CEREBROPROTECTIVE EFFECT OF ANGIOTENSIN IV IN EXPERIMENTAL ISCHEMIC STROKE IN THE RAT MEDIATED BY AT_4 RECEPTORS

Physiologie et Pharmacologie Vasculaire et Rénale, Facultés de Médecine et de Pharmacie, Limoges, France - Département de radiologie, Hôpital Universitaire, Limoges

Recent studies have reported potential roles of angiotensins in an adaptative physiological mechanism of protection against cerebral ischemia-induced neurological damages. In the present study, we examined the protective role of angiotensin IV (AngIV) in a rat model of embolic stroke induced by intracarotid injection of calibrated microspheres (50 µm).

Internal carotid infusions of increasing doses of AngIV (0.01, 0.1 and 1 nmol/0.1 mL saline) dose dependently decreased mortality, neurological deficit and cerebral infarct size at 24 hours. With the highest dose of AngIV, mortality was reduced from 55 % in saline infused controls to 10 % (p=0.003), neurological deficit was reduced from 3.8 ± 0.3 to 1.4 ± 0.3 (p<0.0001) and cerebral infarct size at 24 hours was decreased from 432 ± 26 mm^3 to 185 ± 19 (p=0.0001). The AT_4 antagonist divalinal-AngIV (10^{-9} mol/0.1 mL), or pretreatment with L-NAME (10^{-7} mol/0.1 mL), both completely abolished the protective effect of AngIV (1 nmol). The AT_2 antagonist PD123319 (10^{-7} mol/0.1 mL) partially prevented the protective effect of AngIV on the neurological score. Sequential cerebral arteriographies revealed that AngIV induced a redistribution of blood flow to the ischemic areas within minutes. These results suggest that pharmacological doses of AngIV are protective against acute cerebral ischemia by triggering an AT_4-mediated, NO-dependent intracerebral hemodynamic mechanism.

**Keywords:** Angiotensin IV, AT_4 receptor, stroke, rat

INTRODUCTION

The pressor hormone angiotensin II (AngII) is the main effector peptide of the renin angiotensin system. In the circulation, AngII is rapidly cleaved to
angiotensin(2-8) (AngIII), which in turn is cleaved to angiotensin(3-8) (AngIV) by aminopeptidases. AngIV is the ligand of a specific binding site, termed \( \text{AT}_4 \) receptor, expressed in many tissues including the brain, the kidney, the heart and the vessels (1). The \( \text{AT}_4 \) receptor has recently been identified as an insulin-regulated aminopeptidase (IRAP) (2), a membrane anchored zinc-dependent metallopeptidase, originally identified in fat and muscle cells (3). In mammalian blood vessels, \( \text{AT}_4 \) receptor is present in both endothelial (4) and smooth muscle cells (5), strongly suggesting that AngIV subserves physiological functions in the regulation of vascular tone but the contribution of the \( \text{AT}_4 \) receptor to its vasoactive effects is unclear.

AngII, acting through its \( \text{AT}_1 \) receptor, regulates cerebral blood flow by stimulating cerebral vasoconstriction (6). With regard to the regulation of cerebral blood flow, AngII and AngIV have opposite effects: AngII reduces cerebral blood flow while AngIV increases it. In vivo, intra-arterial infusion of pharmacological doses of AngIV increases blood flow in the renal cortex (7, 8) and the brain (9, 10), implying a vasodilator effect, that was ascribed to \( \text{AT}_4 \) receptor since it was blocked by the specific AngIV antagonist divalinal-AngIV, but unaltered by \( \text{AT}_1 \) or \( \text{AT}_2 \) antagonists.

