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Angiotensin-(1–7) blocks the angiotensin II-stimulated superoxide production

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Abstract

Angiotensin (Ang)-(1–7), a bioactive compound of the renin–angiotensin system, exerts effects leading to blood pressure reduction which counterbalance Ang II pressor actions. The present study was conducted to examine Ang-(1–7) and Ang II effects on superoxide anion production in rat aorta using the lucigenin chemiluminescence method. Ang II dose-dependently increased superoxide anion formation when compared to control levels; a maximal increase (2.5-fold) was observed with 1×10^{-10} M peptide concentration. The Ang II-stimulated superoxide formation was blocked by 1×10^{-10} M losartan, the specific AT_1 receptor antagonist, but not by 1×10^{-10} M PD 123319, the AT_2 receptor antagonist, suggesting that the increased superoxide levels caused by Ang II are mediated through AT_1 receptors activation. The Ang II-stimulated superoxide production was not modified by 2×10^{-8} M allopurinol or 1×10^{-7} M indomethacin, but was completely abolished by NAD(P)H oxidase inhibitors: 1×10^{-8} M diphenylene iodonium, or 2×10^{-8} M apocynin, demonstrating that NAD(P)H oxidase participates in such response.

In contrast to Ang II, Ang-(1–7) concentrations ranging 1×10^{-12} to 1×10^{-6} M did not modify superoxide anion levels, but prevented the Ang II-enhanced superoxide production.

In conclusion, we demonstrated that Ang-(1–7) blocks the pro-oxidant effects of Ang II, thus reducing the superoxide anion production and delaying the hypertension development.

Keywords: Oxidative stress; NAD(P)H oxidase; Angiotensin-(1–7); Superoxide anion; Angiotensin receptors; Angiotensin II

1. Introduction

The renin–angiotensin system (RAS) is a very important regulatory system in cardiovascular and blood pressure homeostasis. Although angiotensin (Ang) II has been considered the major effector peptide of the RAS, Ang-(1–7), the heptapeptide lacking the C-terminal phenylalanine of the Ang II sequence, is also an important biologically active component of the system. Processing of Ang II by prolyl-endopeptidase or endopeptidases produces Ang-(1–7) [1,2]. In addition, Ang I via an independent pathway by the action of angiotensin-converting enzyme (ACE) or tissue-specific endopeptidases contributes to the heptapeptide formation [2–4]. It was reported that ACE2, a new RAS regulatory enzyme, produces Ang-(1–7) from Ang II [5,6]. Ang-(1–7) lacks the vasoconstrictor, aldosterone secretagogue or dipsogenic effects of Ang II [2,7]. In contrast, it causes natriuresis and diuresis, vasodilation and inhibits angiogenesis and cellular growth, suggesting that in many cases this peptide may act as an endogenous antagonist of Ang II [2,7–9]. In fact, an antihypertensive as well as a counterbalancing role to the pressor and proliferative actions of Ang II have been suggested for Ang-(1–7), since some of its effects opposite to those produced by Ang II are enhanced in rat models of hypertension [7].

It is known that reactive oxygen species (ROS) contribute to the pathogenesis of numerous cardiovascular diseases including hypertension, atherosclerosis, cardiac hypertrophy, heart failure and restenosis, being NAD(P)H oxidase the predominant source of ROS. Activation of this enzyme leads to a variety of intracellular signaling events that ultimately promote endothelium dysfunction, vascular smooth muscle cells proliferation, pro-inflammatory genes expression and reconstruction of the extracellular matrix [10]. Ang II, via activation of the AT_1 recep-

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tor, stimulates NAD(P)H oxidases activity in vascular smooth muscle cells increasing superoxide anion formation and nitric oxide inactivation, effects associated with the pathogenesis of hypertension [11,12]. Furthermore, many Ang II effects seem to be mediated by enhanced ROS production [13].

In this study, we investigate in aortic thoracic rings Ang-(1–7) and Ang II effects on superoxide anion levels. Since it has been suggested that Ang-(1–7) counter-regulates the pressor and trophic actions of Ang II [7], we hypothesize that Ang-(1–7) opposes the Ang II-stimulated superoxide production.

