Evidence that the vasodilator angiotensin-(1–7)-Mas axis plays an important role in erectile function

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doa Costa Gonçalves AC, Leite R, Fraga-Silva RA, Pinheiro SV, Reis AB, Reis FM, Touyz RM, Webb RC, Alenina N, Bader M, Santos RA. Evidence that the vasodilator angiotensin-(1–7)-Mas axis plays an important role in erectile function. Am J Physiol Heart Circ Physiol 293: H2588–H2596, 2007. First published July 6, 2007; doi:10.1152/ajpheart.00173.2007.—The vasodilator/angiotensin-(1–7) (ANG-(1–7)) receptor blocker A-779 and the antiproliferative peptide angiotensin-(1–7) [ANG-(1–7)] is released into the corpus cavernosum sinuses, but its role in erectile function has yet to be defined. In this study, we sought to determine whether ANG-(1–7) and its receptor Mas play a role in erectile function. The ANG-(1–7) receptor Mas was immunolocalized in rat corpus cavernosum by confocal microscopy. Infusion of ANG-(1–7) into corpus cavernosum at a rate of 15.5 pmol·kg−1·min−1 potentiated the elevation of the corpus cavernosum pressure induced by electrical stimulation of the major pelvic ganglion (MPG) in rats. The facilitatory effect of ANG-(1–7) was completely blunted by the specific ANG-(1–7) receptor blocker A-779 and Nα-nitro-L-arginine methyl ester. Nitric oxide (NO) release in the corpus cavernosum was evaluated with the fluorescent dye 4-amino-5 methylamino-2′,7′-difluorofluorescin diacetate. Electrical stimulated-release of NO in rat corpus cavernosum was potentiated by ANG-(1–7). Furthermore, incubation of rat and mouse corpus cavernosum strips with ANG-(1–7) at 10 nmol/l resulted in an increase of NO release. This effect was completely abolished in mas-deficient mice. More importantly, genetic deletion of Mas resulted in compromised erectile function as demonstrated by penile fibrosis and severely depressed response to electrical stimulation of the MPG. Furthermore, the attenuated erectile function of DOCA-salt hypertensive rats was fully restored by ANG-(1–7) administration. Together these data provide strong evidence for a key role of the ANG-(1–7)-Mas axis in erectile function.

THE NITRIC OXIDE (NO)/cGMP pathway is considered the most important intracellular mechanism responsible for smooth muscle relaxation leading to erection (16, 17). In the absence of an activated NO/cGMP pathway, the cavernosal smooth muscle cells remain in the contracted state. Adrenergic nerves are mainly responsible for the detumescence of the erect penis and also contribute to the maintenance of the flaccid state through the release of norepinephrine. Other vasoconstrictors such as endothelin-1 and angiotensin II (ANG II) may additionally be involved in the detumescence process (3, 7, 34).

There is evidence that a local renin-angiotensin system (RAS) exists within the corpus cavernosum (3) and that ANG II, which is produced and secreted into the corpus cavernosum in physiological amounts, limits erection (19). Indeed, drugs that block either the action or synthesis of ANG II apparently are not associated with erectile dysfunction, a side effect variably observed with other classes of antihypertensive agents. On the contrary, there is some evidence suggesting that this class of drugs could even facilitate penile erection (11, 14, 24).

It is now generally accepted that ANG II is not the only active peptide of the RAS. Other members, including ANG-(2–8), ANG-(3–8) and ANG-(1–7) may also mediate the actions of this system. It is becoming increasingly evident that the renin-angiotensin system comprises two major arms: a vasoconstrictor/proliferative arm in which the main mediator is ANG II acting on angiotensin AT1 receptors, and a vasodilator/antiproliferative arm in which the major effector is ANG-(1–7) acting via the G protein-coupled receptor Mas (29). RAS peptides, including the vasodilator ANG-(1–7), are present in the human corpus cavernosum (19). Because of its vasodilatory properties, we hypothesized that the ANG-(1–7)-Mas axis plays an important role in penile erection. Here we document the presence of Mas in rat corpus cavernosum, and the effect of its stimulation by ANG-(1–7) on NO release in rat and mouse corpus cavernosum. Furthermore, we explored the in vivo relevance of the ANG-(1–7)-Mas axis in penile erection using Mas knockout mice (Mas−/−) and using normotensive and DOCA-salt hypertensive rats.