Several studies have reported potential roles of angiotensins in an adaptative physiological mechanism of protection against cerebral ischemia-induced neurological damages. A series of seminal basic research has demonstrated that the protective effects of AngII, independently of its blood pressure effect, in the gerbil, is mediated by non-\( \text{AT}_1 \) receptors (11-13). A growing body of evidence suggests that the type 2 receptor, \( \text{AT}_2 \), is involved in this paradoxical protective action of AngII (14-16). Iwai et al (17) reported that in \( \text{AT}_2 \)-null transgenic mice, permanent middle cerebral artery occlusion resulted in more severe infarct size than in the wild-type controls. Pretreatment with the \( \text{AT}_1 \) blocker valsartan at a non-hypotensive dose for ten days reduced the ischemic area and the neurological deficit, but this protective effect was weaker in \( \text{AT}_2 \)-null than in wild-type mice. This study further supports that the \( \text{AT}_2 \) receptor plays a pivotal role in mediating the AngII protective effects and that stimulation of unopposed \( \text{AT}_2 \) contributes to the beneficial effect of \( \text{AT}_1 \) blockers on ischemic brain lesions. However, that the protective effect of valsartan was only diminished but not completely blunted in \( \text{AT}_2 \)-null mice leaves open the possibility that along with \( \text{AT}_2 \), an additional non-\( \text{AT}_1 \) non-\( \text{AT}_2 \) receptor may be involved in the neuroprotective effect of AngII.

Since in the gerbil, the protective effect of AngII is associated with an increase in cerebral blood flow in the ischemic hemisphere (18), the vasodilatory action of AngIV in the cerebral vasculature raised the possibility that AngIV contributes to the cerebral protection in the setting of acute ischemia. The present study was conducted to examine this hypothesis and to clarify the implication of the \( \text{AT}_4 \)/IRAP receptor.
MATERIALS AND METHODS

The study was conducted on male Sprague-Dawley rats weighing 200-250 g (Depre, Saint-Doulchard, France) housed in temperature and humidity-controlled room. Surgical procedure was performed after anaesthesia with thiopenthal (50 mg/kg i.p.). Experimental procedures were in accordance with the guidelines for ethical care of experimental animals of the European Community and were approved by the French Agriculture Ministery (authorization n°B-00889).

AT receptors mRNA measurements by RT–PCR

Total RNA was extracted from biological samples using a RNeasy Mini Kit. 20 basilar arteries were pooled and they were first lysed and homogenized in the presence of a highly denaturating guanidine isothiocyanate-containing buffer, which immediately inactivated RNases. Ethanol was added to provide appropriate binding conditions on a silica-gel-based membrane column. Contaminants were efficiently washed away and high quality RNA was then eluted in 30 µL of water.

RT was performed for 1 hour at 37 °C using 2 µg of total RNA and in buffer containing 100 pmoles of hexamers, 20 U of RNase inhibitor, 50 nmoles of dNTP, 200 nmoles of 1,4-dithiothreitol (DTT) and 20 U of MMLuV-Rtase, in a total volume of 20 µL. 2 RT were performed for each RNA extraction. A negative RT without RNA was performed as negative control. PCR were performed in a MgCl₂ buffer with 1 U of Taq polymerase, 1.875 nmoles of dNTP, 3/20 of the RT mixture and 10 pmoles of each primer. The primers used were (5’-CGT CAT CCA TGA CTG TAA AAT TTC) and (5’-GGG CAT TAC ATT GCC AGT GTG) for AT₁A R, (5’-CAT TAT CCG TGA CTG TGA AAT TG) and (5’-GCT GCT T AG CCC AAA TGG TCC) for AT₁B R, (5’-GGA GCG AGC ACA GAA TTG AAA GC) and (5’-TGC CCA GAG AGG AAG GGT TGC) for AT₂ R (19) and (5’-GTC TTG GTG AGC ATG AGA TGG) and (5’-CTA AGG TCC TGG CAG AGG GTA) for IRAP (20). The final predicted sizes of the RT-PCR products for AT₁A R, AT₁B R, AT₂ R and IRAP were respectively 306, 344, 446 and 165 bp. The thermal cycling conditions included 5 min at 94 °C, followed by 35 cycles at 94 °C for 1 minute, 64 °C for 1 minute (60 °C for IRAP), 72 °C for 1 minute and at the end 72 °C for 7 minutes (19), then held at 4 °C with the help of a thermocycler TECHGENE. The negative RT was used as negative control for each gene expression. 5 µL of the PCR products were separated on ethidium bromide-stained 1 % agarose gel. A molecular size marker was run in parallel. Bands were visualized by UV transillumination and digitally photographed with a digital CDD camera (DC 290, Kodak).

