On Endogenous Angiotensin II Antagonism in Hypertension
The Role of Dipeptidyl Peptidase III

María Paz Ocaranza, Jorge E. Jalil

See related article, pp 630–641

During the past 40 years, most of the new developed and effective antihypertensive drugs target the classic renin–angiotensin–aldosterone system (RAAS) by blocking receptors (such as the mineralocorticoid receptor or the angiotensin II type 1 receptor) and also by inhibiting enzymes within the angiotensin II (Ang II) formation pathway, such as the angiotensin-converting enzyme I (ACE I) and renin as well. More recently, a different group of targets within the RAAS has been explored as antihypertensive molecules. This parallel RAAS has been described as the natural vasodilatory and counterregulatory pathway of the RAAS. By year 2000, this pathway was discovered consisting of ACE2, angiotensin 1–9 (Ang1–9), Ang(1–7) and recently, alamandine. These endogenous molecules display biological effects opposed to Ang II; thus, their activation induces vasodilation, blood pressure reduction, anti hypertrophy, and antihyperplasia, suggesting a plausible antihypertensive role of this axis. In this issue of Hypertension, the role of dipeptidyl peptidase III (DPP III) as a new antihypertensive candidate has been characterized in the mice.1

Similar to DPP III, the carboxy peptidase ACE2 mediates degradation from Ang II to Ang (1–7) and also from Ang I to Ang (1–9). Both peptides contribute to the antihypertensive/vasoprotective effects of the counterregulatory RAAS pathway (Figure). Interest in ACE2 as a therapeutic target has led to the synthesis of small ACE2 activator molecules, which lower blood pressure, improve myocardial function, and reverse myocardial and perivascular fibrosis in the spontaneously hypertensive rat. As an alternative to pharmacological ACE2 activation, recombinant human ACE2 has been shown to lower blood pressure in experimental hypertension. A phase I study in healthy volunteers demonstrated sustained (>24 hours) suppression of circulating Ang II levels after a single intravenous injection of recombinant human ACE2.2

As a direct enzymatic product of ACE2, Ang (1–7) has been studied profusely, and it has shown to activate the G-protein–coupled Mas receptor, which triggers a signaling cascade that results in vasodilation, oxidative stress reduction, and antihypertrophic and antifibrotic effects. A cyclic Ang (1–7) analog containing a thioether bridge that makes it resistant to enzymatic digestion and also a formulation of Ang (1–7) incorporated to hydroxypropyl-β-cyclodextrin/Ang (1–7) have been synthesized, and both have shown to be cardioprotective in animal models of myocardial infarction and insulin resistance/type 2 diabetes mellitus.3,4

More recently, it has been shown that chronic Ang (1–9) administration decreases blood pressure in experimental hypertension induced by Ang II infusion and also in the Goldblatt model (2K-1C).5 Ang (1–9) also reduces cardiovascular damage induced both by hypertension6 and myocardial infarction.7 These beneficial effects of Ang (1–9) are mediated directly through binding to the AT2 receptor.5

A novel member of the Ang family, Ala1-Ang (1–7) (alamandine), has been isolated both from the human plasma and the rat heart.7 Alamandine is a product of decarboxylation of the N-terminal Asp residue of Ang II to form Ala, which has been demonstrated in the heart, followed by hydrolysis of Ala1-Ang II by ACE2. Alamandine is similar in structure to Ang (1–7) except for the replacement of its N-terminal Asp residue by Ala. This heptapeptide has antihypertensive, antifibrotic, and central cardiovascular effects similar to those of Ang (1–7), but it works through a different receptor, the Mas-related G-protein–coupled receptor member D.7 Alamandine incorporated into β-cyclodextrin (alamandine/hydroxypropyl-β-cyclodextrin) is orally active, reduces blood pressure in the spontaneously hypertensive rat, and inhibits cardiac fibrosis in isoproterenol-treated rats.7 The oral bioavailability of alamandine/hydroxypropyl-β-cyclodextrin has revived prospects for exploring the therapeutic antihypertensive potential of Ang (1–7)–related peptides.

Most research about the DPP family has been focused on DPP IV and its family members (Table) in several biological paths and different pathologies, such as glucose homeostasis and diabetes mellitus, the immune system and inflammation, and atherosclerosis.8 Within this group, there is a significant connection between their members about substrate specificity, inhibitors, and functions.9 In Dahl salt-sensitive rats, high-salt diet for 7 days increases blood pressure, and treatment with the DPP IV inhibitor vildagliptin reduces circulating DPP-4 activity, increases plasma glucagon-like peptide 1, and reduces the development of salt-induced hypertension, which is associated to increased urine sodium excretion.9 Together with ACE2 and the abovementioned vasoactive peptides Ang (1–7), Ang (1–9), and alamandine, DPP III is...
a multifaceted cytosolic oligopeptide N-end cutter that regulates, among others, the Ang II and Ang IV turnover (Figure). Thereby, this enzyme can regulate several physiological and pathological processes associated with these peptides. DPP III is an ubiquitous peptidase, and its activity has also been detected in extracellular fluids, such as retroplacental serum, seminal plasma, and cerebrospinal fluid. The mechanism of entry of this peptidase into these fluids is not clear (it could be the result of its release as a consequence of injury or death of the cell of its origin or by secretion via prostasome-like membranous bodies). Tetrapeptides to octapeptides have proved to be their best substrates, and any modification of the C-terminal carboxyl group does not affect the catalytic efficacy of the peptidase.

