arrest in their study, our study used arterial clamping. Second, adrenaline was injected into the right atrium in the Ristagno's study and continuously injected into the cranial window in this study. Lats, pigs were used in Ristagno's study, whereas rabbits

were used in this study. These differences may have affected the results. Contrary to their report, our study suggested that adrenaline did not impair cerebral circulation during the reperfusion period.

This study has some limitations. First, we administered adrenaline and vasopressin into the cranial window to elucidate the direct pharmacological effects of both drugs. The systemic effects of adrenaline and vasopressin can be avoided by topical application; however, our results may not be directly adaptable to clinical situations. Second, cerebral blood flow was not measured in this study, due to Poiseuille's law, cerebral blood flow should be proportional to the vessel radius. Thus, it is probable that cerebral blood flow increases and decreases the with arteriolar diameter.

In conclusion, adrenaline did not act directly on, while vasopressin may have dilator and constrictor effects on, cerebral pial arterioles in the normal state. In the ischemia-reperfusion period, adrenaline increased cerebral pial arterial diameter at 60, 80, 100, and 120 min, in comparison with vasopressin and the control. Adrenaline may thus counteract cerebral vasoconstriction during global brain the ischemia-reperfusion period.

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Conflicts of interest: None

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Table 1. Physiologic measurements during topical application of adrenaline

	MAP (mmHg)	HR (beats/min)	pH	BE (mmol/L)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Na (mEq/L)	K (mEq/L)	Glucose (mg/dL)
10^{-9} mol/L	96 ± 11	299 ± 39	7.44 ± 0.06	2.5 ± 2.8	39.1 ± 4.4	215 ± 28	141 ± 2	3.4 ± 0.3	144 ± 20
10^{-7} mol/L	96 ± 10	297 ± 42	7.44 ± 0.05	3.1 ±2.3	38.5 ± 4.7	213 ± 29	142 ± 2	3.5 ± 0.3	145 ± 23
10^{-5} mol/L	93± 11	294 ± 42	7.46 ± 0.06	2.4 ± 3.0	37.1 ± 4.1	205 ± 23	141 ± 3	3.5 ± 0.4	145 ± 19
10^{-4} mol/L	93 ± 10	298 ± 32	7.46 ± 0.05	2.9 ± 1.6	37.9 ± 2.9	206 ± 29	141 ± 2	3.5 ± 0.3	147 ± 24

Values are mean ± SD.
MAP, mean arterial blood pressure; HR, heart rate; BE, base excess; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen.

Table 2. Physiologic measurements during topical application of vasopressin

	MAP (mmHg)	HR (beats/min)	pH	BE (mmol/L)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Na (mEq/L)	K (mEq/L)	Glucose (mg/dL)
10^{-11} mol/L	96 ± 11	311 ± 30	7.42 ± 0.04	2.2 ± 2.3	39.0 ± 3.7	199 ± 27	141 ± 2	3.3 ± 0.2	142 ± 15
10^{-9} mol/L	101 ± 14	307 ± 30	7.45 ± 0.07	3.1 ± 3.0	39.1 ± 3.6	208 ± 27	140 ± 2	3.4 ± 0.2	145 ± 17
10^{-7} mol/L	96 ± 10	300 ± 38	7.43 ± 0.04	2.8 ± 2.1	39.0 ± 3.6	209 ± 32	140 ± 2	3.5 ± 0.3	147 ± 19
10^{-6} mol/L	101 ± 11	296 ± 37	7.44 ± 0.05	3.6 ± 2.5	40.3 ± 3.1	203 ± 35	141 ± 2	3.5 ± 0.3	144 ± 21

Values are mean ± SD.
MAP, mean arterial blood pressure; HR, heart rate; BE, base excess; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen.

Table 3. Physiologic measurements during the ischemia-reperfusion injury in the control group

