Guanylin peptides regulate electrolyte and fluid transport in the Gulf toadfish ("Opsanus beta") posterior intestine

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Ruhr IM, Bodinier C, Mager EM, Esbaugh AJ, Williams C, Takei Y, Grosell M. Guanylin peptides regulate electrolyte and fluid transport in the Gulf toadfish ("Opsanus beta") posterior intestine. Am J Physiol Regul Integr Comp Physiol 307: R1167–R1179, 2014. First published August 6, 2014; doi:10.1152/ajpregu.00188.2014.—The physiological effects of guanylin (GN) and uroguanylin (UGN) on fluid and electrolyte transport in the toadfish intestine have yet to be thoroughly investigated. In the present study, the effects of GN, UGN, and renoguanylin (RGN; a GN and UGN homolog) on short-circuit current (Isc) and the transport of Cl−, Na+, bicarbonate (HCO3−), and fluid in the Gulf toadfish ("Opsanus beta") intestine were determined using Ussing chambers, pH-stat titration, and intestinal sac experiments. GN, UGN, and RGN reversed the Isc of the posterior intestine (absorbptive-to-secretory), but not of the anterior intestine. RGN decreased baseline HCO3− secretion, but increased Cl− and fluid secretion in the posterior intestine. The secretory response of the posterior intestine coincides with the presence of basolateral NKCC1 and apical cystic fibrosis transmembrane conductance regulator (CFTR), the latter of which is lacking in the anterior intestine and is not permeable to HCO3− in the posterior intestine. However, the response to RGN by the posterior intestine is counterintuitive given the known role of the marine teleost intestine as a salt- and water-absorbing organ. These data demonstrate that marine teleosts possess a tissue-specific secretory response, apparently associated with seawater adaptation, the exact role of which remains to be determined.

Guanylin; uroguanylin; HCO3−; CFTR; osmoregulation; fluid secretion

REGULATION OF SALT, WATER, and acid-base balance by the osmoregulatory organs of teleost fish is a vital process for maintaining homeostasis in a diverse range of habitats and is accomplished through various compensatory responses. To combat diffusive fluid loss, marine teleosts drink seawater (SW) and absorb both salts and water in the intestine (56). It is well established that the absorptive-type Na+/K+2Cl− cotransporter NKCC2 (SLC12a1) transports salts down their electrochemical gradient from the intestinal lumen across the apical membrane into the enterocytes (18, 33, 62). More recently, an additional pathway for Cl− absorption, by SLC26a6 (an apical Cl−/HCO3− antiporter), was identified to exchange intestinal Cl− for intracellular HCO3− and is responsible for up to 70% of Cl− uptake (26, 29, 43, 63). Fluid absorption follows absorption of osmolytes via these two ion-uptake pathways. In addition, titration of HCO3− (to form CO2 and H2O) by protons (H+) extruded by an apical v-type H+−ATPase (VHA) and precipitation of HCO3− with intestinal Ca2+ and Mg2+ (to form Ca- and MgCO3 precipitates) facilitates fluid absorption by lowering intestinal fluid osmolality (21, 23, 25, 29, 64–66). Ultimately, absorption of salts, coupled to base secretion, allows for solute-coupled and osmotic fluid absorption.

Although the marine teleost intestine is vital to salt and fluid absorption, it also exhibits secretory characteristics with respect to Cl− and fluid. Studies have identified possible pathways for both Cl− secretion, via an apical cystic fibrosis transmembrane conductance regulator (CFTR) channel, and fluid secretion into the intestinal lumen by marine teleosts (48, 58, 68), both of which are common in terrestrial animals (16, 32). It has been proposed that the function of apical CFTR is to facilitate Cl− and fluid secretion for the elimination of toxins from the intestine (48, 55). However, neither the exact function nor the regulatory pathways responsible for switching intestinal ion and fluid absorption to secretion are known.

Guanylin (GN) and uroguanylin (UGN) are upstream regulators of apical CFTR and modulate intestinal Na+, Cl−, fluid, and HCO3− fluxes in mammals (3, 50, 61). GN and UGN bind to guanylyl cyclase-C (GC-C), a transmembrane receptor on the apical membrane of intestinal tissues (50, 52, 53), causing an intracellular transduction cascade that increases the formation of cyclic guanosine monophosphate (cGMP). cGMP can either stimulate cGMP-dependent protein kinase (PKG) or lead to increases in cyclic adenosine monophosphate (cAMP) levels that stimulate cAMP-dependent protein kinase (PKA); both PKG and PKA activate CFTR (3, 4, 9, 13, 34, 42). In mammals, this leads to Cl−, fluid, and HCO3− secretion into the intestinal lumen to help neutralize the effects of acidic chyme (2, 5, 30, 54). This process is especially important after the ingestion of food, where GN and UGN are secreted into the intestine, bind GC-C, and elevate the levels of intracellular cGMP in enterocytes (3).