In this issue Pang et al, in an elegant proof of concept study in mice, have shown that DPP III administered during 4 weeks (3× per week by intravenous injection), through catalytic reduction of Ang II levels, significantly diminished systolic blood pressure, cardiac hypertrophy, and myocardial fibrosis induced by Ang II in an extent at least similar to the effect of the angiotensin receptor blocker candesartan in effective antihypertensive doses. In the same experiments, they observed that DPP III diminished urine albumin excretion, kidney damage, and the renal protein levels of the pro-inflammatory molecule monocyte chemoattractant protein-1 and the procoagulant platelet activator inhibitor-1.

The focus of this study was on the capability of DPP III to hydrolyze Ang II, the main effector of the RAAS. In addition, this enzyme is able to hydrolyze Ang IV implicated in hypertrophy, the nuclear factor kappa-light-chain-enhancer of activated B cells activation and also in increased levels of platelet activator inhibitor-1, monocyte chemoattractant protein-1, interleukin-6, and tumor necrosis factor-α. Moreover, Ang IV does regulate cell growth in cardiac fibroblasts, endothelial cells, and vascular smooth muscle cells as well.

Future aspects to be answered to precise the antihypertensive role of DPP III starting from this study are reproducibility, efficacy, and safety in different experimental models of hypertension; its effect on hypertension independent of Ang II—in this study, no antihypertensive effect was observed in noradrenaline-induced hypertension; DPP III delivery and bioavailability; and also the simultaneous measurements of levels of other vasodilatory peptides from the RAAS, such as Ang (1–7), Ang (1–9), and bradykinins. Another interesting issue, which has been preliminary explored in this study, is the possible synergistic effect by combining DPP III with an angiotensin II receptor blocker (or with an ACE inhibitor) in terms of reversing residual hypertensive cardiovascular and renal damage.

Besides, as the in vitro findings here on the kinetics of its hydrolytic activity show that Ang II is cleaved by DPP III with a $K_m=3.7 \mu\text{mol/L}$, a higher value compared with ACE2 (with a $K_m=2 \mu\text{mol/L}$), these data could indicate a lesser catalytic efficiency of DPP III with respect to ACE2. Furthermore, it is well known that ACE2 activity can be regulated by ACE inhibitors and angiotensin II receptor blockers. Therefore, it seems relevant to determine whether conventional hypertension treatment could regulate the activity of DPP III.

This study using DPP III was aimed to effectively reduce Ang II levels by enzymatic cleavage to Ang IV and to C-terminal tetrapeptide sequence. This peptidase belongs to

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**Table. The DPP Family and Some Known Relevant Functions**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPP IV</td>
<td>Glucose homeostasis</td>
</tr>
<tr>
<td>Fibroblast activation protein α</td>
<td>Fibronolysis, extracellular matrix remodeling, inflammation, and tumor growth</td>
</tr>
<tr>
<td>DPP8</td>
<td>Not well known</td>
</tr>
<tr>
<td>DPP9</td>
<td>Not well known</td>
</tr>
<tr>
<td>DPP II</td>
<td>Not well known</td>
</tr>
<tr>
<td>Prolyl carboxypeptidase</td>
<td>Angiotensin II inactivation</td>
</tr>
<tr>
<td>Prolyl oligopeptidase</td>
<td>In vitro inactivation of several neuropeptides</td>
</tr>
</tbody>
</table>

DPP indicates dipeptidyl peptidase.
a rather new concept to treat hypertension based on increasing endogenous Ang II antagonists, the beneficial molecules, by different mechanisms. ACE2, Ang (1–7), alamandine, and Ang (1–9) now belong to this group of new endogenous angiotensin II antagonism. Although further research is necessary to establish the antihypertensive role of DPP III, this study\(^1\) has shown that DPP III possesses many attributes to become a next musketeer.

**Sources of Funding**
This work is partially supported by Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), Chile: grants FONDEF D11I1022 to M.P.O and J.E.J, FONDAP 15130011 to Dr Ocaranza and Fondecyt 1161739 to Dr Jalil.

**Disclosures**
None.

**References**


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Hypertension. 2016;68:552-554; originally published online July 25, 2016; doi: 10.1161/HYPERTENSIONAHA.116.07471

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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