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Research paper

The presence of teleost-type angiotensin components in lamprey buccal gland suggests a role in endocrine mimicry

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ABSTRACT

Previous characterization of a native lamprey angiotensin II (LpAng II) that possesses a different sequence and function than teleost-type angiotensin II (Ang II) has raised a question as to the role of teleost-type angiotensin peptides in lampreys. In this study, teleost-type angiotensin like-peptides were identified in the buccal gland of lampreys by immunoassays and immunohistochemistry. The possible sources of angiotensin like-peptides were investigated in lampreys by manipulating their choice of host and food. Ang II immunoreactivity (irAng II) was detected in the buccal gland and plasma of feeding phase sea lampreys exposed to Atlantic cod, but was mostly absent in fasting lamprey. Qualitatively, the HPLC profiles of irAng II observed in the plasma, when present, were highly similar to those in buccal gland, implying that the buccal gland could be a source of plasma Ang II. Japanese lampreys force-fed with dogfish blood had significantly elevated concentrations of irAng II in their buccal glands when compared to unfed individuals, suggesting that feeding stimuli may have enhanced buccal gland activity. Teleost-type Ang II-containing proteins, other than angiotensinogen, are present in the buccal gland as trypsinization generated Ang II *in vitro*, and the HPLC profile of these irAng II was highly comparable to those naturally present in the buccal gland. [Asn^{1}, Val^{5}, Thr^{9}]-Ang I that was identified in the buccal gland of Japanese lampreys has the same amino acid sequence to those previously isolated from the incubation of plasma and kidney extract, providing an alternative explanation for the previous isolation of teleost-type Ang I in lampreys. irAng I and irAng II were localized in the granule-like structures in the apical region of the secretory epithelia, suggesting that these peptides may be active components of lamphredin. The teleost-type angiotensin peptides in the buccal gland secretion suggested that these host-specific peptides could be part of the endocrine mimicry strategy used by lampreys to evade host immune responses and reduce immune-rejection.

1. Introduction

Fishes respond to external and internal parasitism using both the innate [1] and complement immune systems [2]. The evolutionary arms race between parasites and fish hosts has produced fascinating immunological strategies, including intracellular disguise, behavioral migration, and anti-immune mechanisms, to maintain an ecological balance [3]. Successful parasites can remain attached to their host for a long period of time, maximizing the gain of nutrition and protection; however, they have to cope with the hosts' immune responses and rejection [4]. Parasites such as leeches and schistosomes possess immuno-evasion mechanisms to cope with host rejection [5]. These parasites can secrete hormones that are homologous to those of the host, a phenomenon known as endocrine mimicry [6]. The presence of homologous hormones at the interface between parasite and host may allow the parasite to be overlooked by the host immune systems [4]. As shown in schistosomes, the source of homologous proteins may be a result of horizontal gene transfer [7].

Lampreys are one of the most basal vertebrate lineages [8]. There are parasitic and non-parasitic species of lampreys. Parasitic lampreys begin their life as filter-feeding larvae and remain in this

Abbreviations: LpAng II, lamprey angiotensin II; Ang I, angiotensin I; Ang II, angiotensin II; ir, immunoreactive; RAS, renin-angiotensin system; ACE, angiotensin-converting enzyme; MS-222, ethyl 3-aminobenzoate methanesulfonate; RIA, radioimmunoassay; TFA, trifluoroacetic acid; DAB, 3,3′-diaminobenzidine; TRH, thyrotropin-releasing hormone.

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stage for 2–7 years after which they undergo a metamorphosis developing into a parasitic lamprey that is able to feed on fish – this stage lasts about 2–3 years. After this period, they cease feeding and migrate into streams where they undergo spawning and then die. Endocrine mimicry has not been examined in lampreys, however, if endocrine mimicry does exist in lampreys, fish-type hormones should be present in their salivary secretion. During metamorphosis when a lamprey transforms from the larval phase (ammocetes) to the parasitic phase, a pair of buccal glands develops and produces a secretion called lamphredin into the buccal cavity [9]. Non-parasitic species of lampreys also possess buccal glands at adult stage and the presence of these glands is likely a common ancestral character [10]. The secretory epithelia of the buccal gland in pouched lamprey (*Geotria autralis*) secrete lamphredin into the lumen through merocrine and apocrine pathways [11]. The majority of buccal gland studies focused on the anti-coagulating effects of lamphredin [10,12–14] but it was recently shown that the lamphredin of Japanese lamprey (*Lethenteron japonicum*) contains a large amount of unknown peptides [15]. Besides anti-coagulating factors, other proteins such as peroxiredoxin and translationally controlled tumor protein, which may be involved in immunosuppression, were also identified from the buccal gland of lamprey [16–18].

Several reports have shown that parasites may exploit their hosts' endocrine signals and incorporate the hosts' growth factors and hormones for their own development [19–23]. Teleost-type immunoreactive angiotensins were previously measured in the plasma of river lamprey (*Lamptera fluiatilis*) [24]; and incubation of plasma and kidney extract generated [Asn^1, Val^5, Thr^9]-Ang I in river lamprey [25] and sea lamprey [26], indicating that teleost-type angiotensins are present in lamprey. Since then, we have identified and characterized a native lamprey angiotensin II (LpAng II) by molecular cloning and biochemical analyses, and the data from this study suggest that teleost-type angiotensins may not be the native hormones in lamprey [27]. Therefore, the presence of teleost-type angiotensins in lamprey could be an adaptation related to parasitism on teleosts as a result of potential horizontal gene transfer or an exploitation of host proteins.