GN, UGN, and renoguanylin (RGN) are also present in teleost fish; intestinal tissues express GN and UGN, while renal tissue express UGN and RGN (10, 12, 39, 67). Although the physiological function of the guanylin peptides in teleosts is still uncertain, studies have shown that they may play a role in long-term salinity adaptation. Indeed, GN and UGN mRNA expression increase following a 24-h transfer from freshwater (FW) to SW by at least twofold in the Japanese eel (Anguilla japonica) (12, 39, 67), a pattern also observed in the rat after salt loading of intestinal tissues (8, 40, 45). In mammalian tissues, GN and UGN either increase the secretory short-circuit current (Isc) of the intestine or reverse an absorptive Isc, while in teleosts, these peptides have no effect on the anterior intestine of the Japanese eel, but do reverse the Isc of the mid and posterior intestine, resulting in a net serosa-to-mucosa flux of, presumably, Cl− ions (38, 54, 68).
However, considering that the intestine of marine teleosts must absorb water to avoid dehydration and that the guanylin peptides cause fluid secretion in mammalian intestinal cells, would marine teleosts respond to these peptides in a similar manner? Evidence from the Japanese eel suggests the view of net fluid secretion into the intestinal lumen by the reversal of the $I_{sc}$ in the mid and posterior intestine by GN, UGN, and RGN (68). These observations would suggest that marine teleosts secrete fluid into the intestinal lumen via the guanylin peptide-induced activation of the intracellular cascade that occurs in mammals. However, it remains to be confirmed that the secretory current is conducted by $Cl^-$ and whether this current promotes fluid secretion. Moreover, the molecular components of the secretory ion transport pathway have yet to be firmly established.

The purpose of this study was to further characterize the physiological effects of the guanylin peptides in marine teleost fish by investigating their effects on Gulf toadfish ($Opsanus beta$) intestinal epithelium. A secretory short-circuit current response to luminal application of the guanylin peptides was anticipated and hypothesized to 1) be most pronounced in the posterior intestine, 2) be driven by net secretion of $Cl^-$, and 3) be associated with net fluid secretion and enhanced $HCO_3^-$ secretion. Furthermore, it was hypothesized that 4) a secretory pathway would be present in the guanylin-responsive segments of the intestine.

### MATERIALS AND METHODS

**Experimental animals.** Gulf toadfish ($Opsanus beta$) were caught as bycatch from a local shrimp fisherman in Biscayne Bay, FL. Immediately upon arrival in the laboratory, the Gulf toadfish were briefly placed in a FW bath (3 min) and then treated with malachite green to remove ectoparasites, following the protocol from McDonald et al. (49). Gulf toadfish were separated by size, and 8–10 fish were placed in 62-liter tanks with a continuous flow-through of sand-filtered SW from Biscayne Bay (30–34 ppt salinity, 26–29°C). Gulf toadfish were fed to satiation weekly, but food was withheld for 72 h before experimentation. Fish husbandry and experimental procedures were performed according to an approved University of Miami Animal Care Protocol (Institutional Animal Care and Use Committee No. 10–293, renewal 02). All Gulf toadfish used for experimentation were killed using 0.2 g/l MS-222 (Argent) solution buffered with 0.3 g/l NaHCO$_3$, followed by severing of the spinal cord at the cervical vertebra.

**cDNA cloning.** Cloning was performed using cDNA constructed from homogenized Gulf toadfish intestinal tissues (60). Partial cDNAs of a GN-like and a UGN-like peptide were cloned by 3'- and 5'-rapid amplification of cDNA ends (RACE) using a universal primer and designed-sense primers (Table 1). PCR amplifications were performed according to a modified protocol from Yuge et al. (67) using Taq DNA polymerase and buffer (Invitrogen). After 3' and 5'-RACE procedures were performed, gene-specific primers (Table 1) were used in PCR reactions to determine the full sequence of the GN and UGN prohormones.

**Composition of salines, hormones, and inhibitors.** Japanese eel GN, UGN, and RGN peptides were resuspended into $10^{-3}$ mol/l stock solutions in nano-pure water. The final concentrations for the guanylin peptides in the luminal saline were $10^{-9}$–$10^{-5}$ mol/l. Luminal ($pH$ 7.0) and serosal ($pH$ 7.8) salines were prepared as per Table 2. When using the pH-stat titration system, luminal $pH$ was maintained at 7.8 by titration using a 0.005 N HCl solution. Batiflomycin (Enzo Life Sciences) was used to inhibit the apical VHA and prepared in DMSO at 1.0 mmol/l for application in a final concentration of 2 µmol/l and 0.1% DMSO, which has been previously used in the Gulf toadfish (27, 31). CFTRinh-172 (Sigma) was used to inhibit a putative apical CFTR channel (1, 6, 7, 48) and prepared in DMSO at 1.0 mmol/l for application in a final concentration of 10 µmol/l and 0.1% DMSO, which is in the mid-range solubility limit for this blocker (1).

**In vitro short-circuit current, transepithelial potential, and $HCO_3^-$ secretion experiments on isolated intestinal tissue: effects of Japanese eel guanylin peptides.** To measure the effects of Japanese eel guanylin peptides (GN, UGN, and RGN) on $I_{sc}$, transepithelial potential ($TEP$), and transepithelial conductance ($G_{EA}$), Ussing chambers (Physiological Instruments) were employed. Segments of anterior and posterior intestine from each experimental Gulf toadfish (ranging from 20 to 30 g) were excised, cut open, and mounted onto tissue holders (model P2413; Physiological Instruments), which exposed 0.71 cm$^2$ of excised tissue, and were positioned between two half-chambers (model 2400; Physiological Instruments). Each half-chamber contained 2 ml of appropriately gassed mucosal or serosal saline (Table 2). Salines in each half-chamber were continually mixed by airlift gassing with either 100% O$_2$ (mucosal) or 0.3% CO$_2$ in O$_2$ (serosal) (Table 2), as described in Genz and Grosell (19). The temperatures of the salines in the chambers were maintained at 25°C. Current and voltage electrodes were connected to amplifiers (model VCC660; Physiological Instruments) and, depending on the experiment, recorded either differences in $I_{sc}$ under voltage-clamp conditions at 0.0 mV, with 3 s of 1-mV pulses (mucosal-to-serosal) at 60-s intervals, or differences in $TEP$ under current-clamp conditions at 0.0 µA, with 3 s of 10-µA pulses (mucosal-to-serosal), also at 60-s intervals. Epithelial conductance