METHODS

Animals

Male Wistar rats (12–14 wk, 220–250 g body wt), FVB/N male mice (12–15 wk, 25–30 g body wt), Mas knockout (Mas−/−; 12–15 wk) male mice on the pure genetic background C57BL/6, and wild-type C57BL/6 control mice (Mas+/+) bred at the animal facility of the Biological Science Institute (CEBIO, Federal University of Minas Gerais, Minas Gerais, Brazil) were used in this study. The animals were divided into two major groups: animals used for I) in vivo and
in vitro experiments. They were allowed free access to standard chow and drinking water, and they were kept on a 12:12-h light-dark cycle. Experimental protocols used in this study were approved by the Animal Care Committee of Federal University of Minas Gerais (Minas Gerais, Brazil).

**DOCA-Salt Hypertension**

Male Wistar rats (130–150 g) were uninephrectomized and implanted with a subcutaneous silicone pellet containing DOCA (200 mg/kg body wt in silicone rubber) under trichloroethanol anesthesia as described previously (1). Animals were maintained on drinking water containing 1.0% NaCl and 0.2% KCl for 4 wk. Control animals were uninephrectomized and had free access to drink water. Systolic blood pressure was measured using standard tail-cuff procedures.

**In Vivo Experiments**

**Surgical procedure.** Normotensive and DOCA-salt hypertensive rats and Mas−/− and Mas+/+ mice were anesthetized with intraperitoneal urethane (1,400 mg/kg). The rat left femoral artery and mouse carotid artery were cannulated for continuous monitoring of mean arterial pressure (MAP). The shaft of the penis was freed of skin and fascia, and the right corpus cavernosum was cannulated by insertion of a 30-gauge needle connected to a pressure transducer, permitting continuous monitoring of corpus cavernosum pressure (CCP) as described elsewhere (22). The rat left corpus cavernosum was cannulated with a 30-gauge needle attached to a 10-μl syringe via a short length of polyethylene (PE)-10 tubing to administer vasoactive drugs. The abdominal cavity was opened, the right major pelvic ganglion (MPG) was exposed, and silver bipolar electrodes were properly positioned for electrical stimulation. The MPG was stimulated with a range of voltages (0.5, 0.75, 1.0, 1.2, 1.5, 2.0, 2.5, and 3.0 V, pulse duration of 5 ms at a frequency of 12 Hz) for 30-s duration and 1-min interval between stimulation periods.

**Effect of ANG-(1–7) on erectile response.** To examine the effect of ANG-(1–7) on ganglionic-stimulated changes in the ratio of CCP/MAP in rats, the MPG was stimulated with a range of voltages as the control stimulation. Ten minutes were allowed for the recovery of normal erectile function of the animals. Thereafter, intracavernosal infusion of ANG-(1–7) at a dose of 1.5, 15.5, and 150 pmol kg−1 min−1 or vehicle (both at a rate of 0.5 μl/min) was performed. After 3 min of infusion, ganglionic stimulation was repeated. Subsequent experimental trials were performed using the intermediate dose of ANG-(1–7), 15.5 pmol kg−1 min−1.

**Effect of A-779 on the action of ANG-(1–7).** To examine the effect of Mas antagonism in the facilitatory effect of ANG-(1–7) on the ganglionic-induced CCP/MAP increase in rats, 10 min after the initial measurements of CCP and MAP were taken, the ANG-(1–7) antagonist A-779 (26) at 155 pmol kg−1 min−1 and ANG-(1–7) at 15.5 pmol kg−1 min−1 were simultaneously administered, and 3 min later ganglionic stimulation was repeated.

