Synthesis of Nonmammalian Angiotensins and Their Comparative Pressor Properties in Dogfish Shark, Domestic Chicken, and Rat

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SUMMARY To understand how vertebrates utilize angiotensins during evolutionary development, we undertook studies to synthesize and/or characterize angiotensin-like peptides from nonmammalian species. The present study describes the synthesis of [Asp⁵,Val⁵,Asn⁹] angiotensin I (bull frog, Rana Catesbeiana) (I), [Asn⁵,Val⁵,His⁹] angiotensin I (Japanese goosefish, Lophius litulon) (II), [Asn⁵,Val⁵,Asn⁹] angiotensin I (chum salmon, Oncorhynchus keta) (III), and [Asn⁵,Val⁵,Tyr⁹] angiotensin I (related to native angiotensin in snake, Elaphe climocophora) (IV). Pressor properties of these peptides were compared with the peptides isolated from other species and related synthetic analogs in one representative species from three distinct classes of vertebrates: 1) elasmobranchs: spiny dogfish shark; 2) birds: domestic chicken; and 3) mammals: rat. The effect of angiotensins on short circuit current (to compare sodium and water permeability) was studied by adding these on the dermal side of the isolated frog skin.

In the rat pressor bioassays, the above peptides possessed, respectively, I, 87.8%; II, 51.5%; III, 65.2%; and IV, 60.3% pressor activity of [Ile⁵] angiotensin II, which was blocked with a converting enzyme inhibitor, captopril. In the conscious dogfish shark, the percentage increase of blood pressure based on preinjection level (= 100) in the dorsal aortic pressure was 35% to 60% for [Asp⁵,Ile⁵,His⁹] angiotensin I (human) (3 μg/kg), [Asp⁵,Val⁵,Ser⁹] angiotensin I (chicken) (3 μg/kg), [Asp⁵,Ile⁵] angiotensin II (3.6 μg/kg), and [Asn⁵,Val⁵] angiotensin II (6 μg/kg). Likewise, a 30% to 35% increase in blood pressure was obtained with angiotensin III (3 μg/kg), [Ile⁸] angiotensin II (4.4 μg/kg), and [Ile⁸] angiotensin II (9.1 μg/kg). [Sar¹,Ile⁸] angiotensin I and that of [Asp⁵,Val⁵,Ser⁹] angiotensin I was totally blocked by SQ 20,881, while the pressor response produced by all the above angiotensins and analogs was completely abolished by prior treatment of dogfish with phentolamine. As compared to the results in mammalian species (e.g., rat), [Asn⁵,Val⁵] angiotensin II, [Ile⁸] angiotensin II, and angiotensin III produced much reduced pressor activity in the anesthetized chicken.

The results suggest: 1) pressor activity of angiotensin I-like peptides in rat and dogfish is due to their conversion into the corresponding angiotensin II; 2) pressor response to angiotensins I, II, III, [Ile⁸] angiotensin II, and [Ile⁸] angiotensin II in the dogfish shark may be mediated by catecholamines; 3) presence of aspartic acid residue in position I of angiotensin II is important for its pressor action in the chicken; and 4) Ala-Pro-Gly-[Ile⁵,Val⁵] angiotensin II, characterized from the skin of the Australian frog (Crinia georgiana), has properties that may prove to be compatible with a role in the regulation of salt and water in amphibians. (Hypertension 5 (supp V): V-22-V-28, 1983)

KEY WORDS • bull frog • angiotensin I • Japanese goosefish • chum salmon • snake • blood pressure • elasmobranchs • birds • mammals • frog skin • evolution

From the Research Division, Cleveland Clinic Foundation (Drs. Khosla and Bumpus), Cleveland, Ohio; the University of Tennessee Center for the Health Sciences (Dr. Nishimura), Memphis, Tennessee; the School of Medicine, East Carolina University (Dr. Opdyke), Greenville, North Carolina; and the National University of Tucuman (Dr. Coviello), Tucuman, Argentina. Supported in part by the National Institutes of Health Research Grants HL-6835 and HL-22674-02.

