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ORIGINAL ARTICLE

Contribution of Apelin-17 to Collateral Circulation Following Cerebral Ischemic Stroke

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Abstract

Apelin, an essential mediator of homeostasis, is crucially involved in cardiovascular diseases, including ischemic stroke. However, the functional roles of apelin-17 in cerebral collateral circulation and ischemic stroke protection are unknown. Here, we investigated the association between plasma apelin-17 levels and collateral circulation in patients with ischemic stroke and examined the mechanism undergirding the effects of apelin-17 on cerebral artery contraction and ischemic stroke protection in an animal model. Plasma nitric oxide (NO), apelin-17, and apelin-36 levels were assessed by enzyme-linked immunosorbent assays in ischemic stroke patients with good or poor collateral circulation and in healthy participants. Additionally, the effects of apelin-17 on rat basilar artery contractions (in vitro) and cerebral ischemia (in vivo) were determined using vessel tension measurements and nuclear magnetic resonance, respectively. Patients with good collateral circulation had significantly higher plasma apelin-17 and apelin-36 levels than both patients with poor collateral circulation and healthy participants and plasma NO levels significantly higher than those in healthy participants. In vitro, apelin-17 pretreatment markedly attenuated U46619-induced rat basilar artery contractions in an endothelium-dependent manner. Additionally, NO production or guanylyl cyclase inhibitors abolished the apelin-17 effect on U46619-induced vascular contraction. Intravenous pretreatment of rats with apelin-17 markedly reduced cerebral infarct volume at 24 h after middle cerebral artery occlusion. Plasma apelin-17 levels in ischemic stroke patients were positively associated with enhanced collateral circulation, which our animal study data suggested may have resulted from an apelin-17-induced cerebral artery dilation mediated through the NO–cGMP pathway.

Keywords Stroke · Apelin-17 · Cerebral collateral circulation · Basilar artery · Endothelium · Nitric oxide · Cerebral ischemic protection

Introduction

Ischemic stroke is a leading cause of death in humans worldwide [1]. When the proximal artery becomes occluded, fresh blood is delivered to the ischemic area through collateral circulation, reducing cerebral damage [2, 3]. A rich supply of blood through collateral circulation can reduce expansion of the ischemic area and improve clinical outcomes [4]. Accumulating clinical evidence suggests that the functional status of the collateral

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circulation is an independent predictor of outcome in response to recanalization therapies in patients with ischemic stroke [5]. The collateral vessels can be visualized using computed tomography angiography (CTA), providing detailed information that includes the site of the occlusion, the status of the collateral circulation, and the viability of the tissue [6]. Another important prognostic criterion in patients with ischemic stroke is their score on the National Institutes of Health Stroke Scale (NIHSS), which rates the initial ischemic severity [7]. Numerous studies have shown that many factors, including hypertension, diabetes mellitus, advanced age, smoking, hyperlipidemia, hyperhomocysteinemia, and thrombosis, are associated with ischemic stroke [8]. However, the exact molecular mechanisms responsible for the regulation of cerebral collateral circulation in patients with ischemic stroke are unclear.

Apelin was first identified as an endogenous ligand of the apelin receptor, an orphan G protein-coupled receptor originally isolated in 1998 from bovine stomach extracts [9]. Apelin is initially secreted from cells as a precursor prepropeptide composed of 77 amino acids and subsequently cleaved into a family of apelin peptides [10]. The dominant apelin peptides found in vivo, apelin-12, apelin-13, apelin-17, and apelin-36, have beneficial associations with many diseases, including diabetes, obesity, and cancer [11–13]. Increasing evidence indicates that apelin is also crucially involved in cardiovascular diseases [14, 15]. For example, apelin reduces resting aortic vascular tone, alleviates myocardial ischemia–reperfusion injury, and increases left-ventricular systolic pressure and stroke volume [16–18]. Moreover, apelin has been reported to increase nitric oxide (NO) production to regulate vascular tone [19]. These protective effects on the cardiovascular system suggest that apelin may be a potential therapeutic target in cardiovascular diseases [12]. Recent studies have shown that several apelin subtypes, including apelin-13 and apelin-36, have a potential neuroprotective role in ischemic stroke, and our previous study also have indicated that the plasma apelin-12 level was substantially higher in moyamoya disease patients than in intracranial atherosclerotic disease patients [20–24]. However, the effect of apelin-17 on ischemic stroke is unclear.

In the present study, we first determined whether serum apelin-17, apelin-36, and NO levels (as assessed using an enzyme-linked immunosorbent assay, ELISA) differentiated ischemic stroke patients with good collateral circulation from those with poor collateral circulation. We then used a rat model of ischemic stroke to examine the functional role of apelin-17 in the endothelial-dependent relaxation of the basilar artery in the brain and the protective effect of apelin-17 in ischemic stroke by determining vessel tension and by using nuclear magnetic resonance (NMR), respectively.

