Contribution of Ras farnesyl transferase, MAP kinase and cytochrome P-450 metabolites to endothelin-1 induced hypertension

Farid Ljuca[1*], Gorazd Drevenšek[2], Enver Zerem[3]

[1] Department of Physiology, University of Tuzla, Faculty of Medicine, Univerzitetska 1, 75 000 Tuzla, Bosnia and Herzegovina. [2] Department of Pharmacology and experimental toxicology, University of Ljubljana, Faculty of Medicine, Vrazov trg 2, 1000 Ljubljana, Slovenia. [3] University Clinical Center Tuzla, Trnovac bb, 75 000 Tuzla, Bosnia and Herzegovina.

Abstract

Endothelin 1 (ET-1) is vasoactive peptide that acts via ET-A receptors coupling inducing vascular smooth muscle cell proliferation and contraction. ET-1 is involved in the development and maintenance of hypertension.

Aim of this study was to determine the contribution of Ras farnesyl transferase, mitogen activated protein kinase (MAP kinase) and cytochrome P¬450 (CYP450) metabolites to ET-1 induced hypertension.

ET-1 (5 pmol/kg per minute) was chronically infused into to the jugular vein by use of mini-osmotic pump for 9 days in male Sprague-Dawley rats. Mean arterial blood pressure (MABP) in ET-1-treated rats was 154±2 mm Hg (hypertensive rats) compared with 98±3 mm Hg in control (normotensive) rats. Infusion of Ras farnesyl transferase inhibitor FPTIII (138 ng/min), MAP kinase inhibitor PD-98059 (694 ng/min) and CYP450 inhibitor 17-ODYA (189 ng/min) significantly attenuated MABP to 115±2.5 mm Hg, 109±3 mm Hg and 118±1.5 mm Hg, respectively. These results suggest that CYP-450 metabolites and Ras/MAP kinase pathway contribute to the development of ET-1 induced hypertension. Further investigation has to be done to confirm whether activation of RAS/MAP kinase pathway by arachidonic acid metabolites plays an important role in the development of ET-1 induced hypertension.

KEY WORDS: endothelin-1, hypertension, cytochrome P-450, Ras farnesyl transferase, mitogen activated protein kinase

INTRODUCTION

There are three mature endothelin isoforms: ET-1, ET-2 and ET-3, of which the most potent isoform is endothelin-1 implicated in the physiological control of vascular smooth muscle cell (VSMC) and myocardial contractility [1]. ET-1 acts via ET-A receptors coupling inducing vascular smooth muscle cell contraction [2]. Endothelin-1, a 21-amino acid peptide, is synthesized by the endothelium and it can induce proliferation of vascular smooth muscle cell and thus be implicated in pathogenesis of hypertension [3-5]. ET-1 induces arachidonic acid release via activation of cytosolic phospholipase A2 (cPLA2) [6]. Arachidonic acid is metabolized by cyclooxygenase into prostaglandins, by lipoxygenase into leukotrienes and by cytochrome P-450 into 12-, 19-, and 20-HETE [7, 8]. It has been reported that Ras GTPase/MAP kinase pathway and cytochrome P-450 metabolites contribute to DOCA-Salt-induced hypertension and angiotension II-induced hypertension, as well [9,10]. Aim of this study was to determine the contribution of Ras farnesyl transferase, mitogen activated protein kinase (MAP kinase) and cytochrome P¬450 (CYP450) metabolites to ET-1 induced hypertension. Since it has been reported that activation of Ras/MAP kinase pathway in angiotensin II- and norepinephrine-induced hypertension is mediated by arachidonic acid metabolites [11, 12, 13], we wanted to analyze whether is the same or similar mechanism in ET-1 induced hypertension.

MATERIALS AND METHODS

Animals

All studies were done in 225 to 250 g male Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc. Animals were housed in a temperature controlled room (23°C) with a 12:12-hour light/dark cycle. All experiment procedures in this study were done in accordance with National Institutes of Health Guidelines for this kind of study. Animals were divided into: 1) control group, 2) ET-1 treated group (5 pmol/kg per minute), 3) ET-1 plus Ras farnesyl transferase inhibitor FPTIII (138 ng/min) treated group, 4) ET-1 plus MAP kinase

* Corresponding author: Farid Ljuca, Department of Physiology, University of Tuzla, Faculty of Medicine, Univerzitetska 1, 75 000 Tuzla, Bosnia and Herzegovina. Tel: +387-35-320-647
e-mail: farid.ljuca@untz.ba

Submitted 27 April 2011/Accepted 10 May 2011

inhibitor PD-98059 (694 ng/min) treated group, 5) ET-1 plus CYP450 inhibitor 17-ODYA (189 ng/min) treated group. Endothelin-1 was obtained from Amersham. Ras farnesyl transferase inhibitor FPTIII, MAP kinase inhibitor PD-98059 and CYP450 inhibitor 17-ODYA were obtained from New England Biolabs.