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fug (Crinia georgiana). The present paper reports the synthesis of peptides characterized in other species, namely, [Asp1,Val5,Asn9] angiotensin I (bullfrog, Rana catesbeiana), [Asn1,Val3,His9] angiotensin I (Japanese goosefish, Lophius litulon), [Asn1,Val5,Asn9] angiotensin I (Chum Salmon, Oncorhynchus Keta), and [Asp1,Val3,Tyr9] angiotensin I (related to native angiotensin in snake, Elaphe climocophora) (table 1). Pressor properties of these peptides have been compared with known angiotensins and related synthetic analogs in one representative species from three distinct classes of vertebrates: 1) elasmobranchs: spiny dogfish shark; 2) birds: domestic chicken; and 3) mammals: rat. In a preliminary investigation, the effect of frog-skin angiotensin II on short circuit current has been compared with that of human angiotensin II and goosefish angiotensin II when these peptides were added to the dermal side of the frog skin.

Methods

Synthesis and Purification of Angiotensins

All the angiotensins were synthesized by the solid-phase procedure. The protocol used for the synthesis was similar to that previously described by Khosla et al. for the synthesis of analogs of angiotensin II. All couplings were carried out through N,N-dicyclohexyl-carbodiimide (DCCI), except for Boc-L-asparagine, which was coupled through its p-nitrophenyl ester. Side-chain functional protecting groups employed were: Asp (β-Bzl), Arg (NO2), Tyr (2,6-Cl2Bzl), and His (Bzl). Alpha-amino groups were blocked with the tert-butyloxycarbonyl group. Quantitative coupling of Boc-His (Bzl) was accomplished by adding 1 M excess of 1-hydroxybenzotriazole during coupling with DCCI. At the end of the synthesis, the peptide was cleaved from the polymer and partially deblocked with HBr/CF3COOH at room temperature. Complete deblocking of the peptide was carried out by hydrogenolysis over 5% palladium/BaSO4 in a mixture of MeOH/ACOH/H2O (5:1:1) under 2 atmospheres (atm) of H2 for 36 to 48 hours in a Parr hydrogenation apparatus. Amino-acid analysis of the crude product was carried out to ensure deblocking of side-chain protecting groups.

The crude product in each case was purified on a column of Bio-Rad anion-exchange resin (AG-1 × 2, 200-400 mesh, acetate form) by eluting with 0.1 M ammonium acetate buffer (pH 8.5). The peptide was eluted from the polymer by partially deblocked with HBr/CF3COOH at room temperature. Complete deblocking of the peptide was carried out by hydrogenolysis over 5% palladium/BaSO4. After the reactions, MeOH/ACOH/H2O (5:1:1) under 2 atmospheres (atm) of H2 for 36 to 48 hours in a Parr hydrogenation apparatus. Amino-acid analysis of the crude product was carried out to ensure deblocking of side-chain protecting groups.

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terns. Fractions in the column chromatography were collected with emphasis on purity rather than on yield. No attempt was made to rechromatograph the minor fractions for identification purposes.

TLC was conducted on silica gel or cellulose supported on glass plates. The solvent systems (upper phase) used were: 1) n-BuOH-AcOH-H$_2$O (BAW), 4:1:5; 2) n-BuOH-AcOH-H$_2$O-pyridine (BAWP), 10:2:5 or 65:35:35; and 3) n-BuOH-pyridine-H$_2$O (BPW), 10:2:5 or 65:35:35. Ionophoresis was carried out on S & S 2043A filter paper strips in a Beckman electrophoresis cell (Durrum type), Model R, Series D, at 400 V using 0.5 M AcOH (pH 2.2) and Beckman barbiturate buffer B-2 (pH 8.6). Histidine was run simultaneously as a reference compound, and E (His) indicates the electrophoretic mobility relative to histidine = 1.00. Location of compounds on chromatograms was carried out with ninhydrin reagent and/or diazotized sulfanilic acid (Pauly’s reagent). Single symmetrical spots were observed for purified compounds.