	MAP (mmHg)	HR (beats/min)	pH	BE (mmol/L)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Na (mEq/L)	K (mEq/L)	Glucose (mg/dL)
Baseline	106 ± 13	285 ± 37	7.40 ± 0.05	-1.9 ± 2.3	37.6 ± 2.9	186.6 ± 20.3	142 ± 3	3.3 ± 0.3	145 ± 22
Ischemia	105 ± 35	276 ± 24	7.40 ± 0.06	-2.9 ± 3.1	35.0 ± 4.3	165.7 ± 40.5	141 ± 4	3.7 ± 0.7	188 ± 54*
Unclamp 5 min	75 ± 41*	261 ± 22	7.29 ± 0.08*	-5.4 ± 2.4	40.5 ± 5.7	170.0 ± 45.9	141 ± 4	3.5 ± 0.4	172 ± 35
Unclamp 10 min	93 ± 17	271 ± 34	7.31 ± 0.06	-5.3 ± 2.5	41.4 ± 5.7	174.6 ± 45.0	140 ± 2	3.5 ± 0.4	166 ± 39
Unclamp 20 min	94 ± 29	265 ± 34	7.31 ± 0.07	-5.1 ± 2.7	42.2 ± 5.0	161.6 ± 37.4	140 ± 3	3.5 ± 0.4	137 ± 25
Unclamp 40 min	86 ± 15	267 ± 25	7.33 ± 0.06	-4.1 ± 3.2	39.3 ± 2.8	161.0 ± 41.3	141 ± 3	3.5 ± 0.5	137 ± 25
Unclamp 60 min	84 ± 14	266 ± 26	7.32 ± 0.07	-4.0 ± 4.3	39.3 ± 2.8	165.8 ± 47.7	140 ± 3	3.6 ± 0.5	124 ± 21
Unclamp 80 min	89 ± 14	269 ± 26	7.34 ± 0.07	-2.4 ± 2.6	38.0 ± 4.6	158.6 ± 37.0	141 ± 2	3.7 ± 0.7	125 ± 21
Unclamp 100 min	90 ± 13	268 ± 28	7.35 ± 0.09	-3.3 ± 4.3	39.4 ± 3.7	169.4 ± 39.0	142 ± 3	3.7 ± 0.8	123 ± 24
Unclamp 120 min	90 ± 14	268 ± 29	7.33 ± 0.10	-3.7 ± 5.2	39.9 ± 3.4	156.9 ± 35.4	141 ± 3	3.9 ± 1	122 ± 28

Values are mean ± SD.
MAP, mean arterial blood pressure; HR, heart rate; BE, base excess; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen.
* $P < 0.05$ compared with the values in the baseline.

Table 4. Physiologic measurements during the ischemia-reperfusion injury in the adrenaline group

	MAP (mmHg)	HR (beats/min)	pH	BE (mmol/L)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Na (mEq/L)	K (mEq/L)	Glucose (mg/dL)
Baseline	108 ± 10	308 ± 22	7.40 ± 0.04	-0.6 ± 2.8	39.8 ± 1.5	178.3 ± 38.8	142 ± 2	3.6 ± 0.3	139 ± 11
Ischemia	124 ± 38	259 ± 51	7.33 ± 0.09	-6.9 ± 3.0†	35.0 ± 8.4	150.6 ± 52.4	143 ± 4	3.8 ± 0.6	233 ± 34†
Unclamp 5 min	76 ± 34	277 ± 57	7.28 ± 0.09*	-8.4 ± 3.8†	40.8 ± 5.9	162.4 ± 71.9	142 ± 3	3.7 ± 0.5	228 ± 53†
Unclamp 10 min	80 ± 25	280 ± 51	7.29 ± 0.07	-8.3 ± 3.4†	40.1 ± 3.5	165.6 ± 51.2	140 ± 3	3.8 ± 0.5	238 ± 66†
Unclamp 20 min	94 ± 12	285 ± 40	7.29 ± 0.07	-9.0 ± 5.1†	40.0 ± 4.3	164.3 ± 46.8	140 ± 3	3.8 ± 0.5	232 ± 85†
Unclamp 40 min	84 ± 13	282 ± 36	7.32 ± 0.09	-6.9 ± 4.7†	38.2 ± 2.9	152.7 ± 52.5	141 ± 4	4.0 ± 0.6	212 ± 94*
Unclamp 60 min	79 ± 15	286 ± 38	7.30 ± 0.11	-6.1 ± 4.3†	40.4 ± 3.0	162.2 ± 59.2	141 ± 4	4.2 ± 0.6	200 ± 93
Unclamp 80 min	74 ± 21*	290 ± 25	7.33 ± 0.09	-6.1 ± 4.6†	38.6 ± 2.8	152.9 ± 63.0	142 ± 4	4.4 ± 0.6	195 ± 98
Unclamp 100 min	76 ± 27	286 ± 21	7.32 ± 0.10	-6.6 ± 5.9†	38.0 ± 3.1	141.1 ± 57.8	142 ± 3	4.4 ± 0.8	192 ±1 05
Unclamp 120 min	78 ± 27	280 ± 11	7.31 ± 0.16	-6.6 ± 6.8†	39.7 ± 5.0	151.4 ± 63.6	142 ± 4	4.5 ± 0.9	172 ± 61

Values are mean ± SD.
MAP, mean arterial blood pressure; HR, heart rate; BE, base excess; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen.
* $P < 0.05$ compared with baseline values.* †$P < 0.01$ compared with baseline values.