**Table 1. Sequences of primers for cDNA cloning**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>GUA-S1 (GN-F)*</td>
<td>5’-ATCTGCGCTTAATGCTGCCAC-3’</td>
</tr>
<tr>
<td>Rev-GUA-S1 (GN-R)*</td>
<td>5’-GTCGAAGACGATTTAGCCAGA-3’</td>
</tr>
<tr>
<td>tUGN-R†</td>
<td>5’-CCGGACCCTTCTGACGCCAGG-3’</td>
</tr>
<tr>
<td>tUGN-F†</td>
<td>5’-TGGCAGAGGCTGAGCGCTGG-3’</td>
</tr>
<tr>
<td>RevGUA-S1 (GN-R)*</td>
<td>5’-CCGGACCCTTCTGACGCCAGG-3’</td>
</tr>
<tr>
<td>Universal primer‡</td>
<td>5’-TTGUGN-R†</td>
</tr>
</tbody>
</table>

*Designed sense primers taken and/or modified from Yuge et al. (67).†Gene-specific primers designed from annotated Gulf toadfish transcriptome.‡Universal primer (Clontech SMARTer RACE cDNA Amplification Kit) consists of both a long and short sequence.

## Table 2. Composition of salines for short-circuit current and pH-stat titration experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mucosal $^a$</th>
<th>Serosal</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl, mmol/l</td>
<td>69.0</td>
<td>151.0</td>
</tr>
<tr>
<td>KCl, mmol/l</td>
<td>5.0</td>
<td>3.0</td>
</tr>
<tr>
<td>MgSO$_4$, mmol/l</td>
<td>77.5</td>
<td>0.88</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$, mmol/l</td>
<td>22.5</td>
<td>0.5</td>
</tr>
<tr>
<td>KH$_2$PO$_4$, mmol/l</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CaCl$_2$, mmol/l</td>
<td>5.0</td>
<td>1.0</td>
</tr>
<tr>
<td>HEPES, free acid, mmol/l</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>HEPES salt, mmol/l</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Urea, mmol/l</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Osmolality, mosmol/kgH$_2$O $^+$</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>pH $\pm$</td>
<td>7.83</td>
<td>7.8</td>
</tr>
<tr>
<td>Gas $%$</td>
<td>100$^b$</td>
<td>0.3% CO$_2$ in O$_2$</td>
</tr>
</tbody>
</table>

* Mucosal application of guanylin, uroguanylin, or renoguanylin (10$^{-9}$–10$^{-5}$ mol/l).†Adjusted with mannitol to ensure transepithelial isosmotic conditions in all experiments.‡pH 7.80 was maintained by pH-stat titration.§Salines were gassed for at least 1 h before experimentation.
(G<sub>HCO</sub>) was determined from inflections in I<sub>sc</sub> and TEP during pulsing using Ohm's law. I<sub>sc</sub> and TEP measurements were recorded on a computer using BIOPAC systems Acqknowledge software (v. 3.8.1).

To simultaneously measure electrophysiological parameters (TEP) and HCO<sub>3</sub><sup>-</sup> secretion on the isolated intestinal tissues, Ussing chambers were set up in tandem with a pH-stat titration system (TIM 854 or 856 Titration Managers; Radiometer), as outlined previously (24). A pH electrode (model PHC4000.8; Radiometer) and a microburette tip (from which acid was delivered) was submersed in the luminal half-chamber to allow for pH readings and pH-stat titrations. Mucosal salines (injected into the luminal half-chamber) were maintained at a physiological pH of 7.8, throughout all experiments, to allow for symmetrical pH conditions on either side of the epithelium. The pH values and rate of acid titrant (0.005 mol/l HCl) additions were recorded onto personal computers using the Titramaster software (v. 5.1.0). Epithelial HCO<sub>3</sub><sup>-</sup> secretion rates were calculated from the rate of titrant addition and its concentration, as described in Grosell and Genz (24).

To determine the dose response and effective concentrations of the three eel peptides, GN, UGN, and RGN were added to the luminal half-chamber in a dose-dependent manner (10<sup>-9</sup>–10<sup>-5</sup> mol/l), as described in Takei and Yuge (59), while I<sub>sc</sub> was recorded. Luminal peptide concentrations were increased once stable I<sub>sc</sub><sub>-</sub> readings were reached. The length of time to reach a stable reading varied according to individual preparations, resulting in a range of time courses for the peptide and intestinal segment studied (GN: AI 189 – 452 min, PI 180 – 369 min; UGN: AI 243 – 399 min, PI 155 – 440 min; and RGN: AI 176 – 372 min, PI 254 – 449 min). All subsequent experiments were performed using only RGN due to limited supplies of GN and UGN.

To determine if the secretory anion flux observed following peptide addition was due to the activation of apical CFTR, the CFTR inhibitor CFTRinh-172 was added to the luminal half-chamber before the addition of RGN, while TEP and HCO<sub>3</sub><sup>-</sup> secretion were recorded. To determine if observed, and unexpected, reductions in HCO<sub>3</sub><sup>-</sup> secretion following peptide addition were due to stimulation of apical H<sup>+</sup> secretion, the VHA inhibitor, baflomycin, was added to the luminal half-chamber simultaneously with RGN. Because the effects of baflomycin can be transient (27, 31), baflomycin and RGN were added concurrently, rather than in sequence, to capture the immediate effects of this inhibitor on the tissues.

Vehicle controls using 0.1% DMSO (n = 5) showed no impact of DMSO on the tissue response to RGN for either I<sub>sc</sub> or HCO<sub>3</sub><sup>-</sup> secretion (data not shown).