Materials and Methods

Patient Population

All participants and their family members provided informed consent for this study, which was approved by the Ethics Committee of the Affiliated Provincial Hospital of Anhui Medical University. All procedures were performed consistent with the Declaration of Helsinki and using Good Clinical Practice.

The study population included patients who were admitted to our hospital with symptoms consistent with acute cerebral ischemia and middle cerebral artery (MCA) M1 and/or M2 segment intracranial atherosclerotic occlusions, with no evidence for occlusion in the contralateral MCA or internal carotid artery. All patients underwent brain computed tomography (CT) or NMR and CTA. Patients with the following were excluded from the study: contraindication to iodinated contrast agent administration, previous revascularization, infection or inflammation or autoimmune diseases, chronic obstructive pulmonary disease, previous diagnosis of malignant neoplasm, diabetes with a history of insulin use, body mass index of ≤ 20 or ≥ 30 kg/m^2, or intracranial hemorrhage. For healthy controls, we recruited 22 healthy subjects from our department staff.

Baseline Measures

All patients underwent standard assessments to determine their demographic characteristics, medical history, prior medications, baseline laboratory tests, and stroke severity (NIHSS score). Baseline non-enhanced head and neck CT and CTA images were evaluated by both an experienced neuroradiologist and a clinical neurologist to determine the site and grade of the arterial occlusion and to characterize the collateral circulation during the selection of the included patients. Evidence of blood flow through the collateral vessels on the symptomatic hemisphere were graded as compared with those on the contralateral (normal) hemisphere as follows: 1, absent; 2, less than the contralateral side; 3, equal to the contralateral side; 4, greater than the contralateral side; and 5, robust. Thus, grades 1 or 2 indicated diminished, 3 adequate, and 4 or 5 augmented collateral circulation. Evidence of blood flow through the anterior communicating artery (ACOM) and the posterior communicating artery (PCOM) was graded as follows: 1, absent; 2, probably absent; 3, hairline; 4, definitely present; 5, robust. Grades 4 and 5 were defined as adequate ACOM and PCOM. The study population was divided into two groups on the basis of the degree of collateral circulation development. Patients with collateral vessels graded 1 or 2 were included in the poor collateral circulation group, whereas patients with vessels graded from 3 to 5 were included in the good collateral circulation group. For all participants, blood

samples were collected in the morning after 8–12 h of fasting. Biochemical and lipid parameters were measured using an automated enzymatic analyzer (Beckman Coulter, AU5800, Japan).

Determination of Plasma NO, Apelin-17, and Apelin-36 Levels

Blood samples were obtained after CTA. For measurement of NO, apelin-17, and apelin-36 levels, blood samples (5 mL) were collected in an EDTA- and heparin-containing tube and centrifuged at 3600 rpm for 10 min. The plasma (1 mL) was collected from each sample, and the levels of NO, apelin-17, and apelin-36 in the plasma samples were determined using ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All ELISA experiments used three groups of samples: blank controls, standards, and plasma samples. After the samples were added to each well of the plate, the plate was incubated at 37 °C for 30 min and then rinsed in wash buffer five times. After the final wash, the liquid was discarded by inverting the plate. Horseradish peroxidase was added to each well. After incubation and washout, 3,3′,5,5′-tetramethylbenzidine was applied for 10 min at 37 °C to visualize the reaction product. The reaction was terminated, and the optical density was detected using a plate reader at a wavelength of 450 nm.

Basilar Artery Tension Measurement

All animal experiments were conducted in accordance with the guidelines provided by the US National Institutes of Health (NIH publication No. 8523) and were approved by the Animal Experimentation Ethics Committee of Anhui Medical University. Adult male Sprague-Dawley rats (body weight, 270 ± 10 g) were housed under controlled environmental conditions, with an ambient temperature of 22 ± 1 °C, relative humidity of 65%, and 12-h light/dark cycle, and had free access to food and water. Vessel tension measurements were performed as previously described [25]. Briefly, rats were killed by an overdose of inhaled CO_2. The brain was dissected and placed in oxygenated ice-cold Krebs–Henseleit solution containing in mmol/L 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$ ($7H_2O$), 25.2 $NaHCO_3$, and 11.1 glucose. The basilar artery was rapidly dissected. and the connective tissue surrounding the artery was removed under a dissecting microscope. The artery was cut into rings (2 mm in length). The vessel rings were mounted in a myograph (DMT model 610M, Danish Myo Technology, Aarhus, Denmark) chamber, which was filled with 5 mL of Krebs solution. The chamber was warmed to 37 °C and bubbled with a gas mixture of 95% O_2 and 5% CO_2 to maintain pH 7.4. An optimal preload tension of 2 mN was applied to the vessel rings. After a 20-min equilibration period, the vessel rings were contracted using a high-K^+ (60 mmol/L) solution prepared by replacing NaCl with an equimolar amount of KCl. After being rinsed, the vessel ring was contracted using U46619 (100 nmol/L) for 10 min, and acetylcholine (10 μmol/L) was subsequently used to relax the ring to verify that the endothelium had been removed. The rings were washed again, and the contractile response to U46619 (10^{-9}–10^{-6} mol/L) was obtained by cumulative addition of U46619 to the bath solution with or without pretreatment of apelin-17 (1 μmol/L). In some experiments, The NO production inhibitor L-NAME (50 μmol/L) or the guanylyl cyclase inhibitor ODQ (1 μmol/L) was also used to pretreat the vessel rings with or without apelin-17. For the endothelium-denuded vessels, the endothelial layer was removed by gently rotating a steel wire in the vessel lumen. The successful removal of the endothelial layer was identified by the lack of a relaxation response to acetylcholine.