Table 5. Physiologic measurements during the ischemia-reperfusion injury in the vasopressin group

	MAP (mmHg)	HR (beats/min)	pH	BE (mmol/L)	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Na (mEq/L)	K (mEq/L)	Glucose (mg/dL)
Baseline	109 ± 18	278 ± 44	7.40 ± 0.03	-0.6 ± 1.9	39.6 ± 4.0	200.0 ± 36.2	143 ± 2	3.4 ± 0.2	138 ± 15
Ischemia	103 ± 37	290 ± 64	7.36 ± 0.06	-4.3 ± 3.3*	37.3 ± 8.7	166.0 ± 79.2	143 ± 3	3.8 ± 0.5	199 ± 39
Unclamp 5 min	75 ± 29*	269 ± 44	7.31 ± 0.06†	-5.4 ± 3.5†	41.6 ± 3.0	179.7 ± 60.3	142 ± 3	3.7 ± 0.3	199 ± 57
Unclamp 10 min	78 ± 30*	265 ± 31	7.31 ± 0.07†	-5.4 ± 3.7†	41.4 ± 5.1	183.4 ± 60.4	141 ± 3	3.6 ± 0.5	197 ± 71
Unclamp 20 min	81 ± 29	266 ± 33	7.31 ± 0.06†	-5.0 ± 4.0*	42.1 ± 6.3	191.0 ± 35.4	140 ± 3	3.6 ± 0.5	196 ± 101
Unclamp 40 min	91 ± 28	275 ± 27	7.33 ± 0.07	-4.0 ± 3.5	41.5 ± 3.6	182.3 ± 31.6	141 ± 3	3.8 ± 0.6	195 ± 121
Unclamp 60 min	89 ± 24	287 ± 30	7.35 ± 0.06	-4.0 ± 2.9	39.9 ± 3.9	183.4 ± 18.6	142 ± 4	4.1 ± 0.7	187 ± 89
Unclamp 80 min	90 ± 31	290 ± 34	7.34 ± 0.06	-4.0 ± 2.6	40.0 ± 4.7	158.0 ± 27.5	141 ± 3	4.1 ± 0.8	187 ± 80
Unclamp 100 min	92 ± 27	287 ± 33	7.35 ± 0.05	-3.4 ± 2.4	40.1 ± 4.3	156.3 ± 27.4*	141 ± 3	4.3 ± 0.8	181 ± 75
Unclamp 120 min	88 ± 28	284 ± 36	7.37 ± 0.06	-2.7 ± 3.1	39.5 ± 5.1	166.1 ± 36.7	142 ± 4	4.3 ± 0.9	174 ± 73

Values are mean ± SD.
MAP, mean arterial blood pressure; HR, heart rate; BE, base excess; $PaCO_2$, partial pressure of carbon dioxide; PaO_2, partial pressure of oxygen.
* $P < 0.05$ compared with baseline values.* †$P < 0.01$ compared with baseline values.

Figure legends

Figure 1: The effects of adrenaline (A) and vasopressin (B) on pial arterioles in the normal state. Values are mean ± SD. Data represent percent change in diameter in comparison with pre-drug measurements. Adrenaline had no effects on pial arterioles. Vasopressin at 10^{-11} mol/L tended to induce pial arteriolar dilation (P = 0.1567), and at 10^{-7} mol/L, it tended to induce pial arteriolar constriction (P = 0.1604).

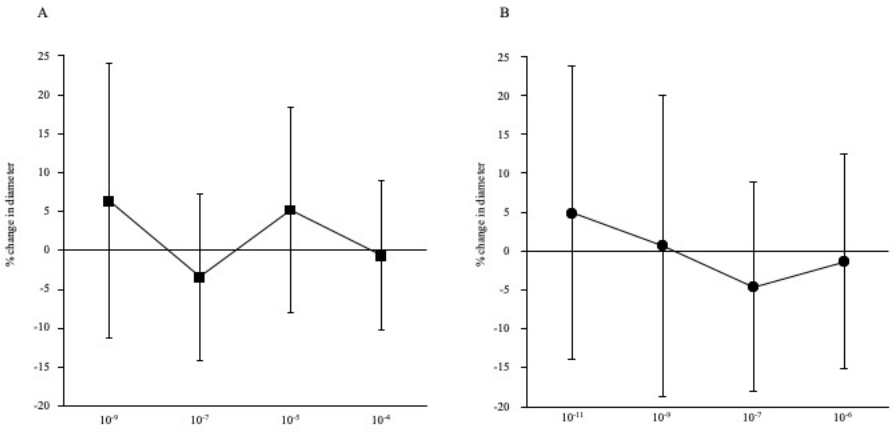

Figure 1

Figure 2: The effects of aCSF (control) (A), adrenaline (B), and vasopressin (C) on pial arterioles during the global brain ischemia-reperfusion period. Values are mean ± SD. Data represent the percent change in diameters from the baseline. In the ischemia-reperfusion period, pial arteriolar diameter decreased during the reperfusion period in the control and the vasopressin groups. Adrenaline increased cerebral pial arteriolar diameter in comparison with

the vasopressin and control groups. Ba = baseline. * $P < 0.05$ compared with baseline, † $P < 0.01$ compared with baseline, ‡ $P < 0.01$ compared with control, ¶ $P < 0.05$ compared with vasopressin, # $P < 0.05$ compared with vasopressin.