Cytosol and membrane preparation

Basilar arteries were removed and perfused for 15 minutes in Krebs solution. Then, arteries were infused or not with insulin (0.4 mU/mL) and disrupted with a Polytron (UltraTurrax T8, IKA Labortechnik) at low speed for 60 seconds into homogenization buffer (20 mM Mops, pH 7.2, 0.25 M sucrose, 1 mM DTT, with protease inhibitors (1 mM EGTA, 100 µM leupeptin, 1 mM PMSF and 1 µM pepstatin) and phosphatase inhibitors (1 mM sodium orthovanadate and 200 µM sodium pyrophosphate) (21). Tissue homogenate was centrifuged at 800 g in a Beckman TL100 ultracentrifuge for 10 minutes at 4 °C to obtain the low-speed pellet which contained nuclei and unhomogenized tissue. The supernatant was re-centrifuged at 100000 g at 4 °C for 60 minutes to yield the membrane pellet and cytoplasmic supernatant. Each pellet was washed twice with TBS and incubated in lysis buffer (50 mmol/L Tris-HCl pH 8.0, 150 mmol/L NaCl, 0.02 % sodium azide, 100 µg/mL phenylmethylsulfonyl fluoride, 1 µg/mL aprotinin, and 1 % Triton X-100).
**SDS/PAGE and Western analysis**

Cytosolic or membranes proteins (40 µg) were separated by 10 % SDS/PAGE and transferred to nitrocellulose by electoblotting for the Western blotting analysis of IRAP. The blots were washed for 1 hour in blocking buffer (TBS pH 7.6, 8 g/L non fat dried milk, 0.05 % Tween 20) at room temperature and then incubated with anti-IRAP rabbit polyclonal antibody (Alpha Diagnostic International, San Antonio, USA) (3) diluted in 200 volumes TBS (10 mmol/L Tris-HCl pH 8.0, 150 mmol/L NaCl, et 0.05 % Tween 20) overnight at 4 °C. The blots were washed thoroughly and incubated for 1 hour with peroxidase conjugated goat anti-rabbit IgG (BioRad Laboratories) diluted in 3000 volumes TBS with 15 % non fat dried milk at room temperature. Immunostained proteins were then washed 5 times with TBS for 5 minutes, and visualized using the ECL detection (ECL reaction, Amersham). Bands were digitally photographed with a digital CDD camera (DC 290, Kodak) and semi-quantified with a Kodak 1D software (version 3.5).

**Experimental procedure: embolic stroke model.**

The experimental model of embolic stroke was adapted from Bralet et al (22). The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed through a ventral midline incision in the neck. The ECA was ligated with a 5-0 suture. A 0.15 mL suspension of calibrated polystyrene microspheres (diameter, 50.0 µm; 15 000/mL of suspension, Distrilab, Netherlands) in isotonic saline solution was injected into the ICA through PE-10 polyethylene tubing previously inserted into the CCA and was flushed with 0.2 mL of saline. During the injection, the proximal portion of the CCA was occluded temporarily by a clamp. After the injection, the tubing was removed, and the CCA was permanently ligated with the 3-0 suture. Body temperature was monitored by a rectal probe and maintained at 37.0 ± 0.5 °C using a homeothermic blanket control unit (Harvard Apparatus, South Natick, MA, USA).