To evaluate the possible role of ANG-(1–7) released endogenously, using a subset of animals we first determined the minimal stimulus at which the maximal value of the CCP/MAP was achieved (such stimulus is particular for each animal). To determine the submaximal stimulus, ganglionic stimulation was performed as described above. Afterward, CCP/MAP values were analyzed. The minimal voltage that evoked the maximal value of CCP/MAP was selected as the submaximal stimulus. MPG was stimulated using this voltage during 2 min (control). Ten minutes after control stimulation, A-779 (155 pmol kg−1 min−1) or vehicle (saline) was infused into cavernosal sinuses, and after 3 min of infusion the stimulation was repeated.

**Role of NO in the effect of ANG-(1–7) on the ganglionic-stimulated CCP/MAP increase in rats.** The role of NO in the ganglionic-stimulated CCP/MAP was evaluated using the NO synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). A submaximal stimulus was determined as described previously and applied to the MPG for 2 min duration. Ten minutes were allowed for the recovery of normal function of the animals. Subsequently, L-NAME (200 μg/kg) was intracavernously injected, and after 10 min the ganglionic stimulation was repeated. After 10 min of recovery, ANG-(1–7) (15.5 pmol kg−1 min−1) or vehicle was infused. Three minutes after the beginning of infusion, the submaximal stimulus was applied again while the CCP and MAP were continuously recorded.

In one group of animals (n = 7), ANG-(1–7) was infused into the cavernous sinuses to determine its ability in potentiate the erectile response induced by submaximal ganglionic stimulation.

**In Vitro Experiments**

**Antiserum.** Polyclonal antiseras directed against Mas, were produced in Mas knockout mice using an antigen, a 12-amino acid peptide (LAEKKAMNTSSR) corresponding to the NH2-terminal domain of the mouse Mas protein. This sequence has 100% homology with mouse and 91.6% homology with rat Mas (see: fasta protein database at www.ebi.ac.uk/fasta33). This antibody was used in a recent study (26) in which an anti-human Mas commercially available antibody was also used for comparison.

**Mas localization.** Rats were euthanized with an overdose of tribromoethanol and were perfused transcardially with PBS (0.02 M, pH 7.4) for 2 min followed by 4% paraformaldehyde in PBS (0.02 M, pH 7.4) for 10 min. Thereafter, the corpus cavernosum were excised (8), cut into strips, and placed in 4% paraformaldehyde for 2 h. Subsequently, 10-μm adjacent tissue sections were made using a cryostat (6 sections per sample). The sections were then incubated in PBS, 0.2% (wt/vol) Tween, and 5% BSA, each for 15 min, and then sequentially incubated with either primary antibody (1:500 dilution) or control solution [containing of primary antibody (1:500 dilution) preincubated for 24 h at 4°C with 50 μg of blocking peptide corresponding to the NH2-terminus of the Mas protein] for 24 h at 4°C. After incubation, the sections were washed in PBS (3 times for 5 min) and subsequently incubated with a secondary antibody (1:400 dilution, Alexa 594, Molecular Probes) for 2 h at ambient temperature. The reaction was stopped by washing with 50 mmol/l Tris buffer, and the tissues were mounted on chrome-alum-coated slides, air dried, and covered with glycerol-Tris mounting media and coverslips. Fluorescent images were obtained using a Zeiss 510 meta laser scanning confocal microscope equipped with an oil-immersion objective lens (×63).

**4-Amino-5-methylamino-2′,7′-difluorofluorescein diacetate NO analysis.** Mouse (Mas+/+ and Mas−/−) corpus cavernous som strips were loaded with 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate (DAF-FM) or DAF-FM plus ANG-(1–7) for 10 min at ambient temperature (DAF-FM: 1.0 μM and ANG-(1–7): 10 nM). Subsequently, the strips were washed in PBS (3 times for 5 min) and frozen overnight at −80°C. Fluorescent images were obtained as previously described.