2. Materials and methods

2.1. Chemicals

NADPH, diphenylene iodonium (DPI), indomethacin, PD123319, N^w-nitro-L-arginine methyl ester (L-NAME), allopurinol and Ang II were purchased from Sigma–Aldrich (St. Louis, MO). Apocynin was obtained from Calbiochem, CA and losartan was from Dupont-Merck, Wilmington, DE. Ang-(1–7) and [D-Ala^7]Ang-(1–7) were synthesized in our laboratory by the Merrifield solid-phase procedure, as described previously [14]. All other chemicals were of analytical grade.

2.2. Experimental procedure

Male Sprague–Dawley rats weighing 200 g were killed by decapitation and thoracic aortas were isolated and placed in chilled modified Krebs/Hepes buffer containing (in mM): 99.01 NaCl, 4.69 KCl, 1.87 $CaCl_2$, 1.20 $MgSO_4$, 1.03 K_2HPO_4, 25.00 $NaHCO_3$, 20.00 Na Hepes, and 11.1 glucose (pH 7.4). The aortas were cleaned of excessive adventitial tissue and cut into 5-mm ring segments.

Superoxide anion production was measured by the lucigenin chemiluminescence method [15]. Briefly, aortic rings were incubated during 30 min at 37 °C in Krebs/Hepes buffer either with no addition (control) or in the presence of different concentrations of Ang II or Ang-(1–7) or a combination of both peptides. To test the effect of antagonists, rings were handled as described above, after preincubation for 30 min with or without antagonist. Following incubation, scintillation vials containing 2 ml Krebs/Hepes buffer with 5×10^{-6} M lucigenin and 1×10^{-8} M NADH were placed into a scintillation counter switched to the out-of-coincidence mode. After dark adaptation, background counts were recorded and the vascular segment was added to the vial. Light production induced by superoxide anion attack on lucigenin was recorded every minute during 30 min and the respective background counts were substracted. At the end of the incubation, rings were removed and dried in a 90 °C oven for 24 h. Chemiluminescence produced by lucigenin in rings with or without additions was expressed as cpm per mg of dried aortic tissue.

2.3. Statistical analysis

All data were expressed as mean ± S.E.M. where n is the number of rats used (seven animals/group). Statistical comparisons were performed using one-way ANOVA. When significance was indicated, a Student–Newman–Keuls post hoc analysis was applied.

3. Results

As shown in Fig. 1, Ang II dose-dependently increased superoxide anion formation when compared to control levels; a maximal increase (2.5-fold) was observed with 1×10^{-10} M peptide concentration. Higher Ang II concentrations produced a smaller increase in superoxide production (Fig. 1). The Ang II-stimulated superoxide formation was blocked by 1×10^{-10} M losartan, the specific AT_1 receptor antagonist, but not by 1×10^{-10} M PD 123319, the AT_2 receptor antagonist (Fig. 2),

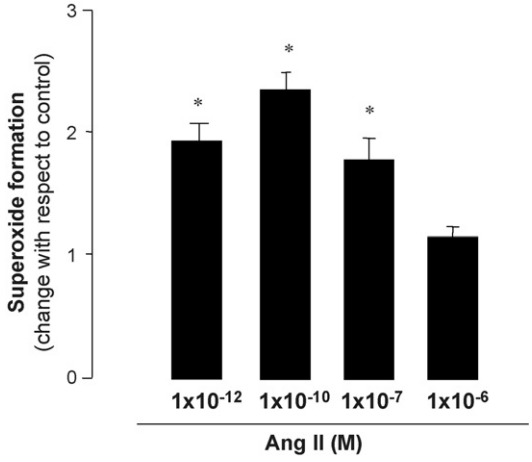

Fig. 1. Effects of Ang II on superoxide production in thoracic aortic rings. Results indicate the ratio between chemiluminescence produced by lucigenin with and without peptide additions and are expressed as means ± S.E.M. ($n = 7$) in each group. *$p < 0.01$ vs. control group.