Methods

Endothelin-1 induced hypertension and blood pressure measurement

ET-1 (5 pmol/kg per minute) was chronically infused into to the jugular vein by use of mini-osmotic pump (Alzet model 2002) for 9 days in male Sprague-Dawley rats. Catheters (PE-60) connected to a mini-osmotic pump were inserted into the jugular veins under isoflurane anesthesia. Inhibitors of Ras farnesyl transferase FPTIII (138 ng/min), MAP kinase PD-98059 (694 ng/min), COX indomethacin (145 ng/min), LO baicalein, (124 ng/min) and CYP450 17-ODYA (189 ng/min) were administered by a mini-osmotic pump. Animals were anesthetized with sodium pentobarbital (60 mg/kg), and left femoral artery was exposed surgically. A small incision was made in the femoral artery. And a catheter was inserted and connected to a pressure transducer (Grass model 7m, Grass Instruments) for blood pressure measurement. Blood pressure was recorded on a polygraph (model 7D; Grass polygraph). Mean arterial blood pressure (MABP) was expressed as mm Hg.

Statistical analysis

Values are expressed as mean ± SEM. The data were analyzed by 1-way ANOVA, and difference between the means for multiple comparisons was determined by the Newman-Keuls test and a value difference of $p<0.05$ was considered statistically significant.

RESULTS

Chronic infusion of ET-1 at the rate of 5 pmol/kg per minute in conscious rats for 9 days significantly increased MABP (154±2 mm Hg versus 98±3 mm Hg in the control group, ($p<0.001$). Infusion of Ras farnesyl transferase inhibitor FPTIII (138 ng/min), MAP kinase inhibitor PD-98059 (694 ng/min) during the ET-1 treatment significantly attenuated MABP to 115±2.5 mm Hg, 109±3 mm Hg ($p<0.01$) (Figure 1). To determine whether activities of enzymes cyclooxigenase (COX), lipoxigenase (LO) and cytochrome P-450 (CYP-450) and their metabolites are involved in ET-1 induced hypertension, rats were infused with inhibitors of COX (indomethacin, 145 ng/min), LO (baicalein, 124 ng/min) and CYP-450 (17-ODYA, 189 ng/min) during the treatment by ET-1. CYP-450 inhibitor, but not COX and LO inhibitors, attenuated significantly MABP to 118±1.5 mm Hg ($p<0.01$) (Figure 2).

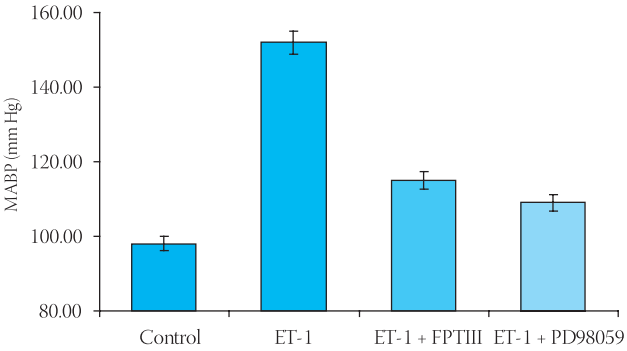

FIGURE 1. Effect of Ras farnesyl transferase inhibitor FPTIII and MAPK inhibitor PD98059 on MABP in ET-1 induced hypertensive rats

DISCUSSION

It has been previously reported that Ras/MAP kinase pathway activation by CYP-450 metabolites contributes to the development of DOCA-Salt-induced hypertension [9]. The present study demonstrates that Ras/MAP kinase pathway activated probably by CYP-450 metabolites contributes to the development of ET-1 induced hypertension, as well. Other vasoactive agents such as Norepinephrine (NE) and angiotensin II (ANG II) increase CaM kinase II and cPLA2 activities and thus release arachidonic acid [11]. The metabolites of arachidonic acid, such as 12- and 20-HETE made via cytocrome P-450 and lipoxygenase, through Ras/MEK/MAPK pathway activation contribute to vascular smooth muscle cell proliferation induced by NE or ANG II [12, 13, 14]. ET-1 promotes vascular smooth muscle

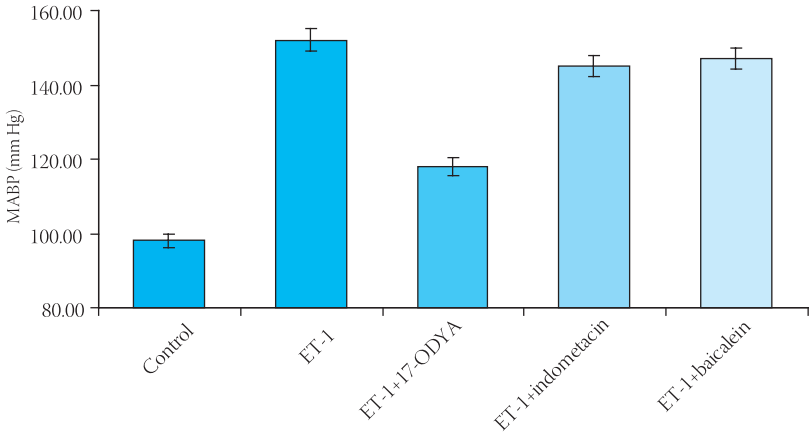

FIGURE 2. Effect of CYP-450 inhibitor 17-ODYA, COX inhibitor indometacin and LO inhibitor baicalein on MABP in ET-1 induced hypertensive rats