Middle Cerebral Artery Occlusion Animal Model of Cerebral Ischemia

A rat model of cerebral ischemia was established using a revised Longa method [26]. Briefly, Sprague-Dawley rats were anesthetized with 5% chloral hydrate (0.7 mL/100 g, intraperitoneal injection), and fixed in the supine position. The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were dissected via a midline incision of the neck. The ICA was first occluded using an artery clip. A small incision was then created in the ICA 5 mm from the CCA bifurcation and a thread embolus was inserted into the ICA. The ICA artery clip was released and the thread embolus was moved forward approximately 1.8 ± 0.2 cm. At this position, the thread embolus reached the start of and blocked blood flow through the MCA. The thread embolus was fixed in place using a gel. During the procedure, the temperature of animal's body was maintained at 25 to 28 °C. The vagus nerve was protected to avoid artificially stimulating it.

Nuclear Magnetic Resonance Imaging and Analysis

Male Sprague–Dawley rats were randomized into control and experimental groups. The rats in the experimental group were pretreated with an intravenous administration of apelin-17 (150 μg/kg) 1 h prior to the middle cerebral artery occlusion (MCAO), while rats in the control group were administered the same volume of physiological saline. Cerebral ischemia was induced by MCAO for 2 h, and then NMR imaging was performed 1 and 48 h after reperfusion.

The NMR scans were obtained using an Achieva system (Philips Healthcare, Best, The Netherlands) with a 3.0-T superconducting magnet and an 80-mT/m gradient system. A phased array head coil (4CH-rat) with a diameter of

50 mm and length of 70 mm was used. Rats were anesthetized with 5% chloral hydrate (0.7 mL/100 g, intraperitoneal injection). During survey scanning, anesthetized rats were fixed in a prone position using a holder that locked their front teeth onto a bite bar. A symmetrical position was obtained using rapid gradient-echo imaging. This step was repeated if a slice correction was necessary. Diffusion-weighted and T2-weighted MR images were obtained for both groups 1 and 48 h after MCAO. Infarct volume was analyzed using a segmentation-based volumetric approach (TeraRecon Inc., Foster City, CA, USA). Each case was measured three times by two experienced radiologists. The infarct volume measurement had high repeatability (Intra-rater intraclass correlation coefficients > 0.99).

Statistical Analysis

All statistical analyses were conducted with SPSS 16.0 statistical software (SPSS, Inc., Chicago, IL, USA) using two-tailed tests. For clinical data, continuous variables are presented as means ± standard deviation (SD) or median ± interquartile range (IQR). Categorical variables are given as percentages. The Kolmogorov–Smirnov test was used to assess the normality of distribution. Differences in categorical variables among the groups were examined using the χ^2 test. Comparisons of continuous variables were analyzed with one-way analysis of variance followed by Mann–Whitney *U*, Kruskal-Wallis, or *t* tests as appropriate. For the animal study, the data are expressed as means ± standard error (SE). The significance of group differences was estimated by independent *t* tests or two-way analysis of variance, as appropriate. For all results, a value of $P < 0.05$ was considered statistically significant.

Results

Participant Characteristics

In total, 60 patients with ischemic stroke having good collateral circulation (Fig. 1a), 58 ischemic stroke patients with poor collateral circulation (Fig. 1b), and 22 healthy participants were included in this study. The characteristics of the participants are shown in Table 1. Given the critical role of NO and apelins [27] in regulating vascular function, we examined the plasma levels of NO, apelin-17, and apelin-36 in patients with ischemic stroke. We found that plasma apelin-17 and apelin-36 levels were significantly higher ($P < 0.05$) in patients with good collateral circulation than those in both the control group and ischemic stroke patients with poor collateral circulation, whereas no significant difference was detected in the plasma levels of apelin-17, apelin-36, or NO between the latter two groups (Fig. 2a–c). The level of NO was significantly higher in patients with good collateral circulation compared with that in participants in the control group but not with that in patients with poor collateral circulation (Fig. 2a–c). No statistically significant differences were found between the groups with good and poor collateral circulation for mean age, body mass index, systolic and diastolic blood pressure, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, or for the other variables examined ($P > 0.05$, Table 1).