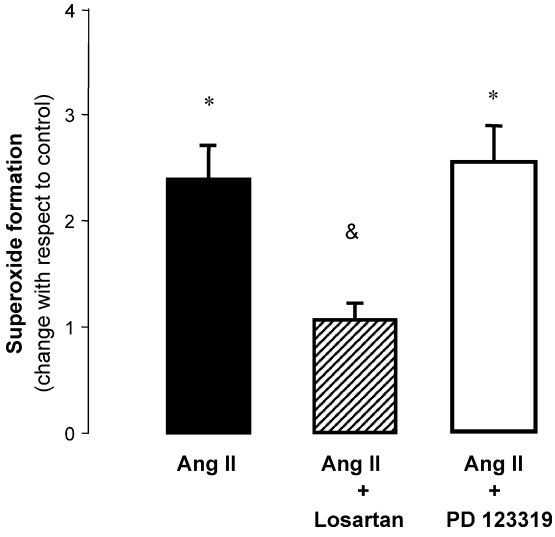

Fig. 2. Effects of pretreatment with 1×10^{-10} M losartan or PD 123319 on superoxide formation by 1×10^{-10} M Ang II in thoracic aortic rings. Results indicate the ratio between chemiluminescence produced by lucigenin with and without additions and are expressed as means ± S.E.M. ($n = 7$) in each group. & $p < 0.01$ vs. Ang II-stimulated group.

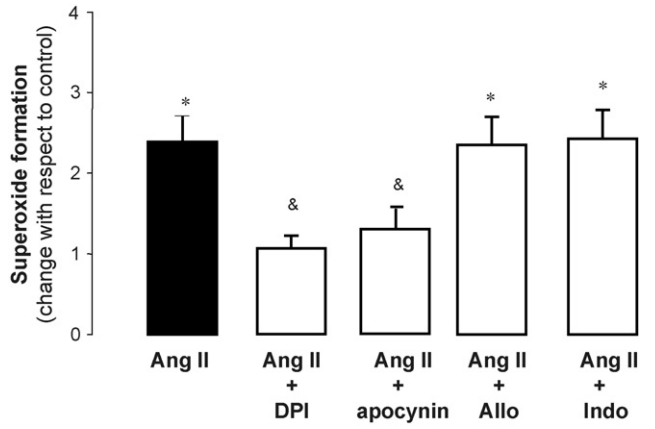

Fig. 3. Effects of pretreatment with either 1×10^{-8} M diphenylene iodonium (DPI), 2×10^{-8} M allopurinol (Allo), 1×10^{-7} M indomethacin (Indo), or 2×10^{-8} M apocynin on superoxide formation by 1×10^{-10} M Ang II in thoracic aortic rings. Results indicate the ratio between chemiluminescence produced by lucigenin with and without additions and are expressed as means ± S.E.M. ($n = 7$) in each group. *$p < 0.01$ vs. control group. & $p < 0.01$ vs. Ang II-stimulated group.

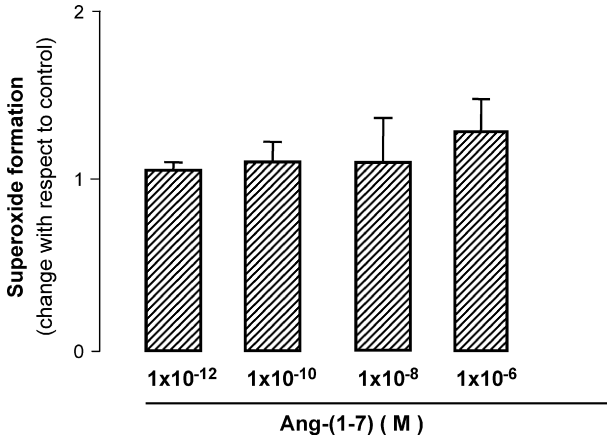

Fig. 4. Effects of Ang-(1–7) on superoxide production in thoracic aortic rings. Results indicate the ratio between chemiluminescence produced by lucigenin with and without peptide addition and are expressed as means ± S.E.M. ($n = 7$) in each group. *$p < 0.01$ vs. control group.

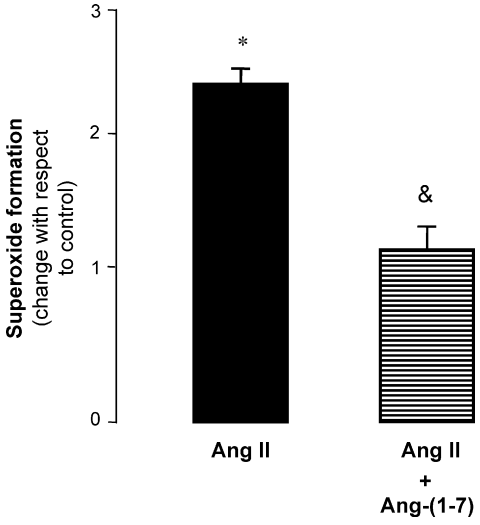

Fig. 5. Effects of pretreatment with 1×10^{-10} M Ang-(1–7) on superoxide formation by 1×10^{-10} M Ang II in thoracic aortic rings. Results indicate the ratio between chemiluminescence produced by lucigenin with and without additions and are expressed as means ± S.E.M. ($n = 7$) in each group. *$p < 0.01$ vs. control group. & $p < 0.01$ vs. Ang II-stimulated group.

suggesting that the increased superoxide levels caused by Ang II are mediated through AT_1 receptors activation.

