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Molecular and evolutionary perspectives of the renin-angiotensin system from lamprey

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ABSTRACT

The recent advance and revision on the renin-angiotensin system in lamprey were summarized and we emphasized that presence of two types of angiotensins (Angs) in lamprey. Due to the parasitic nature on fish blood, teleost-type Angs were produced in their buccal gland and secreted into the lamphredin to evade the host immunorejection. A native lamprey angiotensinogen (AGT) was identified in genome and it retains serine-protease inhibitor activity for thrombin that regulates the blood coagulation pathway. The native lamprey angiotensin II (Lp-Ang II) is hypotensive instead of hypertensive, suggesting a functional divergence on cardiovascular regulation from the main vertebrate groups. The renin gene was absent from the lamprey genome so far, and the mutation on the renin-recognition site on lamprey AGT suggested that other proteases may have replaced the role of renin. Lp-Ang II was shown to bind to AT1 receptor and internalized, but the downstream signaling was still unknown. Molecular and phylogenetic evidence on invertebrate ACE-like proteins indicated that they were not homologous to those in vertebrates and could be acting on other native peptides. Although it was generally believed that the RAS was a well-conserved hormone system in vertebrates and invertebrates, revision by molecular data indicated that invertebrates lack homologous RAS components while lamprey possess an almost complete RAS. This suggests that the hormone cascade system was first evolved around cyclostome emergence and invertebrates could have taken up the RAS components from vertebrates through horizontal gene transfer.

1. History of the RAS discovery in lamprey

It has been a long debate on whether cyclostome including hagfishes and lampreys possess a complete set of the renin-angiotensin system (RAS). In 1970s, it was considered that elasmobranchs, hagfish, and lamprey do not possess a complete RAS as juxtaglomerular granules was not found (Nishimura et al., 1970) and no vasopressor substance was produced from the incubation of plasma (angiotensinogen source) with kidney extract (renin source) with a bioassay using rats. This biochemical method (referred as incubation method below) assumes that all Ang I from vertebrate were vasopressors and certain cross-reactivities were present among different vertebrate species, but it was not the case in lamprey shown by follow-up studies (Wong and Takei, 2011; Kumar et al., 2014). Subsequent studies in Japanese dogfish showed that vasopressor substance was produced by the incubation method using a bioassay in eels, resulting in the identification of [Asn^1, Pro^3, Ile^5, Gln^8] Ang I in an elasmobranch (Takei et al., 1993a). Encouraged by the success in elasmobranchs, great efforts were made to isolate Ang I from cyclostomes in 1990s. As hagfish have only ca. 10 glomeruli on both sides of dorsal wall of peritoneal cavity, lampreys was selected as the experimental animals since true kidney structure is present. Large quantity of plasma (ca. 100 mL) and kidney tissues (ca. 50 g) from two lamprey species (*Lampetra fluviatilis* and *Petromyzon marinus*) were collected for incubation and vasopressor substances were finally produced (Rankin et al., 2004; Takei et al., 2004). Interestingly, the sequence of Ang I produced by the incubation method in two lamprey species was identical to teleost-type [Asn^1, Val^5, Thr^9] Ang I in both lamprey species, although cyclostomes are phylogenetically more distant to teleosts compared with elasmobranchs. Since the incubation in various species were processed in the same laboratory, the reaction efficiency can be compared. The yield of Ang I after the incubation in lamprey was ca. 1/10 of fishes and 1/100 of tetrapods except amphibians (Table 1). As the plasma samples of bowfin was not treated with EDTA and was thawed during shipping from Canada, the Ang I yield from the incubation was exceptionally low (Takei et al., 1998). The low efficiency of the incubation in

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Table 1
Yield of angiotensin I by incubation of plasma with kidney extract from various vertebrates.

Animal	Plasma (mL)	Kidney (g)	Ang I (nmol)	Yield (pmol/mL)	References
River lamprey (*Lampetra fluviatilis*)	125	51	0.13	1	Rankin et al. (2004)
Sea lamprey (*Petromyzon marinus*)	160	40	1.12	7	Takei et al. (2004)
Dogfish (*Scyliorhynus canicula*)	60	8	1.40	23	Takei et al. (1993a)
Bowfin (*Amia caiva*)	105	35	0.72	7	Takei et al. (1998)
Flounder (*Platichtys flesus*)	45	16	1.58	35	Balment et al. (2003)
Lungfish (*Neoceratodus forsteri*)	120	36	2.62	22	Joss et al. (1999)
Axolotl (*Ambystoma mexicanum*)	96	20	4.13	43	Takei et al. (2004)
Alligator (*Alligator mississippinesis*)	4.5	2	1.00	222	Takei et al. (1993b)
Quail (*Coturnix coturnix japonica*)	55	14	10.00	182	Takei and Hasegawa (1990)
Emu (*Dromieceus novaehollandiae*)	30	8	0.48	16	Takei et al. (2004)

lamprey suggested that angiotensinogen (AGT) concentration and/or renin activity were low in lamprey samples. Furthermore, [Asn^1, Val^5, Thr^9] Ang I was not able to induce a vasopressor response while [Asn^1, Val^5] Ang II elicited a weak vasopressor response at high concentration (0.1 μmol/kg) in river lamprey (Rankin et al., 2004). These data raised the question whether the purified [Asn^1, Val^5, Thr^9] Ang I was indeed the native angiotensin in lamprey.