Role of Endothelium in Apelin-17-Induced Cerebral Basilar Artery Relaxation

A previous report showed that apelin-13 relaxes blood vessels in an endothelial-dependent manner [28]. Because our results indicated that plasma apelin-17 levels were significantly higher in patients with good collateral circulation compared with those with poor collateral circulation following acute cerebral ischemia, we hypothesized that apelin-17 may have a protective effect on acute cerebral ischemia by relaxing cerebral arteries to increase collateral circulation. To test this hypothesis, we investigated the effect of apelin-17 on the contraction of the rat basilar artery. We found that a thromboxane mimetic, U46619 (10^{-9}–10^{-6} mol/L), contracted the rat cerebral basilar artery in a concentration-dependent manner (Fig. 3a, b). However, in vessels pretreated with apelin-17 (1 μmol/L) for 10 min, the U46619-induced contraction was significantly suppressed ($P < 0.05$, Fig. 3a, b). Removal of the vascular endothelial layers largely abolished the relaxation effect of apelin-17 on U46619-induced contraction ($P > 0.05$, Fig. 3c, d), suggesting that apelin-17 relaxes the cerebral basilar artery in endothelium-dependent manner.

Role of the NO–cGMP Signaling Pathway in Apelin-17-Induced Cerebral Basilar Artery Relaxation

The results of several studies have suggested that apelin-induced vessel relaxation is closely associated with NO released from the vascular endothelium [16, 28]. Thus, we next investigated the role of NO in apelin-17-induced rat cerebral basilar artery relaxation. The L-arginine analogue L-NAME, an NO synthase inhibitor, was used to inhibit NO release from vascular endothelial cells. We found that L-NAME (50 μmol/L) pretreatment markedly abolished apelin-17-induced rat cerebral basilar artery relaxation (Fig. 4a), suggesting that NO may be responsible for the apelin-17-induced vasodilation. Guanylyl cyclase is a key enzyme involved in NO signaling [29]. We hypothesized that the guanylyl cyclase regulation of 3′,5′-cyclic guanosine monophosphate (cGMP) synthesis from guanosine triphosphate may regulate the vascular smooth muscle contraction. To test this hypothesis, we inhibited guanylyl cyclase with pretreatment of ODQ (1 μmol/L) to suppress cGMP production and found that ODQ significantly abolished apelin-17-induced basilar artery relaxation (Fig. 4b). Hence, our results indicated

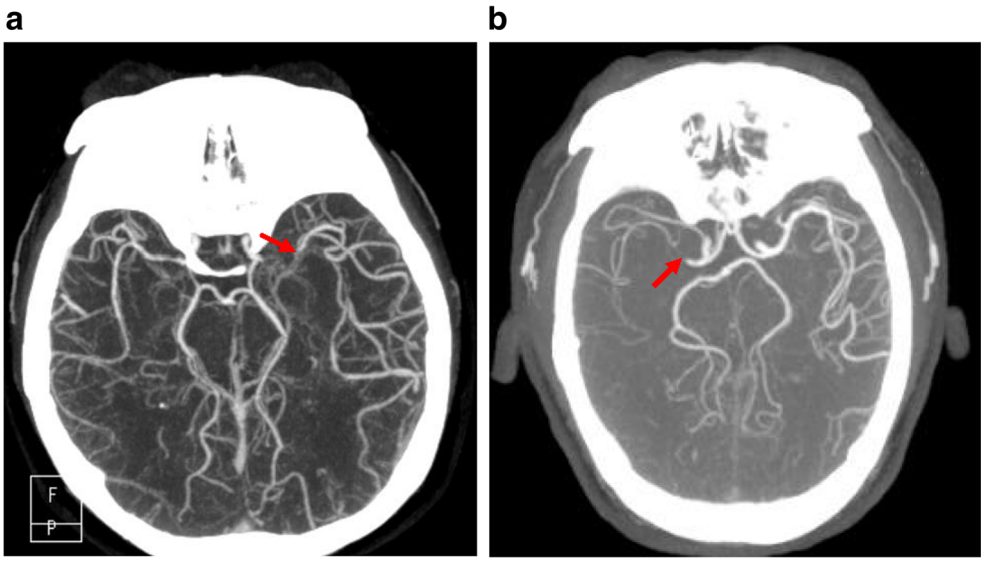

Fig. 1 Middle cerebral artery occlusion and collateral circulation. Computed tomography angiography showing left hemisphere middle cerebral artery occlusion (arrow) in a patient with good collateral circulation (**a**) and in another patient with right hemisphere middle cerebral artery occlusion (arrow) and poor collateral circulation (**b**)

that apelin-17 may have stimulated endothelial cell release of NO to relax vascular smooth muscle via the cGMP-mediated signaling pathway.