Superoxide anion is formed from O_2 mainly by the enzymatic activity of NAD(P)H oxidase, but other oxidant enzymes, such as xanthine oxidase may also be involved [16]. Moreover, it has been demonstrated that arachidonic acid metabolites participate in oxidase activation [17]. In order to test which enzyme mediates the enhanced superoxide anion level caused by Ang II, specific inhibitors of NAD(P)H oxidase, xanthine oxidase and cyclooxygenase were assayed. The Ang II-stimulated superoxide production was not modified by 2×10^{-8} M allopurinol, an specific inhibitor of xanthine oxidase, or by 1×10^{-7} M indomethacin, which inhibits cyclooxygenase (Fig. 3), but was completely abolished in the presence of NAD(P)H inhibitors: diphenylene iodonium (1×10^{-8} M), or apocynin (2×10^{-8} M) (Fig. 3), demonstrating that NAD(P)H oxidase participates in such response.

In contrast to Ang II, Ang-(1–7) concentrations ranging 1×10^{-12} to 1×10^{-6} M did not modify superoxide anion levels (Fig. 4), but prevented the Ang II-enhanced superoxide anion production (Fig. 5).

To test if the decreased Ang II-stimulated superoxide formation caused by Ang-(1–7) was due to the activation of the Ang-(1–7)-sensitive receptor named Mas [18,19], incubation was performed in the presence of the receptor antagonist [D-Ala^7]Ang-(1–7). As shown in Fig. 6, the addition of 1×10^{-10} M [D-Ala^7]Ang-(1–7) did not modify the Ang-(1–7) effect.

Several reports demonstrate that some Ang-(1–7) biological actions are mediated by nitric oxide and/or prostaglandins [2,20]. To investigate whether Ang-(1–7) blocking effect on Ang II-increased superoxide production was mediated by nitric oxide or prostaglandins, incubations were performed with aortic rings pretreated with 1×10^{-10} M L-NAME or indomethacin. No change in the Ang-(1–7) effect was observed (Fig. 7).

4. Discussion

In accordance with previous studies [12,21,22], the present study indicates that in vascular cells Ang II significantly enhanced the production of superoxide anion in a dose-dependent manner. A smaller increase in superoxide formation was observed above 1×10^{-10} M Ang II, probably due to desensitization of AT_1 receptors [23,24]. The fact that the increased superoxide levels caused by Ang II were blocked by DPI and apocynin demonstrates that NAD(P)H oxidases activation is involved.

In our study, the Ang II-augmented superoxide production was totally blocked by losartan, suggesting the involvement of AT_1 receptors (present results). Accordingly, it has been previously demonstrated that Ang II stimulates superoxide production via both AT_{1A} and AT_{1B} receptors in mouse aorta and heart [22]

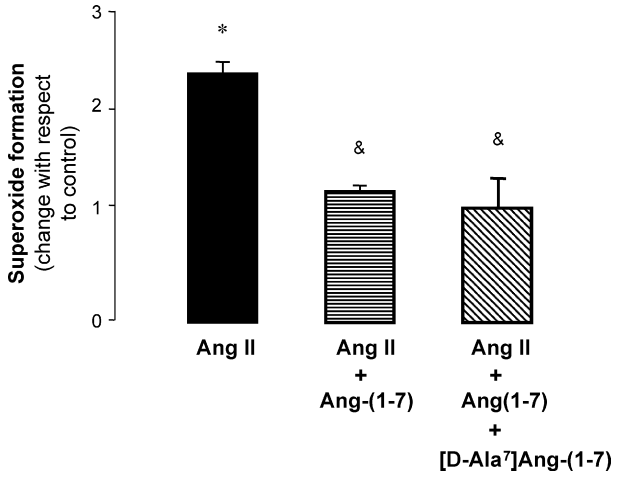

Fig. 6. Effects of 1×10^{-10} M Ang-(1–7) on superoxide formation by 1×10^{-10} M Ang II in thoracic aortic rings pretreated with 1×10^{-10} M [D-Ala^7]Ang-(1–7). Results indicate the ratio between chemiluminescence produced by lucigenin with and without additions and are expressed as means ± S.E.M. ($n = 7$) in each group. *$p < 0.01$ vs. control group. & $p < 0.01$ vs. Ang II-stimulated group.

and that the hypertension induced in rats by chronic infusion of Ang II is correlated with an increased NAD(P)H oxidase-derived superoxide generation and prevented by losartan pretreatment [12]. Furthermore, an increased NADPH oxidase activity mediated through AT_1 receptors has been reported in rats with genetically high ACE levels [25].