To evaluate NO release in vivo during MPG stimulation, rats were divided into four distinct groups: 1 control group, 2 ANG-(1–7), 3 electrical stimulation, and 4 electrical stimulation + ANG-(1–7). The shift of the penis was freed of skin and fascia, and the right corpus cavernosum was cannulated by insertion of a 30-gauge needle attached to a 10-μl syringe via a short length of PE-10 tubing used for infusion of 1.0 μM DAF-FM or 1.0 μM DAF-FM plus 15.5 pmol kg−1 min−1 of ANG-(1–7) solution at a rate of 0.5 μl/min over 5 min (group 1 and 2, respectively). In some animals, the abdominal cavity was opened, the right MPG exposed, and silver bipolar electrodes were properly positioned for electrical stimulation. A submaximal stimulus was applied for 2 min while the DAF (group 3) or DAF and ANG-(1–7) solutions (group 4) were infused into cavernosal sinuses. The animals were then killed, and the corpus cavernosum was removed and frozen at −80°C.

Adjacent rat tissue sections (20 μm, 6 slices per animal) were prepared using a cryostat. Tissues were mounted on chrome-alum-coated slides, air dried, and covered with glycerol-Tris mounting
media and coverslips. Fluorescent images were obtained using a laser scanning confocal microscope excited at 488 nm with argon-ion laser (oil-immersion objective lens ×63). At least one image was captured from each slice.

Quantification was performed using National Institutes of Health Image software, version 1.37v. Three images were randomly selected and submitted to quantification analysis. The RGB confocal images were loaded into the program and converted to 8-bit gray scale before subtracting background fluorescence equivalently for all images (setting the threshold to 50% maximum intensity). Three regions of the captured images were then outlined on the gray-scale pictures, and the fluorescence density was measured. Values are expressed as the mean of fluorescence intensity per square micrometer of the respective images.

Histology

Histological analysis of Mas+/+ and Mas−/− mice corpus cavernosum was performed to evaluate the fibrous tissue content using specific stain. Sections of 20 μm were cut from fresh tissue specimens previously frozen at −80°C and were subjected to Gomori trichrome histological technique. Microscopic data were digitally captured. For quantitative analysis of fibrous tissue content, the intensity of staining in different areas of the corpus cavernosum from Mas+/+ and Mas−/− mice was measured. Images of these areas were captured at 12 bits using a gray scale range of 0–255. These areas were compared and analyzed using the Scion Image software (Scion, Frederick, MD).

Statistical Analysis

All results are expressed as means ± SE. Statistical analysis of the effect of ANG-(1–7) on the ganglionic stimulation-induced increases in CCP/MAP ratio was performed by two-way ANOVA followed by Bonferroni post hoc test. To analyze the intracavernosal effect of A-779 and the influence of L-NAME on the effect of ANG-(1–7), the unpaired Student’s t-test was used. Statistical analysis of fibrous tissue content and the fluorescence intensity of DAF-FM protocols were performed using the unpaired Student’s t-test followed by the Mann-Whitney test. One-way ANOVA followed by Bonferroni post hoc test was used to compare NO release in rat and mouse corpus cavernosum. A value of P < 0.05 was considered significant.

RESULTS

In Vivo Effect of ANG-(1–7) on CCP

Using an in vivo rat model, we examined the effect of intracavernosal infusion of ANG-(1–7) on CCP and MAP. Electrical stimulation of the major pelvic ganglion resulted in a voltage-dependent increase in the CCP/MAP ratio (Fig. 1B), consistent with previous studies on penile erection mechanisms (5, 6, 25). Infusion of ANG-(1–7) produced a small but significant increase in CCP without altering baseline MAP [Fig. 1A, CCP/MAP (mean ± SE); before: 0.17 ± 0.014, n = 10; after ANG-(1–7): 0.25 ± 0.027, n = 10; P < 0.05]. Strikingly, ANG-(1–7) markedly potentiated the...