After the release of lamprey draft genome in 2007, an AGT that contains a putative angiotensin sequence (EEDYDERPYMQPF, Lp-Ang II) was identified via data mining (Table 2). Subsequent cDNA cloning of lamprey AGT revealed that its expression pattern in various tissues was highly similar to those of other vertebrates with liver being the major production site (Wong and Takei, 2011). This is contradicting to the previous biochemical results from incubation method and thus vigorous validation was performed. Homologous RIA was developed and the plasm Lp-Ang II levels were determined, while teleost-type Ang II were undetectable in the same samples (Wong and Takei, 2011). Lp-Ang II is also unique in cardiovascular action as it decreased blood pressure rapidly when injected into lamprey, unlike the general vasopressor effect as in other fish and mammals (Fig. 1). As the previous incubation method relied on the vasopressor bioassay to detect the native Ang I while the Lp-Ang II has no cardiovascular effects when injected into the eels (Wong and Takei, 2011), it is not surprising that the native lamprey Ang I were not identified.

Table 2
Angiotensin I sequences in vertebrates. Shaded residues represent amino acid substitution from human Ang I.

Species	Sequences
Human	-----DRVYIHPFHL-
Rat	-----DRVYIHPFHL-
Bovine	-----DRVYVHPFHL-
Microbat	-----DRLYIHPFHML
Kangaroo rat	-----DRIYVHPFHFL
Opossum	-----DRVYVHPFHL-
Tasmanian devil	-----ERVYVHPFYL-
Platypus	-----DRVYVHPFHFL
Chicken	-----DRVYVHPFSL-
Emu	-----DRVYVHPFNL
Anole lizard	-----DRVYVHPFYL-
Alligator	-----DRVYVHPFAL-
Western clawed frog	-----NRVYIHPFNL-
Coelacanths	-----NRVYVHPFNL-
Lungfish	-----NRVYVHPFTL-
Spotted gar	-----NRVYVHPFKL-
Bowfin	-----NRVYVHPFNL-
Seabream	-----NRVYIHPFHL-
Eel	-----NRVYVHPFGL-
Stingray	-----DRPYIHPFFL-
Little skate	-----YRPYIHPFSL-
Dogfish	-----NRPYIHPFQL-
Elephant shark	-----NRPHIHPFLL-
Sea lamprey	EEDYDDRPYMQPFHL-
	* :::** :

Although the Ang I sequences are relatively conserved among vertebrates, considerable substitution at position 1, 3, 5 were frequently observed among different species (Table 1). [Asp1] dominate the amniotes except [Glu1] is found in Tasmanian devil. Although [Asn1] is mostly found among fishes, the tissue asparaginase activities convert the [Asn1] to [Asp1] in AGT (Wong and Takei, 2012), thus [Asp1] Ang II was present in circulation at certain levels. Although [Asp1, Val5, Asn9]-Ang I was purified from bowfin by the incubation method (Takei et al., 1998), recent molecular data from transcriptome sequencing revealed that the native bowfin Ang I should be [Asn1] instead of [Asp1] (Table 2). Similar to the low incubation efficiency mentioned above, the endogenous asparaginase may have converted the [Asn1] of the bowfin AGT to [Asp1] as the samples were thawed during the shipping. [Pro3] is common among cartilaginous fishes and lamprey with strong codon usage bias, and most other vertebrates possess [Val3] with some exceptions of [Leu3] or [Ile3] in microbat and kangaroo rat respectively. [Met5] and [Gln6] are unique substitutions in lamprey and could be important to determine the species specificity for the Lp-Ang II, which induced no cardiovascular responses in other teleosts. In Lp-Ang II, the N-terminal extension [Glu-Glu-Asp-Tyr-Asp] is also important to determine the hypotensive responses as the deletion of these residues abolished the hypotensive effect of the Lp-Ang II (Wong and Takei, 2011).

Ang II is not always vasopressor in vertebrates. Bird Ang II elicited bi-phasic depressor and pressor responses via nitric oxide and adrenergic pathways respectively (Nishimura et al., 1982). However, a direct comparison between the cardiovascular effects of lamprey and bird is not appropriate because NO is not a vasodilator in lamprey (Evans and Harrie, 2001). The cardiovascular tone in lamprey was not regulated by cholinergic but nicotinic tone (Lukomskaya and Michelson, 1972). Further comparative studies are required to characterize the role of RAS in cardiovascular regulation in lamprey.

2. Teleost-type angiotensins in lamprey buccal glands

Parasitic lamprey secreted fish-type angiotensins in their buccal gland, and the secretion (called lamphredin) could suppress the host immuno-rejection during blood feeding – endocrine mimicry (Wong et al., 2012). Fasted lamprey possessed negligible teleost-type Ang II in their plasma and buccal glands but introduction to fish host increased the teleost-type Ang II significantly in both tissues, suggesting that the peptide production is sensitive to feeding stimuli (Fig. 1). It is unlikely that the lamprey utilized the host proteins to produce teleost-type Ang II because force feeding of shark blood increased teleost-type Ang II but not cartilaginous fish-type Ang II (Wong et al., 2012). Besides teleost-type Ang II, Ang I with the same peptide sequence described in the incubation method studies (Rankin et al., 2004; Takei et al., 2004) was also identified in buccal gland secretion and biochemical characterization suggested that the Ang I and Ang II were generated from different pro-

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