The effects of Ang-(1–7) on superoxide production by NAD(P)H oxidase are poorly documented. In our experimental work, none of the tested concentrations affected superoxide levels, suggesting that Ang-(1–7) did not interact with the vascular NAD(P)H oxidase. Our results are in accordance with those reported by Oudot et al. [26] demonstrating that Ang-(1–7) has no effect on NAD(P)H oxidase activity in isolated perfused rat hearts under non-ischemic conditions. However, pharmacological concentrations of Ang-(1–7) activated myocardial NAD(P)H oxidase after ischemia–reperfusion [26].

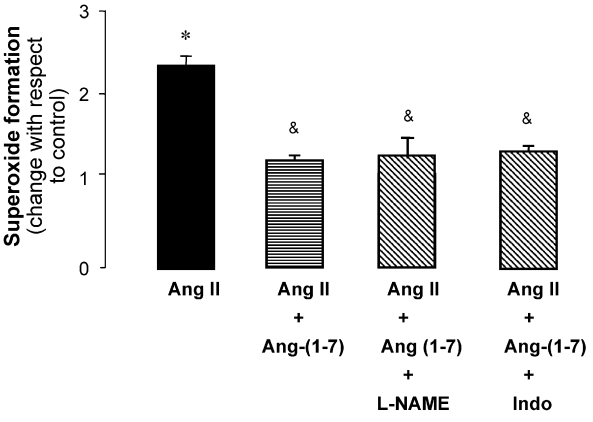

Fig. 7. Effects of 1×10^{-10} M Ang-(1–7) on superoxide formation by 1×10^{-10} M Ang II in thoracic aortic rings pretreated with either 1×10^{-7} M indomethacin, or 3×10^{-7} M L-NAME. Results indicate the ratio between chemiluminescence produced by lucigenin with and without additions and are expressed as means ± S.E.M. ($n = 7$) in each group. *$p < 0.01$ vs. control group. & $p < 0.01$ vs. Ang II-stimulated group.

Since Ang-(1–7) exerts several biological actions through activation of the Mas receptor [18,19] experiments performed in the presence of [D-Ala^7]Ang-(1–7), a selective Ang-(1–7) antagonist, indicated that this receptor is not involved in the Ang-(1–7) effect. Possibly, Ang-(1–7) behaves as an Ang II antagonist by competing for AT_1 receptors. In fact, we have previously demonstrated that Ang-(1–7) releases [^{3}H]norepinephrine in rat atria through activation of AT_1 receptors [27] and elicits high affinity for those receptors in rat glomeruli [28]. Several effects induced by Ang II are blocked by Ang-(1–7). I.e. Zhu et al. [29] demonstrated that Ang-(1–7) shows partial antagonism on the Ang II-induced activation of protein kinase C and extracellular signal-regulated kinases (ERK1/2) in vascular smooth muscle cells. Similarly, the Ang II-stimulated norepinephrine release in hypothalami from SHR was blocked by Ang-(1–7) [30]. Since Ang-(1–7) blocked Ang II-induced pressor responses, it has been suggested that the heptapeptide may act as a specific non-competitive antagonist of AT_1 receptors [31,32]. Moreover, Clark et al. [33] demonstrated in vascular smooth muscle cells that Ang-(1–7) reduces Ang II binding to the AT_1 receptor, as a result of a decreased number of receptors available for subsequent binding with no significant effect on Ang II affinity for the receptor. The down regulation of the Ang II receptor by Ang-(1–7) was blocked by pretreatment with the AT_1 receptor antagonist L158,809 demonstrating a direct interaction of the heptapeptide with the AT_1 receptor [33].