Apelin-17 Decreases Cerebral Infarct Volume In Vivo

On the basis of our clinical findings and the effect of apelin-17 on cerebral basilar artery contraction, we speculated that apelin-17 may have a protective effect on cerebral ischemia in animals pretreated with apelin-17 to enhance brain vascular dilation and collateral circulation. We tested this hypothesis in vivo using NMR to detect the cerebral infarct volume in rats subjected to MCAO, an animal model of cerebral ischemia. We found that intravenous pretreatment with apelin-17 markedly reduced the cerebral infarct volume (Fig. 5a–c), strongly suggesting that apelin-17 plays a critical protective role in cerebral ischemia and may contribute to recovery in patients with ischemic stroke having good collateral circulation.

Table 1 The characteristics of the study groups

Parameters	Healthy group ($n = 22$)	Good collateral group ($n = 60$)	Bad collateral group ($n = 58$)	P value
Age, years[a]	54.86 ± 6.78	60.14 ± 12.21	60.96 ± 12.38	0.124
BMI[a]	23.62 ± 2.42	24.49 ± 4.16	24.33 ± 2.85	0.601
SBP/DBP[a]	1.64 ± 0.13	1.76 ± 0.22	1.75 ± 0.27	0.111
Total cholesterol (mg/dL)[a]	4.29 ± 0.45	3.90 ± 1.07	4.29 ± 1.05	0.078
LDL-cholesterol (mg/dL)[a]	2.65 ± 0.84	2.38 ± 0.81	2.61 ± 0.84	0.205
HDL-cholesterol (mg/dL)[a]	1.26 ± 0.13	1.14 ± 0.34	1.15 ± 0.42	0.374
Triglyceride (mg/dL)[b]	1.52 ± 0.43	1.47 ± 0.85	1.64 ± 1.01	0.229
Lipoprotein a (mmol/L)[b]	218 ± 89.25	164 ± 357	232 ± 281	0.936
Homocysteine (nmol/L)[b]	11.43 ± 2.00	13.70 ± 5.5	12.30 ± 7.72	0.124
Apelin-17 (ng/dL)[b]	34.73 ± 70.10	64.60 ± 89.02[d]	34.32 ± 14.32	< 0.001
Apelin-36 (ng/dL)[b]	285.05 ± 266.93	299.05 ± 235.18	289.97 ± 228.35	0.265
NO (nmol/L)[b]	29.63 ± 49.21	55.98 ± 53.69[d]	33.07 ± 16.86	< 0.001
Sex, male (%)[c]	10/22	41/57	35/52	0.080
Smoking cigarettes (%)[c]	4/22	17/57	22/52	0.106
Consume alcohol (%)[c]	4/22	12/57	10/52	0.950

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *NO* nitric oxide

[a] Data were presented as mean ± SD

[b] Data were presented as median ± IQR

[c] Data were presented as %

[d] Significant differences vs. healthy group and bad collateral group ($P < 0.017$)

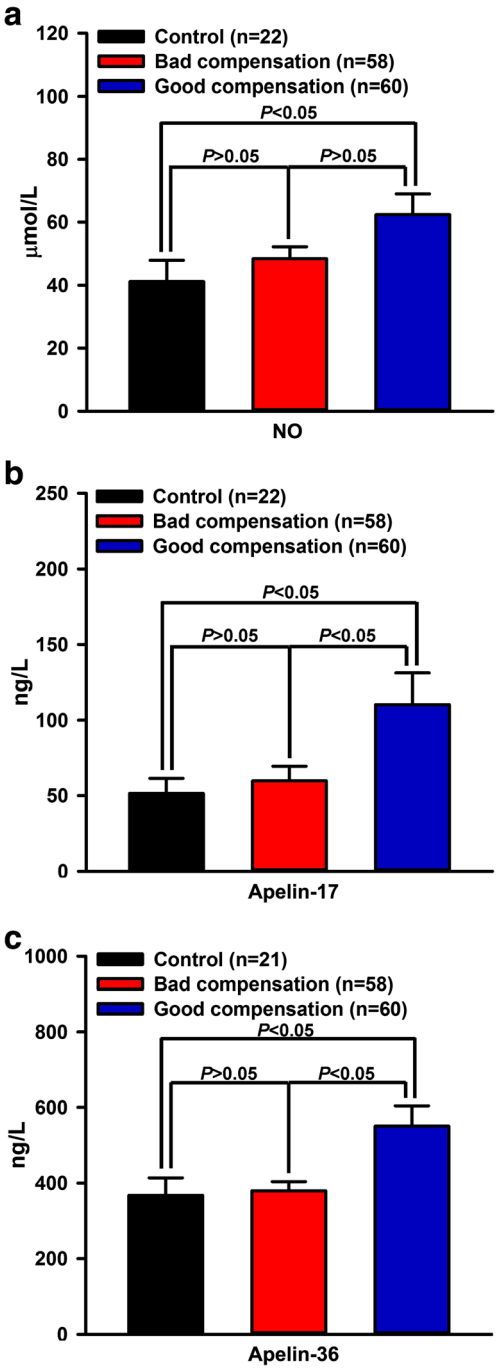

Fig. 2 Plasma nitric oxide (NO), apelin-17, and apelin-36 levels in healthy participants and in patients with middle cerebral artery occlusion. Summary data showing plasma levels of NO (**a**), apelin-17 (**b**), and apelin-36 (**c**) in healthy participants (control) and in patients with an acute complete occlusion and good (good compensation) or poor (bad compensation) collateral circulation. Values shown are the mean ± SEM ($n = 22$–60 each group)