Fig. 1. In vivo effect of ANG-(1–7) on rat ganglionic-stimulated erectile response. A: infusion of angiotensin-(1–7)[ANG-(1–7)] promotes a slightly but significant increase in the corpus cavernosum pressure (CCP)/mean arterial pressure (MAP) ratio (15.5 pmol·kg⁻¹·min⁻¹, n = 10). B: ganglionic stimulation induces an increase in CCP/MAP, which was potentiated by intracavernosal infusion of ANG-(1–7) (15.5 pmol·kg⁻¹·min⁻¹, n = 6). C: A-779 (155 pmol·kg⁻¹·min⁻¹, n = 8) reduced the elevated CCP/MAP induced by the minimal electrical stimulus at which the maximal value of the CCP/MAP was observed compared with control. D: ANG-(1–7)-induced CCP/MAP increase was completely blunted by A-779 infusion (15.5 and 155 pmol·kg⁻¹·min⁻¹, respectively, n = 8). All results are expressed as means ± SE. Statistical analysis was performed with ANOVA followed by Bonferroni post hoc test. **P < 0.05; ***P < 0.01 compared with control, which was recorded 10 min before drug administration. Unpaired Student’s t-test was performed on the A-779 protocols, in which *P < 0.05 is considered significant compared with control.
increase of the CCP/MAP ratio induced by ganglionic stimulation at a doses of 15.5 (Fig. 1B) and 150 pmol·kg⁻¹·min⁻¹ (data not shown). This effect was abolished by coadministration of the Mas antagonist A-779 (27) (Fig. 1D). Notably, intracavernosal infusion of A-779 alone attenuated the CCP/MAP increase induced by the minimal electrical stimulation in which the maximal value of the CCP/MAP was achieved (Fig. 1C), suggesting that ANG-(1–7) endogenously released contributes to the increase in CCP/MAP induced by ganglionic stimulation. Vehicle infusion did not change the ganglionic stimulation-induced increase in CCP/MAP ratio (data not shown).

Immunolocalization of Mas

To further document the participation of Mas in the ANG-(1–7), we next used immunofluorescence to localize the ANG-(1–7) receptor Mas in rat penile structures. Along with functional data, Mas was localized in the bundle of trabecular smooth muscle cells and in arteriolar smooth muscle and endothelial cells of the rats corpus cavernosum (Fig. 2).

**ANG-(1–7) Potentiates Erectile Function by Releasing NO**

To determine whether the potentiating effect of ANG-(1–7) on erectile function was NO dependent, we performed additional experiments in the presence of the NOS inhibitor L-NAME. Figure 3 shows that intracavernosal infusion of ANG-(1–7) significantly increased the CCP/MAP rise induced by submaximal electrical stimulation of the MPG. Administration of L-NAME, at a dose of 200 µg/kg, into the left cavernosal sinuses did not alter baseline CCP or MAP (data not shown). However, as expected (8, 25), L-NAME injection significantly attenuated the increase in CCP/MAP induced by a submaximal stimulus (CCP/MAP: control, 0.57 ± 0.06 vs. L-NAME, 0.38 ± 0.05, n = 8; P < 0.05), which lasted for at least 25 min after administration. Furthermore, the attenuated erectile response induced by NOS inhibition was not altered by administration of ANG-(1–7), suggesting that NO is an impor-

Fig. 3. ANG-(1–7) potentiates penile erection by releasing nitric oxide. A: infusion of ANG-(1–7) potentiated the CCP/MAP rise induced by submaximal ganglionic stimulation (n = 7). N⁶-nitro-L-arginine methyl ester (L-NAME) administration (200 µg/kg) attenuated the increase in CCP/MAP induced by the submaximal electrical stimulation (ES), which was not overcome by ANG-(1–7) (n = 8). Quantification of fluorescence shows that ANG-(1–7) induced nitric oxide release into nonstimulated rat corpus cavernosum [B, ANG-(1–7)]. Submaximal ganglionic stimulation induced nitric oxide release (B; ES), which was potentiated by ANG-(1–7) [B; ES+ANG-(1–7)]. C: representative images of 4-amino-5 methylamino-2,7'-difluorofluorescein diacetate staining in rat corpus cavernosum. Left panels represent nitric oxide release into Wistar control rats corpus cavernosum in the absence (top left) or presence of ANG-(1–7) (bottom left). Right panels represent NO release under submaximal electrical stimulation in the absence (top right) or presence of ANG-(1–7) (bottom right), respectively. All results are expressed as means ± SE. One-way ANOVA followed by Bonferroni post hoc test was used to compare L-NAME (*P < 0.05) and nitric oxide release in the DAF protocol (N < 0.001 compared with (*) control and (**) ANG-(1–7)).
tant downstream mediator of ANG-(1–7) potentiating effect in this experimental model (Fig. 3A).