Free peptides were hydrolyzed in sealed tubes under N$_2$ in the presence of phenol in 6 N HC1 at 110°C for 24 hours. Amino-acid analyses were performed on a Model MM-100 Glenco amino acid analyzer.

**[1-Aspartic Acid, 5-Valine, 9-Asparagine] Angiotensin I (Bull Frog)**

TLC (cellulose) R$_f$ 0.56 (BAW 4:1:5), 0.38 (BPW 65:35:35), 0.65 (BAWP); E (His) 0.60 (pH 2.2), 1.00 (pH 8.6). Amino-acid analysis gave the ratio in the acid hydrolysate: Asp 2.04, Arg 1.01, Val 1.88, Tyr 1.13, His 1.09, Pro 1.06, Phe 0.98, Leu 1.00.

**[1-Asparagine, 5-Valine, 9-Histidine] Angiotensin I (Japanese Goosefish)**

TLC (cellulose) R$_f$ 0.40 (BAW 4:1:5), 0.45 (BWP 65:35:35), R$_f$ 0.65 (BAWP); E (His) 0.86 (pH 2.2), 0.95 (pH 8.6). Amino-acid analysis gave the ratio in the acid hydrolysate: Asp 1.02, Val 1.98, Tyr 0.93, His 2.05, Phe 0.98, Leu 1.00.

**Pressor Activity in Rat**

Pressor activity was determined in anesthetized ganglion-blocked, vagotomized rats by using the following modified procedure of Pickens et al. Sprague-Dawley female rats (weighing approximately 200 g) were injected successively with 5% sodium amytal (Eli Lilly Company, Indianapolis, Indiana) solution in saline (0.4 ml/ip), 0.5 mg atropine sulfate (preconstituted by Elkins-Sinn, Cherry Hill, New Jersey) and a 1 ml solution of hexamethonium chloride (ICN Pharmaceuticals, Cleveland, Ohio) containing polyvinyl pyrrolidone (PVP; GAF Corporation, Calvert City, Kentucky) which was prepared by dissolving 20 g PVP and 500 mg hexamethonium chloride in 100 ml water. Sufficient time was allowed after amytal injection for the rats to become fully unconscious. After tracheotomy and vagotomy, the carotid artery and femoral vein were cannulated for blood pressure registration and intravenous injection, respectively.

Pressor activity was determined by comparing the response of four injections of the standard angiotensin II (0.9 ng, 1.8, 2.7, 3.6 ng) with similar doses of the test sample in four groups of rats by alternating doses

<table>
<thead>
<tr>
<th>Species</th>
<th>Angiotensin</th>
<th>Pressor activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>[Asp$^1$,Ile$^3$] ang II (human)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>[Asp$^1$,Leu$^3$,His$^9$] ang I (human)</td>
<td>69.61 ± 1.94</td>
</tr>
<tr>
<td></td>
<td>[Asp$^1$,Val$^9$] ang II (sheep, ox)</td>
<td>102.75 ± 1.18</td>
</tr>
<tr>
<td>Avians</td>
<td>[Asp$^1$,Val$^9$,Ser$^9$] ang I (chicken)</td>
<td>75.0 ± 1.24</td>
</tr>
<tr>
<td>Reptiles</td>
<td>[Asn$^1$,Val$^9$,Tyr$^9$] ang I (snake***)</td>
<td>60.3 ± 2.02</td>
</tr>
<tr>
<td>Amphibians</td>
<td>[Asp$^1$,Val$^9$,Asn$^9$] ang I (bull frog)</td>
<td>87.8 ± 3.17</td>
</tr>
<tr>
<td>Teleosts</td>
<td>Ala,Pro,Gly [Asp$^1$,Ile$^3$,Val$^9$] ang II (frog skin)</td>
<td>90.6 ± 4.99</td>
</tr>
<tr>
<td></td>
<td>[Asn$^1$,Val$^9$,His$^9$] ang I (Japanese goosefish)</td>
<td>51.5 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>[Asn$^1$,Val$^9$,Asn$^9$] ang I (chum salmon)</td>
<td>65.2 ± 1.56</td>
</tr>
<tr>
<td></td>
<td>[Asn$^1$,Val$^9$] ang II</td>
<td>70.03 ± 1.49</td>
</tr>
</tbody>
</table>

*In anesthetized, ganglion blocked vagotomized rats, relative to [Asp$^1$,Ile$^3$] ang II = 100 (weight basis).