It has been described that some Ang-(1–7) effects are mediated by the release of nitric oxide and/or prostaglandins [2,20]. Present results showed that pre-treatment with L-NAME (nitric oxide synthase inhibitor), or indomethacin (cycloxygenase inhibitor) did not modify the blocking effect of Ang-(1–7) on Ang II-stimulated superoxide production, suggesting a nitric oxide and prostaglandin-independent mechanism.

ACE inhibitors have turned to be important tools in the therapy of hypertension, heart failure, cardiac remodeling, postmyocardial infarction and renal diseases. Ang-(1–7) level increases 5–25-fold in blood after administration of an ACE inhibitor, either due to an increased formation from its precursor Ang I or a decreased degradation after ACE blockade [34]. The accumulation of Ang-(1–7) possibly determines its opposition to the pro-oxidative effects of Ang II in the production of superoxide anion via NAD(P)H oxidase and may play a substantial role in cardiovascular and antihypertensive effects of RAS blockade protecting the endothelium [35]. Furthermore, Igase et al. [36] demonstrated that systemic blockade of AT_1 receptors is associated with increased expression of aortic ACE2 mRNA as well as increased immunoreactive Ang-(1–7) in the thoracic aorta of SHR. Since ACE2 cleaves Ang II to Ang-(1–7) with high catalytic efficiency, the therapeutic use of AT_1 blockers, supports the hypothesis that Ang-(1–7) reinforces the decreased superoxide formation promoted by those drugs.

In conclusion, we demonstrated that Ang-(1–7) blocks the pro-oxidant effects of Ang II, thus reducing the superoxide anion production and delaying the hypertension development.