To further evaluate the possibility that ANG-(1–7) releases NO in the corpus cavernosum, the NO indicator DAF-FM was used. In the first set of experiments corpus cavernosum from anesthetized rats were loaded with DAF-FM or with DAF-FM combined with ANG-(1–7) solution. As shown in Fig. 3, B and C, infusion of ANG-(1–7) (15.5 pmol·kg$^{-1}$·min$^{-1}$) induced NO release. Moreover, submaximal electrical stimulation of the MPG resulted in NO release into the rat corpus cavernosum, and a clear potentiating effect of ANG-(1–7) on the NO release was observed (Fig. 3, B and C).

We next tested the effect of ANG-(1–7) on the NO release using corpus cavernosum strips of Mas$^{+/+}$ and Mas$^{-/-}$ mice (33). ANG-(1–7) induced a substantial NO release into the corpus cavernosum of WT mice, which was absent in the corpus cavernosum taken from Mas-knockout mice (Fig. 4, A and B).

**Genetic Deletion of Mas Compromises Erectile Function**

We next tested the in vivo relevance of the Mas receptor in relation to erectile function using Mas$^{-/-}$ mice. As shown in Fig. 5A (top panel), electrical stimulation of Mas$^{-/-}$ mice MPG induced a change in CCP that was significantly attenuated compared with that observed in Mas$^{+/+}$ mice. To test whether a second series of electrical stimulation would promote a similar response as usually observed in wild-type mice, we performed a second stimulation 10 min after the first one. Strikingly, the erectile dysfunction became markedly evident with a second series of stimulations (Fig. 5A, bottom panel), suggesting that other vasoactive mechanisms such as stored nitroso compounds (13) could partially compensate for the substantially compromised erectile response. As shown in Fig. 5B, Gomori trichrome staining demonstrated that genetic deletion of Mas leads to a marked increase in fibrous tissue in the penile corpus cavernosum, which probably is involved in the compromised erectile function observed in these animals.

**ANG-(1–7) Reverses Erectile Dysfunction in DOCA-Salt Hypertensive Rats**

To test whether ANG-(1–7) could ameliorate erectile dysfunction, we further determined its effect in DOCA-salt hyper-

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**Fig. 4.** Nitric oxide released in mouse corpus cavernosum induced by ANG-(1–7). ANG-(1–7) stimulated nitric oxide release in corpus cavernosum of Mas WT mice, which was completely abolished in Mas-knockout mice (A and B). All results are expressed as means ± SE; n = 3. Statistical analysis was performed with one-way ANOVA followed by Bonferroni post hoc test. *P < 0.001 compared with Mas$^{+/+}$ control and Mas$^{-/-}$ control and ANG-(1–7).
tensive rats (MAP: 179 ± 4 mmHg vs. control rats: 116 ± 2
mmHg; *P < 0.01), which present severe erectile dysfunction
(6) and reduced components of the circulating RAS (15, 32).
DOCA-salt hypertensive rats showed reduced erectile function
in our experimental protocol compared with control animals
(CCP/MAP values: 2.5 V, 0.55 ± 0.10 vs. 0.30 ± 0.06; 3.0 V:
0.57 ± 0.11 vs. 0.33 ± 0.09, n = 6 and 9 for control and
DOCA-salt-treated rats, respectively) (Fig. 6, A and B). Local
administration of ANG-(1–7) increased the electrically induced
elevation of CCP/MAP in both control (Fig. 6A) and DOCA-
salt hypertensive rats (Fig. 6B), and it normalized the erectile
function of DOCA-salt rats (Fig. 6C).

DISCUSSION

In this study, we used a multidisciplinary approach to
investigate the role of the ANG-(1–7)/Mas axis in erectile
function. We have obtained substantial evidence that ANG-
(1–7) acts as a mediator of penile erection by activation of Mas
and subsequent NO release. In the absence of Mas, erectile
function was severely compromised as demonstrated by a
markedly depressed response to electrical stimulation of the
MPG associated with penile fibrosis. Furthermore, the severely
depressed erectile function of DOCA-salt hypertensive rats
was essentially normalized by ANG-(1–7) administration.
These data provide strong evidence for a previously unsus-
pected key role of ANG-(1–7) and its receptor Mas in erectile
function.