The peptides and enzymes of the renin-angiotensin system (RAS) such as angiotensin I (Ang I), angiotensin II (Ang II), and angiotensin-converting enzyme (ACE) are commonly found in leech and schistosome secretions [6] and could play a role in immuno-modulation. In vertebrates, Ang II increases blood pressure, thirst, and sodium reabsorption in kidney and the effects are involved in the control of blood pressure and volume [28]. In mammals, Ang II acts through AT1 and AT2 receptors and governs the transcription of pro-inflammatory mediators such as transforming growth factor β, interleukin 1β, tumor necrosis factor α, and plasminogen activator inhibitor type 1, in both resident tissue and infiltrating cells such as macrophages (for review, see [29]). However, no comparable studies are yet available in non-mammalian vertebrates, therefore we aimed to establish the relationship between immunological responses and angiotensins in lamprey, to understand the evolutionary and comparative aspects of vertebrate parasitism.

Intact protein absorption across the intestine in teleost fishes is well-established and fishes are capable of absorbing and transporting macromolecules into their blood stream [30]. Lampreys are early-diverged vertebrates and it is likely that they are also capable of absorbing intact proteins, including angiotensinogen and renin, from their host through the diet. In order to delineate the source of angiotensin, it is important to define hosts that possess angiotensins with distinctive sequences. Therefore, if the host angiotensin or angiotensinogen was absorbed and utilized in lamprey, the host-specific angiotensin sequences could be identified in lamprey blood. In the present study, we investigated the possible sources of angiotensin peptides in lampreys that are homologous to other teleosts through manipulations of their host and food. Our results suggest that the buccal gland of lamprey is a source of teleost-type angiotensins and these host-specific peptides are possibly involved in endocrine mimicry to reduce host immune-rejection.

2. Materials and methods

2.1. Fasting and cod exposure experiment in sea lamprey

Landlocked feeding (parasitic)-phase sea lampreys (*Petromyzon marinus*) ($n = 21$, 27–96 g body weight) were collected from Lake Huron, Michigan, transported to the University of New Hampshire (UNH) and were kept in a temperature-controlled recirculating system at the Aquatic Research Center at UNH. The water temperature was maintained at 12 °C and the animals were exposed to a 12 h:12 h light dark cycle. The lampreys were originally kept in freshwater and gradually acclimated to seawater at a maximum rate of 5‰ increment per day for seven days. The lampreys were finally acclimated to 27‰ but only 9 individuals survived until sampling. Lampreys were not fed for 2 weeks after capture and the salinity acclimation was performed during this fasting period. After 2 weeks of laboratory fasting, six individuals were randomly assigned to the fasting group and they were fasted for another 1 week. Another six lampreys were assigned to the cod group and these lampreys were allowed to feed on the Atlantic cod (*Gadus morhua*) *ad libitum* for 1 week. In this group, only 3 cod-exposed lampreys survived until the final sampling time. The experimental and sampling procedures were approved by UNH IACUC (#090703). Lampreys were anesthetized with 0.1% ethyl 3-aminobenzoate methanesulfonate (MS-222; Sigma, St. Louis, MO) neutralized with sodium bicarbonate until ventilation movement ceased. Blood samples were then obtained by heart puncture using syringes containing an inhibitor cocktail (0.05 M 1,10-phenanthroline, 0.225 M potassium EDTA, and 0.1 TIU aprotinin) that prevents blood clotting and inhibits peptide and protein degradation. Blood samples were centrifuged immediately at 10,000 rpm for 5 min and plasma fractions were collected and frozen at −20 °C until use. Buccal glands were dissected, snap frozen in liquid nitrogen, and stored at −20 °C until use. Buccal glands were boiled (100 °C) in 1 M acetic acid (1:5 w/v) for 5 min and homogenized with a TissueLyser II (Qiagen, MD). The homogenate was centrifuged to remove debris in order to obtain clear buccal gland extract. Plasma or buccal gland homogenates were extracted by equal volumes of acidic acetone (acetone:water:1 M HCl = 40:5:1) [24]. Precipitated proteins were removed by centrifugation and soluble fractions were lyophilized using a rotary evaporator. The freeze-dried samples were then transferred to the Atmosphere and Ocean Research Institute, University of Tokyo for further analysis.

The partially purified plasma and buccal gland extract were resolved by a reverse-phase HPLC system (Tosoh PU-980, Tokyo) attached to a UV-absorbance detector (Tosoh, UV-970, Tokyo) and utilizing an analytical column (Tosoh, ODS 100V column, 5 μm, 4.6 mm I.D. × 25.0 cm, Tokyo). A linear gradient from 15 to 35% acetonitrile in 0.1% trifluoroacetic acid (TFA) over 40 min was used for separation. The column was maintained at 40 °C and flow rate was adjusted to 1 mL/min. The protocol resulted in the elution of a single peptide within 0.4 min. Corresponding 1 mL fractions eluting near the retention time of [Asn^1, Val^5]-Ang II (27 min), [Asn^1, Ile^5]-Ang II (32 min), and LpAng II (32–33 min), were collected and dried by lyophilization. The HPLC resolved fractions were reconstituted in radioimmunoassay (RIA) buffer for the