Discussion

In the present study, we identified an association between plasma apelin-17 levels and cerebral collateral circulation in patients with ischemic stroke as well as demonstrated the role of apelin-17 in endothelial-dependent relaxation of the rat basilar artery and the protective effect of apelin-17 in a rat model of ischemic stroke. Our primary findings were as follows: (1) the plasma levels of apelin-17 and apelin-36 in ischemic stroke patients with good collateral circulation were significantly higher than those in ischemic stroke patients with poor collateral circulation or those in healthy control participants. However, plasma levels of NO in the good collateral circulation group were significantly higher than those in healthy participants but were not different from those in ischemic stroke patients with poor collateral circulation. (2) Apelin-17 significantly attenuated the U46619-induced rat basilar artery contraction in an endothelium-dependent manner and the NO–cGMP signaling pathway may be involved in the process. (3) As determined by NMR, apelin-17 significantly reduced MCAO-induced rat brain ischemia in vivo. Taken together, we suggested that apelin-17 had a beneficial effect in ischemic stroke and that the mechanism for this effect was through the relaxation of brain blood vessels to enhance cerebral collateral circulation.

Apelins, predominately apelin-36, apelin-17, apelin-13, and apelin-12 in humans, were first identified as a peptide isolated from the bovine stomach by Tatemoto et al. [9, 11]. Synthetic apelin-13 and apelin-17 were determined to be short C-terminal fragments of apelin-36. However, the activities of apelin-13 and apelin-17 are much higher (8- to 60-fold) than that of apelin-36 [9, 30]. The results of two studies indicated that apelin-13 and apelin-36 have protective effects on brain ischemia–reperfusion injury through activation of the phosphatidylinositol 3-kinase/AKT signaling pathway [23, 31]. However, the functional role of apelin-17 in nervous system disease remains unclear. The present study provided evidence that apelin-17 was increased in the plasma of ischemic stroke patients with good collateral circulation compared with that in ischemic stroke patients having poor collateral circulation. In animal experiments examining the mechanism underpinning this effect, we demonstrated that apelin-17 markedly attenuated agonist-induced the rat basilar artery contraction in an endothelium-dependent manner. Therefore, we speculated that apelin-17 may relax cerebral blood vessels to enhance the blood supply, thus having a protective effect in ischemic stroke patients with good collateral circulation. To test this hypothesis, we examined the effect of apelin-17 in rat brain ischemia–reperfusion injury in vivo using NMR, a powerful tool for accurately depicting cerebral blood supply in real time. Apelin-17 pretreatment significantly reduced the cerebral infarct volume in rats subjected to MCAO, a widely used animal model of ischemic stroke. Thus, these results indicated that an increased apelin-17 level in the plasma may relax cerebral arteries to attenuate cerebral ischemia–reperfusion injury, offering protection in patients with ischemic stroke.

Several apelins have shown potent effects in cardiovascular diseases, but the mechanisms underlying these effects are