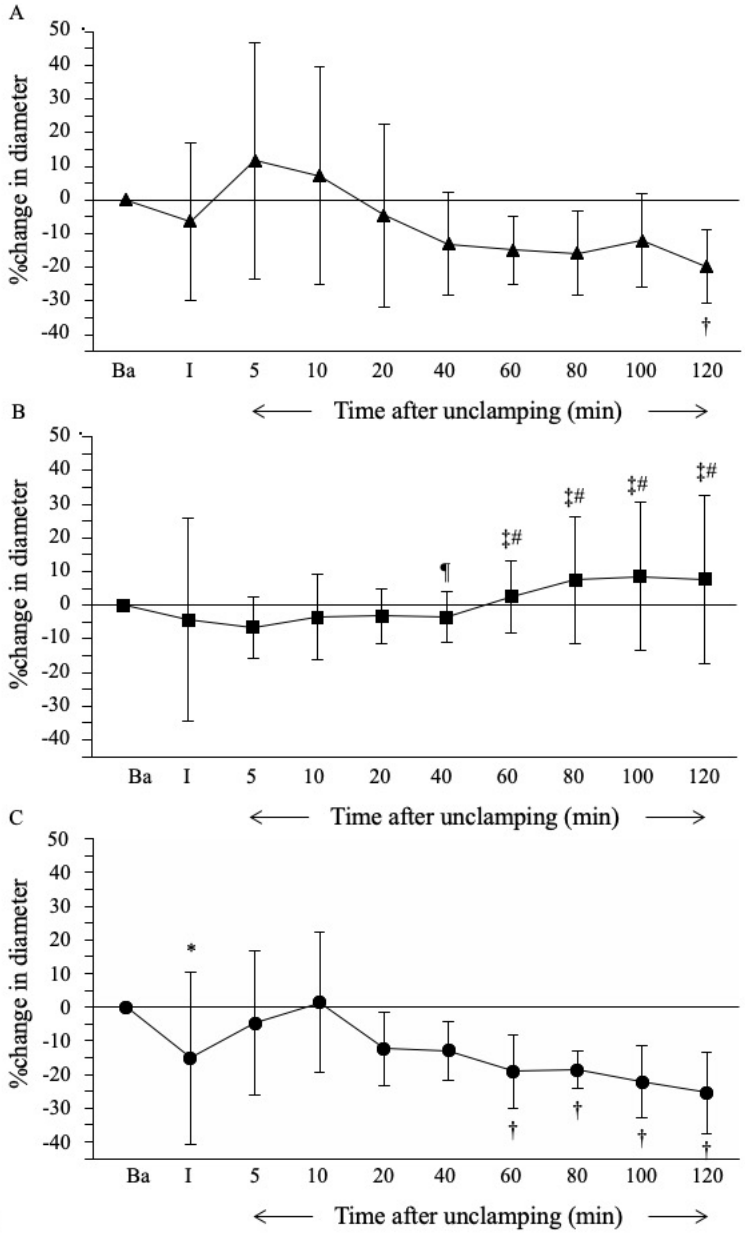

Figure 2

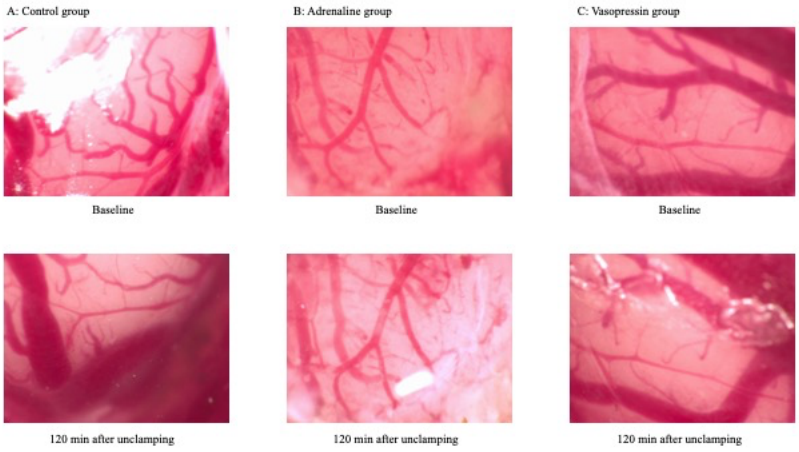

Figure 3

Figure 3: Representative images of pial microvessels before and 120 min after ischemia-reperfusion.