**Dependence of HCO<sub>3</sub><sup>-</sup> secretion on mucosal Cl<sup>-</sup> concentration.** Considering the unexpected reduction in HCO<sub>3</sub><sup>-</sup> secretion following peptide addition, the dependence of HCO<sub>3</sub><sup>-</sup> secretion on mucosal Cl<sup>-</sup> concentrations was examined. Low Cl<sup>-</sup> levels were achieved by replacing Cl<sup>-</sup> salts with corresponding gluconate salts, while higher levels were achieved by partially replacing MgSO<sub>4</sub> with MgCl<sub>2</sub>. The pH-stat titration experiments were limited in time (~1 h per flux period) as serosal-to-mucosal Cl<sup>-</sup> flux, as well as Cl<sup>-</sup> leaking from the pH electrode, contributed to altered Cl<sup>-</sup> concentrations during the flux period, especially during low-Cl<sup>-</sup> conditions. HNO<sub>3</sub>, rather than HCl, was used as titrant in these experiments in an attempt to reduce Cl<sup>-</sup> contamination. All experiments were initiated with a control flux (standard mucosal saline, Table 2, Cl<sup>-</sup> concentration = 129 mmol/l), and then a subsequent flux with Cl<sup>-</sup>-manipulated salines. Subsamples of the mucosal saline during the second flux were obtained at the beginning and end of each flux to verify actual luminal Cl<sup>-</sup> concentrations. Secretion rates of HCO<sub>3</sub><sup>-</sup> from the second flux period are expressed as a fraction of the initial control secretion rates.

**Intestinal sac preparations.** Intestinal sac preparations were used to examine the net flux of water, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, and Na<sup>+</sup> across the anterior and posterior intestinal epithelia, and followed a modified protocol from Genz and Grosell (19). The entire length of the intestine was excised from an adult Gulf toadfish and cut distally to the pyloric sphincter and proximally to the rectal sphincter. A PE50 catheter was inserted into the anterior end of the excised intestine and tied off with a silk suture. The appropriate mucosal saline (Table 2) was then injected into the catheter to rinse the intestine of any debris, precipitates, and intestinal fluids. The anterior portion of the intestine was then tied off with a silk suture and cut to make the anterior sac preparation. The mid intestine was discarded, while a new PE50 catheter was inserted into the posterior portion and the distal end was tied off to make the posterior sac. Two mucosal salines were used for this experiment (Table 2): control mucosal saline and treatment mucosal saline containing 5 × 10<sup>-8</sup> mol/l RGN. The volume of injected mucosal saline was determined by weighing a 5-ml syringe before and after filling (Table 2). After an intestinal preparation was filled with appropriate mucosal saline, the intestinal sac was blot-dried and weighed; it was then placed in a scintillation vial filled with 15 ml of serosal saline gassed with 0.3% CO<sub>2</sub>. The preparations were then weighed and its mucosal saline was collected. The sac tissue itself was then cut down its midline, spread onto tracing paper, and traced to measure its surface area. Subsamples of mucosal saline were collected at the beginning and end of the 2-h flux period.

Water, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, and Na<sup>+</sup> concentration equivalents were calculated by the Henderson-Hasselbalch equation, using mucosal pH values, total CO<sub>2</sub> (measured by a Corning 965 carbon dioxide analyzer) readings from individual intestinal sac preparations, and a pK of 9.46 (29). Concentrations of Na<sup>+</sup> were measured using flame spectrometry (Varian 220FS), while Cl<sup>-</sup> concentrations were measured using anion chromatography (DIONEX 120). The fluxes for these ions were calculated from the differences between the initial and final amounts of each ion, taking into account the flux period and tissue surface area. Water flux was calculated by taking the difference between the initial and final volume (mass) of each intestinal sac, also taking into account the flux period and tissue surface area.

**Immunohistochemistry.** The anterior and posterior intestinal segments were excised from adult Gulf toadfish, from which CFTR, NKCC (for both NKCC1 and -2), and NKA were immunolocalized following a modified protocol from Bodnier et al. (6, 7). After dissection, intestinal tissues were fixed in 2-step fixation (Anatech) for 48 h, followed by a 1-wk immersion in 70% ethanol. Tissues were then dehydrated through an ascending grade of ethanol (3 washes in 95%, followed by 3 washes in 100%). Following dehydration, tissues were prepared for wax imbedding by immersing them in two washes of butanol, followed by two washes with Histocoice (Amresco). Tissues were then immersed in four washes of paraplast (Mc Cormick Scientific) and imbedded. Serial sections (4 μm) were cut from the tissues using a Leitz microtome (model 1512). Sections were transferred and mounted onto poly-l-lysine-coated slides and dried for 24 h at 37°C. Slides were then prepared for antibody treatment by immersing them into two washes of Histocoice (Amresco), five washes of decreasing alcohol (100% → to 50% ethanol), and one wash in PBS. Subsequently, slides were incubated in 10 mmol/l citric acid solution and placed in a microwave for two 5-min incubations to reveal antigenic sites. Slides were then immersed in 0.01% Tween-20 in PBS, followed by immersion in a 5% skin milk solution, and three washes in PBS. Primary antibody (10 μg/ml) was dissolved in 0.5% skim milk in PBS, added to each slide, and incubated for 1 h at 37°C. The primary antibodies consisted of monoclonal mouse CFTR (R&D Systems), polyclonal goat NKCC1, and polyclonal rabbit NKA (Santa Cruz Biotechnology). After the 1-h incubation, slides were immersed in three PBS washes. The secondary antibodies consisted of anti-mouse IgG, anti-goat IgG, and anti-rabbit IgG (Alexa Fluor 488, 350, and 568, respectively, from Invitrogen). Slides were once again incubated in 37°C for 1 h, followed by three successive washes in PBS. Coverslips were placed on the slides using ProLong Gold Antifade Reagent (Molecular Probes). Control slides were treated with the same conditions, but without primary antibody. Slides were observed with an Olympus fluorescent microscope (u-tvo.5xc–2), with attached
RESULTS