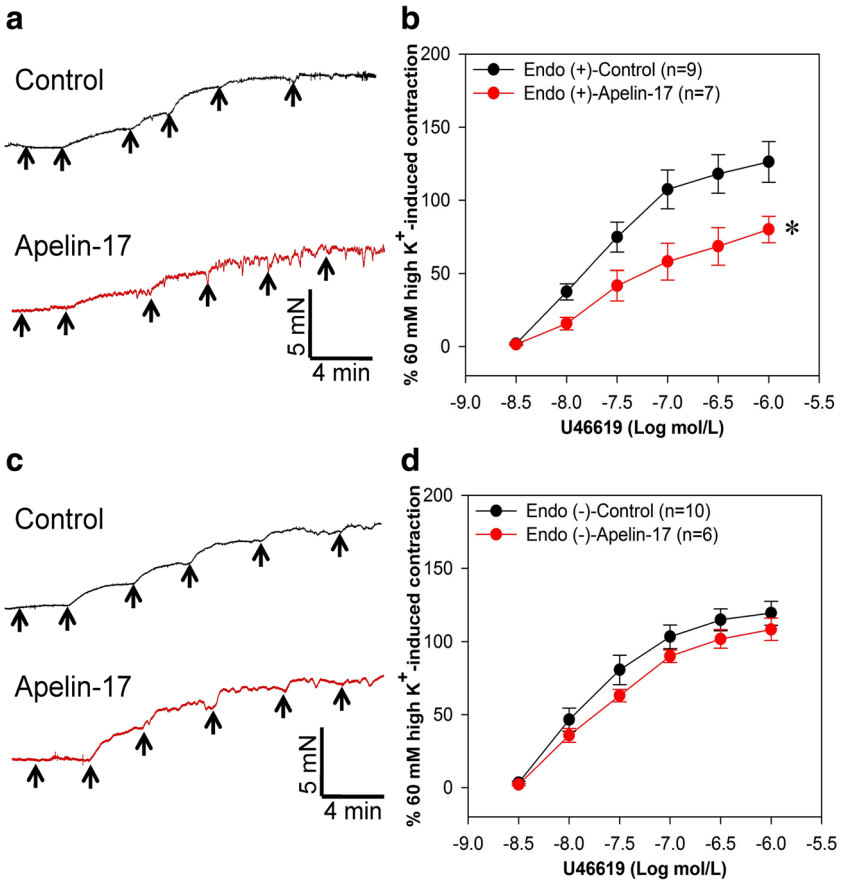

Fig. 3 Effect of apelin-17 on U46619-induced contraction of rat basilar artery. Representative traces (**a**, **c**) and summary data (**b**, **d**) showing the effect of apelin-17 (1 μmol/L) or saline (control) pretreatment for 20 min on the rat basilar artery contraction induced by U46619 (a thromboxane mimetic) with (**a**, **b**, Endo+) or without (**c**, **d**, Endo−) intact endothelium. Values are shown as the mean ± SEM ($n = 6$–10 each group). *$P < 0.05$, control vs. apelin-17 treatment

complex. Some studies, for example, indicate that apelin-12 and apelin-36 lower blood pressure or relax forearm resistance artery blood flow in a NO-dependent manner [19, 32]. However, another study reported that apelin-13 relaxes the mammary artery in an endothelium-dependent manner via enhancing prostacyclin production but not via NO in endothelial cells [28]. Therefore, not only do different apelins display various effects on blood vessel tension regulation but one type of apelin may also have different effects on various types of blood vessels. To the best of our knowledge, the functional role and underlying mechanism of apelin-17 in cerebral artery have not been previously characterized. The basilar artery is representative of cerebral arteries and one that could be practically investigated in vitro using the myography technique. In the present study, we found that apelin-17 attenuated the U46619-induced contraction of rat basilar artery in the

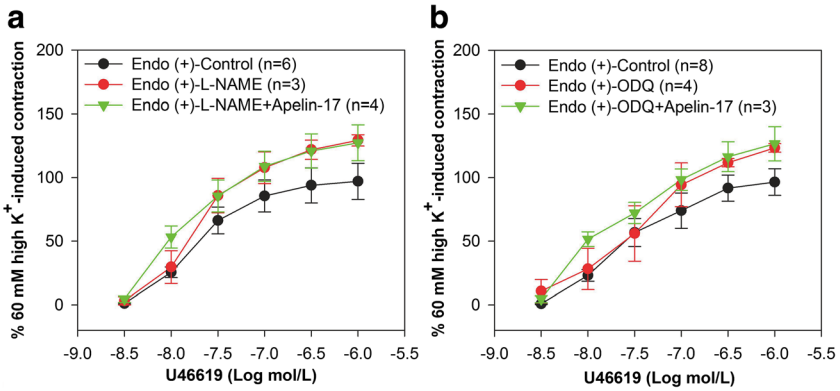

Fig. 4 Role of the nitric oxide (NO)–cGMP signaling pathway in apelin-17-attenuated contraction induced by U46619 in the rat basilar artery. Summary data showing the effect of L-NAME (an analogue of arginine, 50 μmol/L), **a** and ODQ (a guanylyl cyclase inhibitor, 1 μmol/L) **b**) on an apelin-17-attenuated contraction induced by U46619 in the rat basilar artery. After the vessels were pretreated with L-NAME or ODQ in the presence of apelin-17 or saline (control) for 20 min, increasing concentrations of U46619 were applied to induce vessel contractions. Values shown are the mean ± SEM ($n = 3$–8 per group)