**Experimental procedure: pharmacological effect of AngIV**

Rats were randomly assigned to 12 groups of 20 each. In a sham-operated control group (group 1), the same volume of vehicle (saline without microspheres) was injected. Members of group 2 received 0.1 mL saline injected in the CCA 10 minutes after the injection of microspheres and served as controls. Animals of groups 3-5 received AngIV (respectively 10^{-11}, 10^{-10} and 10^{-9} mol/0.1 mL saline) (Neosystem, Strasbourg, France) injected in the CCA 10 minutes after the injection of microspheres. Animals of group 6 received a delayed injection of AngIV (10^{-9} mol/0.1 mL saline) one hour after the microspheres. Anaesthesia was maintained in this group by supplemental injection of thiopental (5 mg/kg at 30 minutes intervals). In animals in groups 7 and 9, and 8 and 10 respectively, the specific AT_{1} or AT_{2} antagonists (PD123319 10^{-7} mol/0.1mL, Sigma Aldrich, St-Louis, USA) or divalinal-AngIV (10^{-9} mol/0.1mL, Pacific Northwest Biotech, USA) were administered along with the microspheres. After 10 minutes, 0.1 mL saline (groups 7 and 8) or 10^{-9}mol/0.1mL AngIV (groups 9 and 10) was injected in the CCA. Animals in groups 11 and 12 were pretreated with L-NAME (10^{-7} mol/0.1mL saline), a nitric oxide (NO) synthesis inhibitor, prior to the surgical procedures, and were treated either with saline or AngIV ten minutes after the injection of microspheres.

**Blood pressure monitoring**

In a subset of animals, PE-50 polyethylene tubing was inserted into the left femoral artery for monitoring blood pressure and for measuring arterial pH, PaCO_{2}, and PaO_{2} before and 15 minutes after microsphere injection.
Neurological deficits

Neurological evaluation was performed 24 hours after the induction of ischemia and scored on a 6-point scale: 0 = no neurological deficit, 1 = failure to extend left forepaw fully, 2 = circling to the left, 3 = falling to the left, 4 = no spontaneous walking with a depressed level of consciousness, and 5 = death (23).

Infarct volume

Twenty four hours after surgery, the animals were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg i.p.) and decapitated. The brains were quickly removed and sectioned coronally into 8 slices, each with a 2 mm thickness. The brain slices were incubated for 30 minutes in a 2 % solution of 2,3,4-triphenyltetrazolium chloride (Sigma, Saint-Louis, USA) at 37 °C and fixed by immersion in 10 % buffered formalin solution. The 8 brain sections were photographed with a coupled CCD camera (ECD-1000HR computer camera, ELECTRIM Corp). With the use of an image-analysis program (Perfect Image), the unstained areas of both hemispheres were calculated for each slice. The infarct volume was then calculated, without correction for brain oedema, by multiplying the area by the slice thickness and summing the volumes from all slices. Measurements of infarct volume were done without knowledge of group assignment.

Cerebral arteriographies

A cerebral angiography was performed in 4 rats. The abdominal aorta was exposed and punctured with a 22-Gauge needle. A standard microcatheter and microguidewire (Excel 14 and Transsend 14, Boston Sc, Fremont, USA) were introduced and navigated in one CCA up to the carotid bifurcation. In order to obtain a selective injection of the calibrated microspheres in the ICA, the microguidewire was used to achieve vasospasm on the branches of the ECA. At this point, 0.15 mL of the solution containing the microspheres was injected, followed after 5 minutes by injection of AngIV (10^{-9} mol/0.1 mL). Selective angiograms with anterior-posterior and lateral projections were achieved before and after vasospasm, after injection of microspheres, after a control injection of saline, and at 1, 5, and 10 minutes after injection of AngIV.

Statistical analysis

The values presented in this study are mean ± SEM. A statistical analysis was performed by 2-way ANOVA followed by post hoc analysis to detect differences between the groups for the physiological variables, the neurological scores and infarction volumes. A Kaplan-Meyer analysis followed by a log-rank test was used for comparison of mortality. Results were considered significantly different at values of p<0.05.