cell proliferation through extracellular signal-regulated kinase activation mediated by cPLA2 [6]. ET-1 is involved in the development and maintenance of hypertension [4, 5]. In our study, administration of ET-1 produced significant rise in MABP compared with that in control (normotensive) rats. Infusion of Ras farnesyl transferase inhibitor FPTIII, MAP kinase inhibitor PD-98059 and CYP450 inhibitor 17-ODYA, but not COX inhibitor indometacin or LO inhibitor baicalein, significantly attenuated MABP. Our findings suggest that ET-1 induced hypertension is mediated by CYP-450 metabolites and Ras/MAP kinase pathway activation. Similar results have been reported in DOCA-Salt-, norepinephrine- and Angiotensin II-induced hypertension, as well [9,10,12].

CONCLUSION

The present study demonstrates that Ras/MAP kinase pathway activated probably by CYP-450 metabolites contributes to the development of ET-1 induced hypertension. It seems to be common mechanism by which ET-1, ANG II and NE induce hypertension. Further investigation has to be done to confirm whether activation of RAS/MAP kinase signal transduction pathway induced by different vasoactive agents by arachidonic acid metabolites is a universal mechanism in the development of hypertension.

DECLARATION OF INTEREST

Authors report no conflict of interest related to this study.

REFERENCES

[1] Rubany GM, Polokoff MA. Endothelin: biology, biochemistry, pharmacology, physiology and pathophysiology. Pharmacol Rev 1994; 46:325-415.

[2] Masaki T, Miwa S, Sawamura T, Ninomiya H, Okamoto Y. Subcellular mechanism of endothelin action in vascular system. Eur J Pharmacol 1999; 375: 133-138

[3] Ljuca F, Drevenšek G. Endothelin-1 induced vascular smooth muscle cell proliferation is mediated by cytochrome P-450 arachidonic acid metabolites. Bosn J Basic Med Sci 2010; 10 (3): 223-226

[4] Mortensen LH, Pawlovski CM, Kanagy NL, Fink GD. Chronic hypertension produced by infusion of endothelin in rats. Hypertension 1990; 15: 729-733

[5] Sedeek MH, Llinas HD, Fortepiani L, Abram SR, Alexander BT, Reckelhoff JF, Granger JP. Role of oxidative species in Endothelin-1 induced hypertension. Hypertension 2003; 42: 806-810.

[6] Trevisi L, Bova S, Cargnelli G, Ceolotto G, Luciani S. Endothelin-1 induced arachidonic acid release by cytosolic phospholipase A2 activation in rat vascular smooth muscle cell via extracellualar signal-regulated kinases pathway. Biochem Pharmacol 2002; 64: 425-431.

[7] McGiff J.C. Prostaglandins, prostacyclin and thromboxanes Annu Rev Pharmacol Toxicol 1991; 31: 339-369.

[8] McGiff JC, Steinberg M, Guilly J. CYP-450 arachidonate products, a new class of lipid mediators. Trends Cardiovasc Med 1996; 6: 4-10.

[9] Muthalif MM, Benter IF, Khandekar Z, Gaber L, Estes A, Malik S, Parmentier JH, Manee V, Malik KU. Contribution of Ras GTPase/Mitogen-activated protein kinase and cytochrome P450 metabolites to Deoxycorticosterone-salt-induced hypertension. Hypertension 2000; 35: 457-463.

[10] Muthalif MM, Karzoun NA, Gaber L, Khandekar Z, Benter IF, Saeed AE, Parmentier JH, Estes A, Malik KU. Angiotensin II-induced hypertension: contribution of Ras GTPase/Mitogen-activated protein kinase and cytochrome P450 metabolites. Hypertension 2000; 36(4):604-609.

[11] Muthalif MM, Benter IF, Uddin MR, Malik KU. Calcium/calmodulin-dependent protein kinase IIalpha mediates activation of mitogen-activated protein kinase and cytosolic phospholipase A2 in norepinephrine-induced arachidonic acid release in rabbit aortic smooth muscle cells. J Biol Chem 1996; 271(47):30149-30157.

[12] Uddin MR, Muthalif MM, Karzoun NA, Benter IF, Malik KU. Cytochrome P-450 metabolites mediate norepinephrine-induced mitogenic signaling. Hypertension. 1998; 31(1 Pt 2): 242-247.

[13] Muthalif MM, Karzoun NA, Benter IF, Gaber L, Ljuca F, Uddin MR, Khandekar Z, Estes A, Malik K.U Functional significance of activation of calcium/calmodulin-dependent protein kinase II in angiotensin II-induced vascular hyperplasia and hypertension. Hypertension 2002; 39(2 Pt 2):704-709.

[14] Muthalif MM, Benter IF, Karzoun N, Fatima S, Harper J, Uddin MR, Malik KU.20-Hydroxyeicosatetraenoic acid mediates calcium/calmodulin-dependent protein kinase II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. Proc Natl Acad Sci USA 1998; 95 (21): 12701-12706.