It has been proposed that penile erection is driven by two
distinct hemodynamic phases. The first one is dependent on
NO release from nonadrenergic, noncholinergic (NANC)
nerves in the penis causing dilation of arterioles and expansion
of sinusoids. This phase is followed by the release of endothe-
lial-derived relaxing factor from endothelial cells surrounding
corpus cavernosum trabecules in response to shear stress. It is
generally accepted that NO derived from endothelial NOS is
the major vasodilator for this second stage of erection (4, 17).
Our results do not allow consider how ANG-(1–7) influenced
these two stages. It is not clear whether ANG-(1–7) acts to
stimulate NO release from NANC nerve terminals, to facilitate
the release of acetylcholine from pre- and postsynaptic para-
sympathetic nerves (2), or to sensitize the corpus cavernosum
endothelial cells in the increase of shear stress (10). Additional
studies are necessary to unmask at what stage ANG-(1–7)
facilitates penile erection.

Evidence for the involvement of Mas in the proerectile effect
of ANG-(1–7) was provided by the blockade of its potentiating
effect on the erectile response induced by MPG stimulation, by
the receptor Mas antagonist A-779 and by the absence of NO

Fig. 5. Effect of Mas deletion on erectile performance and penile structure.
A: increase in CCP/MAP induced by ganglionic stimulation in Mas−/− mice
was significantly lower than in Mas+/+ mice (top). The increase in CCP/MAP
induced by a second series of ganglionic stimulations performed 10 min after
the first was further attenuated in the Mas−− mice (bottom). B: Mas−/− mice
stained with Gomori trichrome showed increased fibrous tissue compared with
Mas+/+ mice, indicating increased collagen content (top). Representative
images of Gomori trichrome staining in Mas−/− and Mas+/+ mice corpus
cavernosum (bottom). All results are expressed as means ± SE. WT, wild type;
KO, knockout. Statistical analysis was performed with ANOVA followed by
Bonferroni post hoc test. Mann-Whitney U-test was applied for quantification
of collagen content. *P < 0.05; ** P < 0.01; *** P < 0.001 compared with
Mas−/− (A) or Mas+/+ (B).
release in response to ANG-(1–7) in corpus cavernosum strips taken from Mas−/− mice. Furthermore we have documented the presence of Mas, by immunofluorescence, in smooth muscle and endothelial cells from rat corpus cavernosum. These observations are in keeping with the recent report by Sampaio and coworkers (26) that ANG-(1–7) produces NO release from human endothelial cells through activation of the phosphatidylinositol-3 kinase/Akt pathway, which has endothelial NOS as one of the important downstream targets.

As mentioned above, it is widely accepted that NO released from NANC nerves and from endothelial cells lining corpus cavernosal sinuses is the most important mediator of cavernosal smooth muscle relaxation to initiate and maintain erection (4). Endothelial integrity is also required because the earliest detectable changes in vascular disease states associated with erectile dysfunction are abnormalities in the endothelium, causing a loss of its homeostatic mechanisms (21). In our in vivo experiments, we observed that L-NAME, a nonspecific NOS inhibitor, completely blocked the potentiation of ganglionic-stimulated CCP/MAP increase induced by ANG-(1–7), which strongly suggests the importance of NO on its effect. Indeed, using a specific marker for NO (DAF), we showed in this study that ANG-(1–7) induced NO release in rat and mouse corpus cavernosum, and also potentiated NO release during ganglionic stimulation in rats.

To get new insight into the possible physiological relevance of ANG-(1–7) and Mas receptor on erectile function, we performed in vivo experiments using Mas deficient mice. Strikingly, the increase in CCP/MAP induced by ganglionic stimulation was substantially reduced in Mas−/− mice. The severely compromised erectile function in Mas−/− mice became even more evident with a second series of electrical stimulation. This last observation suggests that other vasoactive mechanisms such as stored nitroso compounds (13) could partially compensate for the decreased erectile response in the first MPG series of electrical stimulation. Further studies are necessary to confirm this possibility.