cDNA cloning. Two distinct guanylin-like peptides and their prepropeptides were cloned from Gulf toadfish intestinal cDNA, revealing peptides of 108 and 109 amino acids (Fig. 1). The two cloned peptide variants differ at amino acid residues 1, 3, 9–11, and 13 (Fig. 1). They were sufficiently different from one another to identify them as Gulf toadfish GN (tfGN) and Gulf toadfish UGN (tfUGN), after comparing them to the sequences of birds, mammals, reptiles, and teleosts (Fig. 2). In addition, these putative tfGN and tfUGN peptides were verified by comparing their nucleotide sequences (Fig. 1) to identical sequences found in the intestinal transcriptome of the Gulf toadfish.

Moreover, tfGN differed from Japanese eel GN and RGN at residues 1, 3, and 8, and at residues 1 and 3, respectively. tfUGN exhibited greater variation than GN and differed from Japanese eel UGN and RGN at residues 1, 3, 10, 11, and 13, and at residues 1, 9–11, and 13, respectively (Fig. 2).

Effects of eel guanylin peptides on I_{sc}. The effects of eel GN, UGN, and RGN on dissected Gulf toadfish tissues mounted in Ussing chambers are clear. A one-way, repeated-measures ANOVA revealed that the I_{sc} of the anterior intestine (n = 6 for all treatments) remained unchanged (Fig. 3, A–C), while in the posterior intestine, there was a significant reversal of the I_{sc} (from mucosa-to-serosa to serosa-to-mucosa), due to the eel guanylin peptides, in a dose-dependent manner (Table 3). The maximal ΔI_{sc} in the posterior intestine due to eel GN, UGN, and RGN (n = 5, 6, and 6, respectively) occurred at concentrations of ≥10^{-7}, 10^{-7}, and 2 × 10^{-7} mol/l (Fig. 3, D–F), respectively, with EC_{50} values of 5.82 × 10^{-8} (UGN), 4.90 × 10^{-8} (UGN), and 1.16 × 10^{-8} (RGN) mol/l. The differences observed in response to the eel peptides in the posterior intestine are in agreement with those from a previous report (68). Differences in G_{m} values between the anterior and posterior intestinal segments, independent of the dose of the three eel guanylin peptides, were significant (Table 4). No differences in G_{m} across doses in the anterior intestine were observed, but G_{m} increased in the posterior intestine in a dose-dependent manner for all three eel guanylin peptides, matching the changes in observed I_{sc} (Table 4).

Effects of eel RGN on HCO_{3}^{-} secretion. Surprisingly, mucosal application of eel RGN to the posterior intestinal tissue, mounted in an Ussing chamber-pH-stat titration system, significantly reduced HCO_{3}^{-} secretion, depressing the mean secretion rate between 13.3 and 21.2% (Fig. 4A). Additionally, the TEP also decreased significantly 30 min after exposure to RGN (Fig. 4B). However, G_{m} remained unaffected after mucosal application of RGN, with a mean value of 5.6 ± 0.1 mS/cm^{2}.

To examine if the observed inhibition of HCO_{3}^{-} secretion in the posterior intestine due to RGN may have been caused by a downstream effect of RGN acting on the apical VHA, both bafilomycin and RGN were applied to the mucosal saline in an Ussing chamber-pH-stat titration system. Following the results of the previous experiment, a one-way, repeated-measures ANOVA revealed that this treatment reduced the mean HCO_{3}^{-} secretion rate by 12.9–34.5% after mucosal application (Fig. 5A). The TEP also decreased significantly 30 min after treatment with RGN and bafilomycin (Fig. 5B). Similarly to the previous experiment, G_{m} remained unaffected after treatment (4.68 ± 0.12 mS/cm^{2}). Mean HCO_{3}^{-} secretion rates in Figs. 4A and 5A, in the pre-RGN-treated (control) tissues, fall within the range previously reported for the Gulf toadfish posterior intestine (21, 31).

Intestinal sac preparations: Na^{+}, Cl^{−}, water, and HCO_{3}^{-} fluxes. As hypothesized, a one-tailed test revealed that eel RGN resulted in a significant stimulation of a secretory Cl^{−} flux in the posterior intestine (Fig. 6B), while no effect was present in either the control tissues or in the eel RGN-treated anterior intestinal tissues. However, in both the anterior and posterior intestinal sac preparations, there were no differences in Na^{+}...
Japanese Eel GN
European Eel GN
Trout GN
Salmon GN
Catfish GN
Toadfish GN
Zebrafish GN
Medaka GN
Human GN
Rat GN
Pig GN
Walrus GN
Zebra Finch GN
Mallard GN
Green Sea Turtle GN
American Alligator GN
Japanese Eel RGN
Trout UGN
Salmon UGN
Medaka UGN
Toadfish UGN
European Eel UGN
Japanese Eel UGN
Catfish UGN
Zebrafish UGN
Human UGN
Pig UGN
Rat UGN
Walrus UGN
Soft-shelled Turtle UGN

flux between control and eel RGN-treated tissues (Fig. 6A). As for water, a one-tailed test revealed that eel RGN stimulation resulted in significant net fluid secretion in the posterior intestinal sacs (Fig. 6D), while no effects were present in control tissues or eel RGN-stimulated anterior intestinal sacs. Contrary to our hypothesis, however, a one-tailed test revealed that eel RGN significantly decreased HCO\textsubscript{3}/H\textsubscript{2}CO\textsubscript{3} secretion (similarly to the pH-stat titration experiment) by roughly 34% in the posterior intestinal sacs (Fig. 6C) compared with controls or eel RGN-treated anterior intestinal sacs.