**Position I substituted with unidentified N-blocked Asp or Asn.
of the standard and the test sample and vice versa. Doses of the standard and the test angiotensin that gave 10, 15, 20 and 25 mm Hg rise in blood pressure were determined from the dose-response curves and mean pressor activity calculated relative of [Asp1,He5] ang II = 100. All solutions were prepared in 0.1% aqueous bacitracin (weight basis) and the results were expressed as a percentage of the pressor activity of angiotensin II (table 2).

Blockade of Pressor Activity of Angiotensins I with the Converting-Enzyme Inhibitor, Captopril, in the Rat

Pressor Bioassays

After injecting angiotensin II (0.9 ng, 1.8 ng, 3.6 ng), [Ile3] angiotensin I (7.2 ng) and test sample of the nonmammalian angiotensin (table 2; 5.4 or 7.2 ng), captopril (5 mg in 0.5 ml saline) was injected intravenously into the rat for 1 minute and the baseline was allowed to stabilize. Angiotensin I (7.2 ng) was injected again to see if its response was totally blocked. This was followed by the injections of the test sample of the nonmammalian angiotensin and angiotensin II, respectively. Determination of the angiotensin II dose-response curve was included in this study to judge the sensitivity of the rat before and after treatment with captopril.

Pressor Activity in the Dogfish Shark (Squalus Acanthias)

Dogfish were caught on trawl-lines in Frenchman Bay, Maine and submerged in sea-water live-cars until used 2–5 days after capture. Fish of both sexes were used. Males weighed between 1.5 and 2.5 kg and females between 3.5 and 8 kg. The fish were in a fasted state at the time of use. A polyethylene catheter (Intramedic, PE 60) was introduced percutaneously into the dorsal aorta via the caudal artery. The fish were then placed dorsal side up in a long narrow box provided with a constant flow of cold sea-water (13°–15°C). Injections of the test compounds (1 ml solution) were made through the dorsal aortic catheter, which also served to record systolic and diastolic dorsal aortic pressure. Further details of this procedure were described previously.12 Wherever the blood pressure response is reported here in terms of percent change (preinjection level = 100%), the calculation was derived from diastolic dorsal aortic pressure measurements (table 3).

Effects of Frog-Skin Angiotensin II in Amphibians

Details of this procedure are described by Coviello et al.13

Pressor and Vasodepressor Actions of Angiotensins in the Conscious or Anesthetized Chickens

Angiotensins were tested in chronically cannulated adult female chickens as described previously by Nakamura et al.14 and Nishimura et al.15

Results

Pressor Activity in Rat

In general, the pressor activity determined in rat of all the angiotensin I-like peptides isolated from the various nonmammalian species was between 50% and 88% of [Ile3] angiotensin II (table 2). Fruchter et al.16 reported that, in the bullfrog and mudpuppy, pressor activity of human angiotensin I is inhibited by captopril, a converting-enzyme inhibitor. As is the case with mammalian angiotensins I, the pressor activity of [Asp1,Val5,Asn9]-, [Asn1,Val5,Asn9]-, [Asn1,Val5, Tyr9]- and [Asn1,Val5,His9] angiotensin I was inhibited by pretreatment of the rats with captopril.