Using adult rat cardiac fibroblasts, Iwata et al. (18) showed that ANG-(1–7), binding to specific sites distinct from AT1 and AT2 receptors, had an inhibitory effect on collagen synthesis and also inhibited increases in collagen synthesis and mRNA expression of growth factors induced by ANG II. Tallant et al. (31) demonstrated that ANG-(1–7) attenuated MAP kinase activation, which was blocked by an antisense oligonucleotide to the Mas receptor. In addition, Santos et al. (28) demonstrated that Mas knockout mice present a marked change in the heart collagen expression to a profibrotic profile, with increased levels of collagen I, collagen III, and fibronectin and with reduced expression of collagen VI. Based on these findings, we evaluated the fibrous content of the corpus cavernosum of Mas deficient mice. We observed that the genetic deletion of Mas induced an increase in the fibrous tissue content, which might be related to the erectile dysfunction observed in Mas−/− mice. Altogether, these findings point out the importance of ANG-(1–7)-Mas axis in the regulation of collagen content, which is probably related to inhibition of activity and/or expression of MAP kinases.

Finally, we took advantage of the DOCA-salt model of hypertension, which has been described as a good model for rat erectile dysfunction (6), to test whether ANG-(1–7) could be used to attenuate erectile dysfunction. ANG-(1–7) fully restored the erectile function of the DOCA-salt rats. It has also been demonstrated that salt diet associated with mineralocorticoid administration promotes several humoral alterations, including a marked reduction of the circulating RAS activity (23). Therefore, the beneficial effect of ANG-(1–7) on the erectile function of DOCA-salt animals appears to be indepen-
dent of its counterregulatory effects on circulating ANG II. However, we cannot discard the possibility that local ANG II may be increased in DOCA-salt rats. In addition, because ANG-(1–7) was described to downregulate AT1 receptor (9), in association with the fact that the Mas receptor is suggested to be a physiological antagonist of the AT1 receptor (20), we cannot rule out the possibility of a counter-regulatory effect of ANG-(1–7) in different situations in which ANG II plays a major role.

Because ANG II has been associated with penile detumescence (3), drugs that reduce the formation or action of ANG II, such as angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptors blockers (ARBs), have been reported to improve erectile response. Enalapril, but not hydralazine, induces structural remodeling of the penile vasculature and ameliorates blood inflow to the corpus cavernosum in spontaneously hypertensive rats (14). Captopril improves the erectile function of spontaneously hypertensive-stroke prone and normotensive aged rats (11). One could speculate that part of that effect could be due to an increase in the half life of bradykinin (30) but that does not explain the improvement observed with ARBs. Considering that both ACEi and ARBs increase ANG-(1–7) levels in plasma and tissue (12), our findings strongly suggest that the beneficial effects of the RAS blockade on erectile function could be mainly mediated by ANG-(1–7). In addition, our data provide evidence for a novel dual role of the RAS in erectile function: prodetumescence mediated by the ANG II-AT1 axis and proerection mediated by the ANG-(1–7)-Mas axis.

Perspectives

Our study presents a novel finding that the ANG-(1–7)-Mas axis is required for normal erectile function. While acute blockade of Mas with A–779 reduced the maximal erectile response, chronic Mas-deficiency leads to a more severe erectile dysfunction associated with the accumulation of fibrous tissue in the penis. On the other hand, intracavernosal ANG-(1–7) administration ameliorated the erectile dysfunction present in DOCA-salt hypertensive rats. These observations are in line with the NO-releasing activity of ANG-(1–7) in corpus cavernosum and with a Mas-dependent potentiating effect on the erectile response evoked by MPG electrical stimulation. Although further investigations need to be done to fully understand the role of ANG-(1–7)-Mas in erectile function, our study provides support to consider the ANG-(1–7)-Mas axis as a putative target for development of new therapeutics for the treatment of erectile dysfunction.

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