Effects of CFTRinh-172 and RGN on ion transport across the posterior intestine. Neither treatment with CFTRinh-172 nor with subsequent treatment of RGN in the mucosal half-chamber of an Ussing chamber affected the HCO\textsubscript{3} secretion rate in the posterior intestine (Fig. 7A). CFTRinh-172 did not affect posterior intestinal TEP; however, as with previous experiments, RGN did cause a significant decrease in TEP despite being applied after CFTRinh-172 (Fig. 7B). The typical stimulation of \( I_{sc} \) by RGN was prevented by pretreatment with CFTRinh-172 (Fig. 7C). Similarly to the previous experiments, \( G_{te} \) remained unaffected across all treatments with a mean value of 6.08 ± 1.00 mS/cm\textsuperscript{2}. It is worth mentioning that 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), a known CFTR blocker used in the Japanese eel (68), was initially tested. However, application of NPPB alone resulted in a net secretory current, similarly to that produced by the eel peptides (possibly acting as a general Cl\textsuperscript{-}/HCO\textsubscript{3} transporter blocker). Thus, CFTRinh-172 was used in the present study.

Dependence of HCO\textsubscript{3} secretion on mucosal Cl\textsuperscript{-} concentration. Contrary to expectations, intestinal HCO\textsubscript{3} secretion rates were unaffected by luminal Cl\textsuperscript{-} concentrations (Fig. 8) within the range tested (3.8–154.8 mmol/l).

Immunohistochemistry: tissue-specific differences in histology. Differences between the anterior and posterior intestine (n = 3) concerning the presence of apical CFTR, apical NKCC2, basolateral NKA, and basolateral NKCC1 were observed. In the anterior intestine, NKCC1-like, NKCC2-like, and NKA-like immunoreactivity was observed in the enterocytes (Fig. 9A). These same transporters were also observed in the posterior intestine, along with CFTR-like immunoreactivity.
in the apical membrane (Fig. 9, B and C). These results indicate distinct differences in apical CFTR localization between the anterior and posterior intestine and are in agreement with previous reports of other marine teleost species (6, 46, 48).

**DISCUSSION**

**Tissue-specific responses to eel GN, UGN, and RGN.** Despite the evolutionary distances between the selected animal classes listed in Fig. 2, the amino acid sequences of GN and UGN are remarkably well conserved. In the majority of sequences across species and animal classes, the amino acid residues 4–8 (CEICA), 10–12 (AAC), and 14–15 (GC) do not differ. Eel RGN produced effects in the intestine of both the Gulf toadfish and Japanese eel, presumably because of its high degree of similarity to GN from both these species. Thus, despite these minor differences, our initial analyses gave us confidence that the Japanese guanylin peptides could be used to stimulate physiological responses in the Gulf toadfish tissue.

Table 3. **Short-circuit current of the anterior and posterior intestine in response to guanylin, uroguanylin, and renoguanylin**

<table>
<thead>
<tr>
<th>Dose, mol/l</th>
<th>Control</th>
<th>$10^{-9}$</th>
<th>$10^{-8}$</th>
<th>$2 \times 10^{-8}$</th>
<th>$5 \times 10^{-8}$</th>
<th>$10^{-7}$</th>
<th>$10^{-6}$</th>
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<tbody>
<tr>
<td>GN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AI</td>
<td>$-25.4 \pm 16.6$</td>
<td>$-7.5 \pm 2.6$</td>
<td>$-5.5 \pm 2.9$</td>
<td>$-20.3 \pm 17.9$</td>
<td>$-18.3 \pm 18.4$</td>
<td>$-25.5 \pm 23.2$</td>
<td>$-28.0 \pm 26.2$</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>$-22.3 \pm 15.4$</td>
<td>$-18.8 \pm 18.8$</td>
<td>$-18.1 \pm 18.2$</td>
<td>$-14.0 \pm 17.9$</td>
<td>$-5.0 \pm 18.67$</td>
<td>$14.5 \pm 24.9^*$</td>
<td>$17.0 \pm 20.0^*$</td>
<td>$22.2 \pm 20.8^*$</td>
</tr>
<tr>
<td>UGN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AI</td>
<td>$-8.8 \pm 6.8$</td>
<td>$-8.4 \pm 7.3$</td>
<td>$-7.9 \pm 8.0$</td>
<td>$-8.0 \pm 8.1$</td>
<td>$-7.3 \pm 8.4$</td>
<td>$-4.0 \pm 8.8$</td>
<td>$-5.4 \pm 9.4$</td>
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</tr>
<tr>
<td>PI</td>
<td>$-25.9 \pm 4.6$</td>
<td>$-21.0 \pm 4.0$</td>
<td>$-17.7 \pm 3.9$</td>
<td>$-7.0 \pm 8.7$</td>
<td>$-5.0 \pm 8.1$</td>
<td>$-1.5 \pm 6.7^*$</td>
<td>$9.5 \pm 5.3^*$</td>
<td>$12.5 \pm 6.4^*$</td>
</tr>
<tr>
<td>RGN</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AI</td>
<td>$-7.3 \pm 2.5$</td>
<td>$-1.7 \pm 3.7$</td>
<td>$1.4 \pm 3.0$</td>
<td>$-4.5 \pm 5.9$</td>
<td>$-6.1 \pm 5.2$</td>
<td>$-8.6 \pm 5.4$</td>
<td>$-9.3 \pm 5.5$</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>$-30.2 \pm 3.07$</td>
<td>$-23.6 \pm 3.9$</td>
<td>$-13.3 \pm 5.8$</td>
<td>$2.6 \pm 8.4^*$</td>
<td>$12.3 \pm 12.5^*$</td>
<td>$14.7 \pm 12.8^*$</td>
<td>$6.2 \pm 8.0^*$</td>
<td>$18.3 \pm 12.9^*$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 5–6$ for the responses of each anterior and posterior intestinal segment to guanylin (GN), uroguanylin (UGN), and renoguanylin (RGN), respectively. $I_{sc}$, short-circuit current; AI, anterior intestine; PI, posterior intestine. Peptides were applied to the mucosal half-chamber (2-ml volume) of an Ussing chamber in a cumulative manner. Negative mean values denote net absorption, while positive mean values denote net secretion. The time course for each experiment varied among individuals (see text for details). *Significant effects of increasing dose within a tissue from the control.
Table 4. Transepithelial conductance of the anterior and posterior intestine in response to guanylin, uroguanylin, and renoguanylin