Frog skin angiotensin II produced over 90% pressor activity of angiotensin II in the rat.1

Pressor Activity of Angiotensins in the Dogfish Shark

The decapeptides related to human and chicken angiotensins I produced significant pressor response in the conscious dogfish (table 3), which was totally blocked by an angiotensin I converting-enzyme inhibi-

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### Table 3. Pressor Activity of Angiotensins and Analogs in Dogfish Shark (Squalus Acanthias)

<table>
<thead>
<tr>
<th>Angiotensin</th>
<th>Avg. Dose µg/kg</th>
<th>Systolic (%)</th>
<th>Diastolic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Asp1,Ile3,His9] ang I (human)</td>
<td>3</td>
<td>135±5</td>
<td>138±6</td>
</tr>
<tr>
<td>[Asp1,Val5,ser9] ang I (chicken)</td>
<td>3</td>
<td>144±8</td>
<td>152±8</td>
</tr>
<tr>
<td>[Asp1,Ile3] ang II (human)</td>
<td>3.6</td>
<td>148±5</td>
<td>147±5</td>
</tr>
<tr>
<td>[Asn1,Val5] ang II (Japanese goosefish)</td>
<td>6</td>
<td>158±19</td>
<td>145±15</td>
</tr>
<tr>
<td>des-Asp1 [Ile3] ang II (&quot;ang III&quot;)</td>
<td>3.0</td>
<td>133±12</td>
<td>137±10</td>
</tr>
<tr>
<td>[Ile3] ang II</td>
<td>4.4</td>
<td>132±14</td>
<td>119±10</td>
</tr>
<tr>
<td>[Sar1,Ile3] ang II</td>
<td>9.1</td>
<td>131±7</td>
<td>127±4</td>
</tr>
<tr>
<td>[Sar1, Thr9] ang II</td>
<td>7.9</td>
<td>104±11</td>
<td>105±10</td>
</tr>
<tr>
<td>[Ile3] ang I</td>
<td>8.3</td>
<td>105±9</td>
<td>104±10</td>
</tr>
</tbody>
</table>
tor, SQ 20,881 (table 4). These results suggest that the pressor activity of angiotensin I-like peptides is due to their conversion into the corresponding angiotensin II.

Previous studies by Opdyke and coworkers\textsuperscript{12} indicated that the pressor response to angiotensin II is completely abolished by prior treatment of dogfish with phentolamine, an \(\alpha\)-adrenergic blocking agent. Likewise, in the present studies (data not included), phentolamine totally blocked the pressor effects due to: 1) decapetides related to human and chicken angiotensin I; 2) octapeptides related to human and Japanese goosefish angiotensin II; and 3) angiotensin III.

The antagonists of the pressor action of angiotensin II in the mammalian species, e.g., [Ile\textsuperscript{8}] and [Sar\textsuperscript{1},Ile\textsuperscript{8}] angiotensin II, elicited significant pressor response in dogfish shark (table 3). This inherent pressor activity of [Sar\textsuperscript{1},Ile\textsuperscript{8}] angiotensin II was also apparent during its infusion into dogfish. For example, within 3 minutes after beginning an infusion of 10 \(\mu\)g/kg min of this peptide, diastolic dorsal aortic pressure increased to 154\% of control pressure but slowly declined to 118\% of control after 20 minutes of continuous infusion. Neither [Sar\textsuperscript{1},Thr\textsuperscript{8}] angiotensin II nor [Ile\textsuperscript{8}] angiotensin II showed such pressor effects when infused in this way.

The pressor response to [Sar\textsuperscript{1},Ile\textsuperscript{8}] angiotensin II was completely abolished by prior injection of phentolamine (three experiments). This suggests that the pressor response of this peptide may be mediated by catecholamines. Similar results were obtained when catecholamine secretory activity of angiotensin II-antagonists was determined in isolated retrogradely perfused cat adrenal medulla. [Sar\textsuperscript{1},Thr\textsuperscript{8}] angiotensin II was totally devoid of secretory activity while [Sar\textsuperscript{1},Ile\textsuperscript{8}] angiotensin II produced 3\% of adrenal medullary secretory activity of angiotensin II.\textsuperscript{17}

**Pressor Activity of Angiotensins in Domestic Chicken**

Nakamura and coworkers\textsuperscript{14} observed that in the conscious chicken, [Asp\textsuperscript{1},Val\textsuperscript{8}] angiotensin II (which is native to this species) produced sharp and quick depressor responses at lower doses, while at higher doses, a sharp depressor response was followed by a pressor response.