RESULTS

Expression of angiotensin receptors in the rat basilar artery

AT_{1A}R, AT_{1B}R, AT_{2}R and AT_{4} (IRAP) mRNAs were amplified from rat basilar artery total mRNA in 2 different extractions, yielding PCR products of the expected size (Figure 1A). No amplification was found with negative RT for all couples of primers. Two RT were performed for each RNA extraction (not shown).
Figure 1. A: RT-PCR analysis of AT\textsubscript{1A} R, AT\textsubscript{1B} R, AT\textsubscript{2} R and IRAP in the rat basilar artery. RT-PCR products are shown in ethidium bromide-stained agarose gel. A negative RT (RT-) was used as negative control for each gene expression. A molecular size marker was run in parallel (at left). Sizes of the RT-PCR products for AT\textsubscript{1A} R, AT\textsubscript{1B} R, AT\textsubscript{2} R and IRAP were the same as due.

B: Western blot analysis of IRAP
Western blot of membranes (Mb) and cytosolic (Cyt) fractions prepared from basal or insulin (0.4 mU/mL) – treated basilar arteries detected with rabbit anti-IRAP antibody.

C: Semi quantitative evaluation of IRAP
Semi quantitative evaluation of IRAP in the membrane and cytosolic fractions in basal conditions or after treatment with insulin (0.4 mU/mL). The same amount of total proteins was deposed in each well (not shown). Results shown are mean ± SEM for 4 experiments.
Western blot analysis

A major band of 165 kDa (size of IRAP) was visualized both in membranes and cytosolic fractions (Figure 1B). The specificity of the antibody for IRAP was confirmed by the capacity of IRAP to translocate to the membrane in response to insulin (0.4 mU/mL) (Figure 1C).

Effect of exogenous AngIV

In the sham-operated group, animals injected with saline without microspheres all survived for 24 hours and no neurological deficits were observed. By 24 hours after microspheres injection, triphenyltetrazolium chloride–stained brain slices demonstrated patchy, unstained areas of infarcted tissue in the region supplied by the ipsilateral middle cerebral artery (MCA) and anterior cerebral artery, but smaller infarcted areas were also present in the contralateral hemisphere in one third of the rats. The volume of infarction was 29 ± 12 mm³ in the sham-operated animals, and 432 ± 26 mm³ in the control group (microspheres).

Intracarotidian injection of increasing doses of AngIV progressively decreased mortality from 55 % in saline injected controls to respectively 50 %, 35 % and 10 % with 0.01, 0.1 and 1 nmol AngIV, but the beneficial effect of AngIV on survival was only statistically significant with the highest dose (Figure 2A). Consistent with a dose-dependent protective effect of AngIV, increasing doses of AngIV similarly decrease the neurological deficit score from 3.80 ± 0.35 in saline injected controls to respectively 3.40 ± 0.40 with 0.01 nmol AngIV, to

![Graphs showing the effect of AngIV on mortality, neurological deficit, and infarct volume](image-url)

**Figure 2.** Effect of increasing doses of AngIV on mortality, neurological deficit and infarct volume 24 hours after embolic stroke.

A) Ang IV progressively increased survival rate, but the beneficial effect was only significant with the highest dose: *: p=0.003 AngIV 1 nmol versus controls. B) neurological score *: p=0.03 for 0.1 nmol AngIV versus controls; **: p=0.0001 for 1 nmol Ang IV versus controls. C) infarct volume *: p=0.06 for 0.1 nmol AngIV versus controls; **: p<0.0001 for 1 nmol Ang IV versus controls. Data are mean ± SEM, n=20 rats/group.
2.45 ± 0.50 with 0.1 nmol AngIV (p=0.03 versus controls), and to 1.40 ± 0.34 with 1 nmol AngIV (p=0.0001 versus controls) (Figure 2B). The lowest dose of AngIV (0.01 nmol) had no detectable effect on infarct volume (418 ± 35 mm³ versus 432 ± 26 for controls) (Figure 2C), but 0.1 nmol AngIV reduced infarct volume to a border line significant level (351 ± 35 mm³, p=0.06 versus controls), whereas the highest dose of AngIV (1 nmol) further reduced infarct volume to 185 ±19 mm³, p<0.0001 versus controls).