<table>
<thead>
<tr>
<th>Doses, mol/l</th>
<th>Control</th>
<th>$10^{-9}$</th>
<th>$10^{-8}$</th>
<th>$2 \times 10^{-8}$</th>
<th>$5 \times 10^{-8}$</th>
<th>$10^{-7}$</th>
<th>$10^{-6}$</th>
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<td>GN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>8.86 ± 1.06</td>
<td>8.69 ± 1.22</td>
<td>8.41 ± 2.42</td>
<td>8.41 ± 1.68</td>
<td>8.97 ± 1.50</td>
<td>8.46 ± 2.69</td>
<td>9.38 ± 1.89</td>
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</tr>
<tr>
<td>PI</td>
<td>6.62 ± 1.44</td>
<td>7.00 ± 1.25</td>
<td>6.97 ± 1.22</td>
<td>7.37 ± 1.04</td>
<td>7.22 ± 1.16</td>
<td>7.16 ± 1.22</td>
<td>7.34 ± 1.37</td>
<td>8 ± 1.12*</td>
</tr>
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<td>UGN</td>
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</tr>
<tr>
<td>AF</td>
<td>11.03 ± 0.36</td>
<td>11.33 ± 0.54</td>
<td>11.30 ± 0.57</td>
<td>10.98 ± 0.35</td>
<td>11.37 ± 0.43</td>
<td>11.51 ± 0.53</td>
<td>11.59 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>6.29 ± 0.50</td>
<td>6.10 ± 0.50</td>
<td>6.20 ± 0.54</td>
<td>6.22 ± 0.55</td>
<td>6.28 ± 0.63</td>
<td>6.17 ± 0.59</td>
<td>6.41 ± 0.55</td>
<td>7.32 ± 0.64*</td>
</tr>
<tr>
<td>RGN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF</td>
<td>10.44 ± 0.33</td>
<td>10.29 ± 0.31</td>
<td>10.56 ± 0.28</td>
<td>10.63 ± 0.36</td>
<td>11.08 ± 0.26</td>
<td>11.27 ± 0.22</td>
<td>11.43 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>5.94 ± 0.53</td>
<td>5.89 ± 0.53</td>
<td>5.89 ± 0.55</td>
<td>6.42 ± 0.74</td>
<td>6.65 ± 0.92</td>
<td>6.96 ± 0.88</td>
<td>6.63 ± 1.09</td>
<td>7.09 ± 1.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 5–6$ for the responses of each anterior and posterior intestinal segment to GN, UGN, and RGN, respectively. Peptides were applied to the mucosal half-chamber (2-ml volume) of an Ussing chamber in a cumulative manner. *Differences from the control within a tissue; †transepithelial conductance ($G_{te}$) value of the anterior intestine at any given dose is significantly greater than its counterpart value in the posterior intestine ($P < 0.05$).

Indeed, all eel guanylin peptides elicited robust responses from the Gulf toadfish posterior intestine, similarly to those observed in the Japanese eel and, in some cases, to mammalian intestinal epithelia as well. Moreover, the similarities in the amino acid sequences between eel RGN and tGN may also explain the greater than fourfold difference in the $EC_{50}$ value of eel RGN compared with eel GN and UGN.

The present study reveals that only the posterior portion of the Gulf toadfish intestine responds to mucosal application of eel GN, UGN, and RGN by reversing $I_{sc}$, causing net Cl$^-$/HCO$_3^-$ secretion and in TEP ($A$) change. Changes in $I_{sc}$ secretion ($B$) and transepithelial potential (TEP; $A$) of isolated posterior intestinal tissue following mucosal application of RGN (5 $\times$ 10$^{-8}$ mol/l; indicated by the arrow) in Ussing chambers. Values are means ± SE ($n = 6–7$). *Significant differences ($P < 0.05$) in HCO$_3^-$ secretion and in TEP from the control period (0–30 min).