The pressor response in chicken was blocked by phentolamine or phenoxybenzamine or by depletion of catecholamines (caused by pretreatment with reserpine or 6-hydroxydopamine). These results suggest that the pressor response is perhaps due to the release of catecholamines.\textsuperscript{14} The depressor response was not inhibited by atropine, prostaglandin synthetase inhibitors, methysgeride, propranolol, or a vasopressin antagonist, but was blocked markedly with [Sar\textsuperscript{1},Ile\textsuperscript{8}] angiotensin II.\textsuperscript{14} The depressor responses to angiotensin seen in anesthetized, phenoxybenzamine-, reserpine-, or 6-hydroxydopamine-treated chickens appeared to be of the same nature as depressor responses in the conscious chickens.\textsuperscript{15}

Comparison of human and bovine angiotensin II in the anesthetized chicken indicates that they have almost equal potency (table 5).\textsuperscript{15} Pressor potency was reduced to 27\% when aspartic acid in position 1 was replaced with asparagine, or to 37\% when aspartic acid was replaced with sarcosine. Replacement of aspartic acid with a hydrogen atom, as in angiotensin III, almost completely abolished the pressor activity.

**Table 5. Pressor Activity of Angiotensins in Anesthetized Chicken (Gallus gallus v. domesticus)**

<table>
<thead>
<tr>
<th>Angiotensin</th>
<th>Pressor activity (molar basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Asp\textsuperscript{1},Val\textsuperscript{8},Ser\textsuperscript{8}] ang I (chicken)</td>
<td>62</td>
</tr>
<tr>
<td>[Asp\textsuperscript{1},Val\textsuperscript{8},His\textsuperscript{8}] ang I (bovine)</td>
<td>62</td>
</tr>
<tr>
<td>[Asp\textsuperscript{1},Val\textsuperscript{8}] ang II (chicken, bovine)</td>
<td>100</td>
</tr>
<tr>
<td>[Asp\textsuperscript{1},Ile\textsuperscript{8}] ang II (human)</td>
<td>116.7 ± 7.8</td>
</tr>
<tr>
<td>[Asn\textsuperscript{1},Val\textsuperscript{8}] ang II (Japanese goosefish)</td>
<td>26.7 ± 4.3</td>
</tr>
<tr>
<td>[Sar\textsuperscript{1},Ile\textsuperscript{8}] ang II</td>
<td>37.5 ± 4.6</td>
</tr>
<tr>
<td>des-Asp\textsuperscript{1} — ang II (&quot;ang III&quot;)</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

For further details see Nishimura et al.\textsuperscript{15}
Effects of Angiotensins in Amphibians

Frog skin angiotensin II increased the short circuit current significantly (which is correlated with active sodium and chloride transport; after it was added to the dermal side of the isolated skin of the South American frogs Leptodactylus Chaquensis and Ocelatus and the toad Bufo Arenorum in concentration of 10^-6 M (see article by Proto et al. on pp V-101-104 of this issue). In frogs, the effect was significant at 15 minutes and reached 45% over control after 2.5 hours; addition of amiloride (10^-4) blocked the short circuit current response due to frog skin angiotensin. The effect of frog skin angiotensin II was not abolished by the absence of chloride ion in the bathing fluid. Human angiotensin II (10^-6) had no effect on short circuit current in the isolated skin of the summer frogs unless the frogs were pretreated for 14 days with 0.1% NaCl. Goosefish angiotensin II was ineffective at similar concentrations, and none of the angiotensins modified the short circuit current in the toad bladder.

Discussion

As is evident from table 1, except for minor changes in positions 1 and 5, the structure of angiotensin II has been well preserved after the advent of renin-angiotensin system. Although position 9 in angiotensin I appears to be class-specific, all the angiotensin I-like peptides tested in the rat produced pressor response after their conversion into the corresponding angiotensin II. Pressor activity of angiotensins I in the rat varied between 50% to 88% of [Ile^2] angiotensin II (table 2). This large variation in the pressor activity may possibly be due to a difference in the rate of their conversion into the corresponding angiotensin II, which in turn may be dependent upon the nature of substituent in position 9. Angiotensin II-like peptide isolated from frog skin is unique in at least three ways: 1) it is the first angiotensin isolated from a vertebrate in which the amino terminus has been elongated; 2) substituents in positions 3 and 5 have not been interchanged; and 3) the corresponding angiotensin I has not been isolated. However, elongation of the amineterminus or interchange of substituents in positions 3 and 5 did not affect its pressor activity. This undecapeptide was found to be almost equipotent with [Ile^2] angiotensin II in the rat.