A significant positive correlation was observed between the volumes of infarction and the neurological deficits (Y = 67.1 X + 165.5, r² = 0.623, p<0.0001) (not shown). The protective effect of AngIV was not associated with any changes in physiological parameters: no significant differences were detected in rectal temperature, mean arterial blood pressure, arterial pH, PaCO₂, and PaO₂ 10 minutes after injection of AngIV 1 nmol or vehicle (Table 1). Delayed administration of AngIV (1 nmol) one hour after the injection of microspheres had no protective effect (not shown).

The specific AT₄ antagonist divalinal-AngIV alone had no effect, but completely abolished the protective effect of 1 nmol AngIV on mortality and neurological deficit (Figures 3A & B). The specific AT₂ antagonist PD123319 partially prevented the protective effect of 1 nmol AngIV: mortality (35 %) was intermediate, and was neither significantly different from controls nor from AngIV treated rats, and neurological score (2.55 ± 0.48) was significantly different from controls (p<0.02) and from AngIV treated rats (p<0.03).

In microspheres-injected rats pre-treated with L-NAME, there was a non-significant increase in mortality and neurological deficit (70 % and 4.15 ± 0.36, respectively, ns versus controls). Pre-treatment with L-NAME completely abolished the protective effect of 1 nmol AngIV on mortality (75 %) and neurological deficit (4.15 ± 0.37), (p<0.0001) (Figures 3A & B).

**Mechanism of the protective effect of AngIV**

Representative cerebral angiographies performed in four different rats before, after injection of microspheres, and following injection of 1 nmol AngIV are

| Table 1: Effect of AngIV infusion on physiological parameters. |
|-------------------|-----------------|--------|
| Microspheres       | AngIV           | t-test |
| Body temperature (°C) | 36.2 ± 0.6      | 36.2 ± 0.6 | ns |
| PaCO₂ (mm Hg)      | 44.7 ± 2        | 41.3 ± 3  | ns |
| PaO₂ (mm Hg)       | 72.3 ± 10       | 82.3 ± 7  | ns |
| Arterial pH         | 7.41 ± 0.2      | 7.43 ± 0.2 | ns |
| Mean Arterial Blood Pressure (mm Hg) | 109 ± 3        | 109 ± 5  | ns |

Results are mean ± SEM, n = 4. Administration of AngIV in the carotid artery after the injection of microspheres had no effect on body temperature, arterial blood gasometry, pH and mean arterial blood pressure.
shown in Figure 4. Injection of microspheres induced a marked reduction of cerebral perfusion with areas in which distal vessels disappeared. Injection of saline had no effect (not shown), and AngIV consistently induced in the four rats examined a marked vasodilatation of various branches of the external carotid artery and the internal carotid artery within 10 minutes. Arteries that could not be seen on the initial angiogram were visualized after injection of AngIV, and contributed to the rapid reperfusion of the ischemic areas.

**DISCUSSION**

These data add to the handful of recent studies indicating that angiotensin peptides are cerebroprotective in the setting of acute brain ischemia, and points for the first time to AngIV as to one possible effector of this cerebral protection through its specific binding site AT$_4$. Using large, non-physiological doses of AngIV, we found that a single bolus injection in the carotid artery following the microembolic stroke did not change systemic blood pressure, but dose dependently reduced mortality, neurological deficit and brain infarct size. The largest dose of AngIV studied (1 nmol) decreased by more than two fold brain infarct volume, resulting in a marked decrease in mortality from 53 to 10 % at 24 hours, despite the likelihood of its rapid cerebral circulation wash-out and the short systemic half-life of this 6-
Selective cerebral angiograms were obtained in 4 rats before (Basal), after injection of microspheres (MS) and at 10 minutes after injection of AngIV. Sequential representative angiograms with anterior-posterior (Lane 1) or lateral projections (Lanes 2, 3 & 4) are shown. Injection of microspheres was followed by disappearance of parenchymal perfusion in patchy brain areas, whereas reflux contrast enhanced permeant vessels. Following injection of AngIV, a progressive vasodilation was observed within 5 to 10 minutes on various branches of the external carotid artery and the internal carotid artery. Arteries that could not be seen on the initial angiogram were visualized after injection of AngIV, resulting in restoration of blood flow supply to the ischemic areas.
aminoacid peptide. The specific AngIV antagonist divalinal-AngIV blunted this protection. The reported specificity of divalinal-angIV to the AT4 receptor/IRAP in numerous tissues (24, 25) and our results of RT-PCR and Western blot analysis showing in rat basilar artery the presence of respectively, AT4 mRNA and IRAP protein, reinforce our hypothesis of a specific AT4/AngIV protective effect. Pretreatment with L-NAME, an inhibitor of nitric oxide synthases, abolished this protective effect suggesting that Ang-IV triggers a nitric oxide-dependent mechanism. Sequential arteriographies confirmed that the protective effect of AngIV was due to a vasodilating effect, with the opening of pre-existing collateral vessels resulting in an immediate redistribution of blood flow towards ischemic areas. Consistent with this mechanism, delayed administration of AngIV one hour after the embolic stroke was devoid of any protective effect. Our findings are fully consistent with the report by Kramar et al (9, 10) that pharmacological doses of Ang-IV elicits a nitric oxide-dependent increase in cerebral blood flow specifically inhibited by divalinal in the rat.