Acknowledgements

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References

[1] Welches WR, Santos RAS, Chappell MC, Brosnihan KB, Greene LJ, Ferrario CM. Evidence that prolylendopeptidase participates in the processing of brain angiotensin. J Hypertens 1991;9:631–8.
[2] Santos RAS, Campagnole-Santos MJ, Andrade SP. Angiotensin-(1–7): an update. Regul Pept 2000;91:45–62.
[3] Santos RAS, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, et al. Converting enzyme activity and angiotensin metabolism in the dog brainstem. Hypertension 1988;11(Suppl I):153–7.
[4] Chappell MC, Jaiswal R, Brosnihan KB, Ferrario CM. Processing of angiotensin I to angiotensin-(1–7) by vascular smooth muscle cells of spontaneously hypertensive rats. Hypertension 1991;18:385–6.
[5] Burrell LM, Johnston CI, Tikellis C, Cooper ME. ACE_2, a new regulator of the renin–angiotensin system. Trends Endocrinol Metab 2004;15: 166–9.
[6] Ferrario CM. Angiotensin-converting enzyme 2 and angiotensin-(1–7). An evolving story in cardiovascular regulation. Hypertension 2006;47: 515–21.
[7] Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI. Counterregulatory actions of angiotensin-(1–7). Hypertension 1997;30:535–41.
[8] Delli Pizzi AM, Hilchey SD, Bell-Quilley CP. Natriuretic action of angiotensin-(1–7). Br J Pharmacol 1994;111:1–3.
[9] Freeman EJ, Chisolm GM, Ferrario CM, Tallant EA. Angiotensin-(1–7) inhibits vascular smooth muscle cells growth. Hypertension 1996;28: 104–8.
[10] Cai H, Griendling KK, Harrison DG. The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 2003;24:471–8.
[11] Wang HD, Johns DG, Xu S, Cohen RA. Role of superoxide anion in regulating pressor and vascular hypertrophic response to angiotensin II. Am J Physiol Heart Circ Physiol 2002;282:H1697–702.
[12] Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996;97:1916–23.
[13] Griendling KK, Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. Regul Pept 2000;91:21–7.
[14] Lopez Ordieres MG, Gironacci MM, Rodriguez de Lores Arnaiz G, Peña C. Effect of angiotensin-(1–7) on ATPase activities in several tissues. Regul Pept 1998;77:135–9.
[15] Munzel T, Sayegh H, Freeman BA, Tarpey MM, Harrison DG. Evidence for enhanced vascular superoxide anion production in nitrate tolerance. A novel mechanism underlying tolerance and cross-tolerance. J Clin Invest 1995;95:187–94.
[16] Lassègue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression and regulation. Am J Physiol 2003;285:R277–97.
[17] Zafari AM, Ushio-Fukai M, Minieri CA, Akers M, Lassegue B, Griendling KK. Arachidonic acid metabolites mediate angiotensin II-induced NADH/NADPH oxidase activity and hypertrophy in vascular smooth muscle cells. Antioxid Redox Signal 1999;1:167–79.
[18] Santos RAS, Simoes e Silva AC, Maric C, Silva DMR, Machado RP, de Buhr I, et al. Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci USA 2003;100:8258–63.
[19] Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1–7) inhibits growth of cardiac myocytes through activation of the Mas receptor. Am J Physiol Heart Circ Physiol 2005;289:H1560–6.
[20] Brosnihan KB, Li P, Ferrario CM. Angiotensin-(1–7) dilates canine coronary arteries through kinins and nitric oxide. Hypertension 1996;27:523–8.
[21] Kawazoe T, Kosaka H, Yoneyama H, Hata Y. Acute production of vascular superoxide by angiotensin II but not by catecholamines. J Hypertens 2000;18:179–85.
[22] Rahman M, Kimura S, Nishiyama A, Hitomi H, Zhang G, Abe Y. Angiotensin II stimulates superoxide production via both angiotensin AT1A and AT1B receptors in mouse aorta and heart. Eur J Pharmacol 2004;485:243–9.
[23] Muthalif MM, Benter IF, Udin MR, Harper JL, Malik JU. Signal transduction mechanism involved in Ang-(1–7)-stimulated arachidonic acid release and prostanoid synthesis in rabbit aortic smooth muscle cells. J Pharmacol Exp Ther 1998;284:388–98.
[24] Gironacci MM, Fernandez-Tome MDC, Speziale E, Speziale NS, Peña C. Enhancement of phosphatidylcholine biosynthesis by angiotensin-(1–7) in the rat renal cortex. Biochem Pharmacol 2002;63:507–14.
[25] Jalil JE, Perez A, Ocaranza MP, Bargetto J, Galaz A, Lavandero S. Increased aortic NADPH oxidase activity in rats with genetically high angiotensin-converting enzyme levels. Hypertension 2005;46:1362–7.
[26] Oudot A, Vergely C, Ecarnot-Laubriet A, Rochette L. Pharmacological concentration of angiotensin-(1–7) activates NADPH oxidase after ischemia–reperfusion in rat heart through AT_1 receptor stimulation. Regul Pept 2005;127:101–10.
[27] Gironacci MM, Adler-Graschinsky E, Peña C, Enero MA. Effects of angiotensin II and angiotensin-(1–7) on the release of [^{3}H]norepinephrine from rat atria. Hypertension 1994;24:457–60.
[28] Gironacci MM, Coba MP, Peña C. Angiotensin-(1–7) binds at the type 1 angiotensin II receptors in rat renal cortex. Regul Pept 1999;84:51–4.
[29] Zhu Z, Zhong J, Zhu S, Liu D, Van der Giet M, Tepel M. Angiotensin-(1–7) inhibits angiotensin II-induced signal transduction. J Cardiovasc Pharmacol 2002;40:693–700.
[30] Gironacci MM, Valera MS, Yujnovsky I, Peña C. Angiotensin-(1–7) inhibitory mechanism on norepinephrine release in hypertensive rats. Hypertension 2004;44:1–5.
[31] Mahon JM, Carr RD, Nicol AK, Henderson IW. Angiotensin-(1–7) is an antagonist at the type 1 angiotensin II receptor. J Hypertens 1994;12:1377–81.
[32] Höcht C, Opezzo JAW, Gironacci MM, Peña C, Taira CA. Hypothalamic cardiovascular effects of angiotensin-(1–7) in spontaneously hypertensive rats. Regul Pept 2006;135:39–44.
[33] Clark MA, Diz DI, Tallant EA. Angiotensin-(1–7) downregulates the angiotensin II type 1 receptor in vascular smooth muscle cells. Hypertension 2001;37:1141–6.
[34] Yamada K, Iyer SN, Chappell MC, Ganten D, Ferrario CM. Converting enzyme determines plasma clearance of angiotensin-(1–7). Hypertension 1998;32:496–502.
[35] Luque M, Martin P, Martell N, Fernandez C, Brosnihan KB, Ferrario CM. Effects of captopril related to increased levels of prostacyclin and angiotensin-(1–7) in essential hypertension. J Hypertens 1996;14:799–805.
[36] Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM. Angiotensin II AT_1 receptors regulate ACE2 and angiotensin-(1–7) expression in the aorta of spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 2005;289:1013–9.