The renin-angiotensin system seems to have emerged during the early evolution of bony fishes and is present in a variety of teleosts and tetrapods. Historical evidence indicates that elasmobranchs do not possess renal juxtaglomerular granules and are therefore thought to be incapable of producing renin. Besides, incubation of homologous plasma and the kidney extract did not form angiotensin-like substance. Perfusion of an isolated dogfish gut preparation with blood containing angiotensin II caused no increase in vascular resistance, indicating the lack of angiotensin receptors which directly mediate vasoconstriction in the intestinal circulation. Angiotensin II also did not constrict the dogfish celiac artery and anterior intestinal vein in vitro and it failed to alter blood flow in the intestinal mesentery. But in spite of lack of a direct vasoconstrictor effect, intravascular injection of angiotensin II produced a pressor response, which was completely abolished by prior treatment with phentolamine. These results suggested that the pressor response to angiotensin II in the dogfish may be caused solely by catecholamine release. This is further corroborated by the findings that intravenous injection of angiotensin II into the dogfish shark increased plasma levels of catecholamines.

The present results suggest that pressor activity of angiotensin I-like peptides in the conversion into the corresponding angiotensin II which in turn produce a pressor response, presumably due to the release of catecholamines. Angiotensin II antagonist, [Sar^1, Thr^8] angiotensin II did not elicit a pressor response in this species while [Ile^2]- and [Sar^1, Ile^8] angiotensin II produced a weak pressor response which was blocked with phentolamine. These results are in accord with previous findings in isolated retrogradely perfused cat adrenal medulla. It is of interest to note that angiotensin III also produced a moderate pressor response in this species.

Nishimura et al. reported partial inhibition (30%-40%) of the pressor response to [Asn^1, Val^3] angiotensin II in American eel (Anguilla rostrata) by alpha-adrenergic blocking compounds, e.g. phentolamine, phenoxybenzamine and reserpine. Similar results were reported in reptiles, e.g., turtle (Pseudemys scripta). In chronically cannulated chicken (Gallus gallus domesticus), native chicken angiotensin II, [Asp^1, Val^3] angiotensin II caused a biphasic blood pressure response, a depressor response followed by a pressor response. The pressor response appears to be mediated by catecholamines while the depressor response may possibly be a direct action on vascular smooth muscle. 14, 15

Thus, the results reported in the present paper and previous findings by Opdyke et al., Nishimura et al. in bony fishes, and Zehr et al. in reptiles suggest that in relatively more primitive stages of evolution, before the development of renin-angiotensin system (e.g., sharks), the pressor action of exogenous angiotensin II is mediated by the release of catecholamine, while in the mammalian species the pressor action of angiotensin II is primarily through direct vasoconstriction. In the intermediate stages of evolution (bony fishes, reptiles, etc), a 30%-50% pressor action of angiotensin II may be due to release of catecholamines, while the remainder of the pressor action may be by direct vasoconstriction.

In mammalian species [Sar^1] angiotensin II has been found to be at least as potent as angiotensin II while angiotensin III retained 20%-30% pressor activity of angiotensin II. A much reduced pressor activity of these peptides in the anesthetized chicken (table 5) indicates that either the functions of angiotensins II or the nature of its receptors may have changed during the course of evolution. Alternately, this difference could...
also be due to variations in the rate of metabolism of angiotensins in various species. In any case, these results suggest the importance of aspartic acid in position 1 for pressor activity (or catecholamine releasing effect) of angiotensin II in the chicken.

Angiotensin II-like peptide isolated from frog skin has properties which may prove to be compatible with a role in regulation of salt and water in amphibians.

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