The mechanism by which AT4/IRAP stimulation induces cerebrovascular dilation is not known. IRAP, present in intracellular vesicles together with the glucose transporter Glut4, translocates to the plasma membrane in response to endogenous products such as insulin, oxytocin or ET-1 (26, 27). Although the in vivo substrates of IRAP remain to be identified, IRAP cleaves and inactivates in vitro a number of peptides such as arginine-vasopressin, met- and leu-enkephalin, lys-bradykinin and somatostatin (28-30). There is no evidence to date that IRAP is able to transduce classical intracellular cascades of second messengers (31). Aminopeptidase activity towards extracellular peptides increases in insulin- and oxytocin–treated cells, suggesting that one physiological role of AT4/IRAP could be to counter-regulate the actions of circulating or locally released peptides (26). As recent reports have shown that the AT4/IRAP endogenous ligand AngIV inhibits its enzymatic activity, it has been suggested that the action of AngIV could be to prolong the effects of extracellular signaling peptides by slowing their degradation (32). In accordance with these studies, our results suggest that AngIV binding to AT4/IRAP could interfere with certain peptides, in turn responsible for endothelial nitric oxide release and, finally, the observed dilation. Thus, further investigations will be required to establish the precise mechanism of the AngIV dilation.

Whatever this mechanism, in the present study we demonstrate that internal carotid infusion of pharmacological doses of exogenous AngIV dose dependently reduces mortality, neurological deficit and brain infarct size in a rat model of acute embolic stroke, and that this protective effect, mediated by in part the AT4 receptor, seems to be the consequence of the opening of collateral vessels resulting in rapid reperfusion of the ischemic areas.

Although consistent with the hypothesis that AngIV might contribute to the endogenous physiological protective mechanism against acute cerebral ischemia by specifically increasing the blood flow increase to the ischemic area, the very
large dose of AngIV, directly administered in the cerebral circulation, preclude any firm conclusion regarding the physiological relevance of this pharmacological effect. Furthermore, the AngIV-administration one hour after the injection of microspheres had no protective effects showing that a therapeutic use of AngIV is unlikely. However, the observation during the sequential cerebral arteriographies that AngIV removed the vasospasm within 10 minutes might open the way to a clinical application to prevent the catheter-induced vasospasm during interventional neuroradiology.

Acknowledgments: This work was supported by a grant of the Fondation pour la Recherche Médicale, of the Conseil Régional du Limousin, of the Société Française d’Hypertension Artérielle, and of the Club des Jeunes Hypertensiologues.

REFERENCES


Received: February 9, 2006
Accepted: July 31, 2006

Author’s address: Prof Nicole Oudart, Department of Physiology and Pharmacology (EA3841) Faculté de Pharmacie; Université de Limoges, 2 rue du Docteur Marcland, 87025 Limoges (France). Phone: +33 555 43 58 83, Fax: +33 555 43 59 12. E-mail: nicole.oudart@unilim.fr