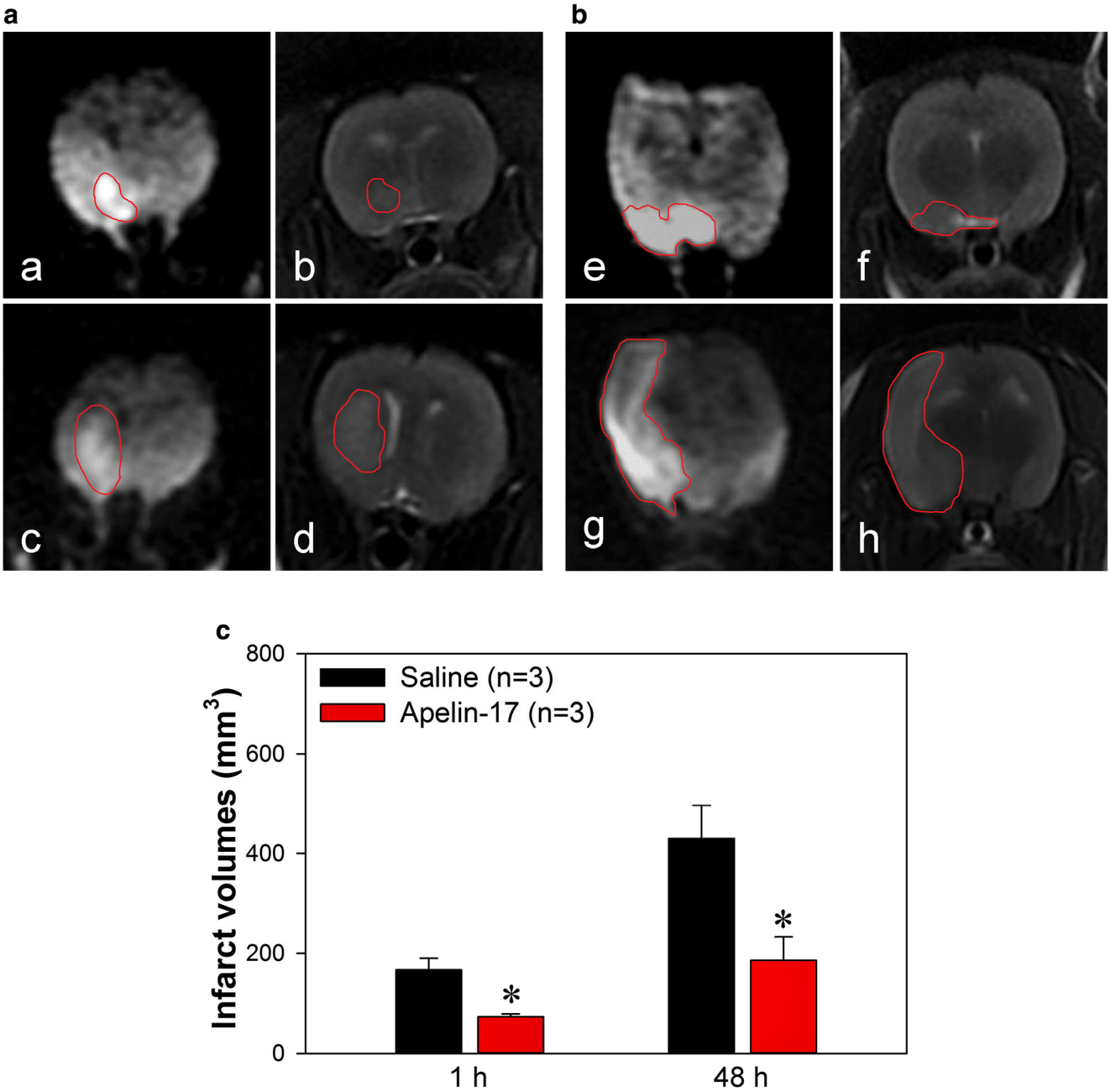

Fig. 5 Role of apelin-17 in cerebral infarction. Representative nuclear magnetic resonance images (**a**, **b**) and summary data (**c**) showing the effect of apelin-17 (150 μg/kg) or saline pretreatment 1 h before middle cerebral artery occlusion on cerebral infarct volume. Images were obtained 1h or 48 h (reperfusion) after middle cerebral artery occlusion for 2 h. **a** Apelin-17-treated group. (a) 1 h, Diffusion-weighted image (DWI); (b) 1 h, T2-weighted image; (c) 48 h, DWI; (d) 48 h, T2-weighted image. **b** Saline-treated group. (e) 1 h, DWI; (f) 1 h, T2-weighted image; (g) 48 h, DWI; (h) 48 h, T2-weighted image. The infarct zone was indicated by red line. Values are the mean ± SEM (n = 3 per group). *$P < 0.05$, apelin-17 vs. saline group

endothelium-intact vessel, but not in the endothelium-denuded vessel. To further investigate the underlying mechanism, we used L-NAME to inhibit NO production and found that L-NAME abolished apelin-17-induced basilar artery relaxation. In addition, we used ODQ, an inhibitor of guanylyl cyclase, to clarify the downstream actions of NO. Our results indicated that ODQ also markedly reduced apelin-17-induced basilar artery relaxation. Taken together, these results provided experimental evidence that apelin-17 has a protective effect against brain ischemia–reperfusion injury, as it relaxes cerebral arteries maybe through the NO–cGMP signaling pathway [33, 34]. Among apelin-17, apelin-36, and NO levels, plasma apelin-17 levels were highest in ischemic stroke patients with good collateral circulation. Apelin-17 may relax cerebral arteries via the NO–cGMP signaling pathway to increase the cerebral blood supply, leading to protection from

brain ischemia–reperfusion injury. Based on the results of the present study, we suggest that plasma apelin-17 levels may be useful as a biomarker for determining clinical prognosis in patients with ischemic stroke and for following the effects of clinical treatments in these patients.

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Compliance with Ethical Standards

Research Involving Animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Conflict of Interest The authors declare that they have no conflict of interest.

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