The Gulf toadfish and Japanese eel responses are in contrast to the effects of GN and UGN in mammals, where both peptides act on the entire mammalian intestinal tract (5, 13, 15, 30, 42). A parsimonious explanation for the region-specific responses in the Gulf toadfish intestine to the guanylin peptides may be the distribution of possibly distinct GC-C receptor subtypes and their different affinities for the guanylin peptides. Indeed, GC-C1 and GC-C2 are two GC-C subtypes so far characterized in the European (Anguilla anguilla) and Japanese eels (11, 39, 69). Two subtypes, OlGC6 and OlGC9, are also expressed in medaka (Oryzias latipes) (36), while only one GC-C type has been found in mammals and it is the predominant GC in the
intestine (4). Nonetheless, stimulation of fish and mammalian GC-Cs expressed in cell culture lines by GN or UGN leads to significant guanylyl cyclase activity and increases in cGMP (10, 13, 15, 36, 53, 69), yet, in the anterior intestine of marine teleosts, these increases may not be occurring in significant amounts (if at all) to produce the downstream effects observed in the posterior intestine.

An equally parsimonious alternative/additional explanation to describe the regional response of the Gulf toadfish intestine to the guanylin peptides may be the observation of both basolateral NKCC1-like and apical CFTR-like immunoreactivity in the posterior intestine. Expression of basolateral NKCC1 and apical CFTR is not uncommon in the posterior intestine of marine and SW-adapted euryhaline teleost species (6, 14, 35, 46, 48), which could form a secretory pathway for Cl\(^-\).

Eel UGN elicited a weaker response from the Gulf toadfish posterior intestine than did eel GN and RGN (despite a lower EC\(_{50}\) value than eel GN), and this difference appears to be more pronounced than that observed in the Japanese eel (68). In the Japanese eel, GC-C1 binds preferentially to UGN, while GC-C2 is the predominant GN receptor (69). Although it is unknown if Gulf toadfish possess multiple GC-Cs, it is possible that the presence of multiple Gulf toadfish GC-Cs bind eel GN and UGN differentially, and it is conceivable that the posterior intestine of the Gulf toadfish expresses more of a GC-C subtype that binds favorably to eel GN and RGN over eel UGN. In addition, it cannot be ruled out that the experimental conditions applied in the present study affected peptide-receptor interactions. In rat intestinal tissues and in T84 cells expressing rat GC-C, for example, tissues were most sensitive to UGN in salines of pH 5.5, with GN being more effective between pH 7.4 and 8.0 (34, 38). It is plausible that UGN elicited a weaker I\(_{sc}\) reversal at the posterior intestine due to the pH of the mucosal saline used in this study (pH 7.8), which would interfere with its binding ability. However, the low pH hypothesis may not apply to teleost guanylin peptides as Yuge et al. (69) performed their experiments by applying eel guanylin peptides to a mucosal saline of pH 7.2, resulting in increases in intracellular cGMP levels in the Japanese eel.

Eel GN, UGN, and RGN reverse electrolyte and fluid transport in the posterior intestine. In the present study, the eel guanylin peptides reversed the I\(_{sc}\) of the Gulf toadfish posterior intestine from net absorptive to net secretory (Table 3). This secretory response is in accordance to those observed in the Japanese eel mid and posterior intestine (68), throughout the rat intestine (38), and in T84 cells (34). Conversely, the I\(_{sc}\) of the Gulf toadfish anterior intestine, as with the Japanese eel (68), was unaffected by either of the eel guanylin peptides (Fig. 3, A–C). The reversal of the posterior intestinal I\(_{sc}\) by eel RGN coincides with a reversal of Cl\(^-\) flux, which results in net Cl\(^-\) and fluid secretion into the intestinal sac preparations (Fig. 6B). This response is similar to that observed in mammalian tissues and in T84 cells, in which mucosal application of GN has been shown to significantly alter the I\(_{sc}\), activate apical CFTR, and cause Cl\(^-\) and fluid secretion [for review in Forte (17)], and agrees with a GN-induced reversal of the I\(_{sc}\) of the Japanese eel mid and posterior intestine (68). Furthermore, in all Ussing chamber experiments performed in this study, mucosal application of eel RGN significantly decreased the serosa negative TEP of the posterior intestine, consistent with anion secretion (Figs. 4B, 5B, and 7B). Moreover, eel GN increased G\(_{an}\) modestly, but significantly, in the I\(_{sc}\) studies on the posterior intestine; however, such increases were not observed in the subsequent studies and may be due to the dose of RGN to which we exposed tissues. In the I\(_{sc}\) experiments, only posterior intestinal tissues exposed to 10\(^{-8}\) mol/l GN, UGN, or RGN demonstrated significant increases in G\(_{an}\). Curiously, while it appears that 5 \(	imes\) 10\(^{-8}\) mol/l RGN was sufficient to inhibit HCO\(_3\) secretion and reverse I\(_{sc}\), it was insufficient to cause changes in G\(_{an}\).

To determine if apical CFTR mediates the reversal of the I\(_{sc}\) at the posterior intestine, via net Cl\(^-\) secretion, we used...
CFTRinh-172, a known mammalian CFTR blocker (41). Yuge and Takei (68) demonstrated that simultaneously exposing Japanese eel intestinal tissues to NPPB, another CFTR inhibitor, and GN did not affect the absorptive $I_{sc}$, suggesting that $\text{Cl}^{-}/\text{HCO}_3^{-}$ is secreted by apical CFTR. In the present study, exposing the Gulf toadfish posterior intestine to CFTRinh-172 prevented the RGN-stimulated change in $I_{sc}$. These results suggest that, as in mammals and in the Japanese eel, apical CFTR plays a primary role in $\text{Cl}^{-}$ secretion by the intestine and is activated by the downstream effects of GC-C stimulation. Consistent with these observations is evidence of apical CFTR-like immunoreactivity in the posterior intestine, but not in the anterior intestine, of the Gulf toadfish.

Apical expression of CFTR in the intestine of marine and SW-adapted teleosts has been documented previously (6, 20, 48), and it has been revealed that apical CFTR expression may be important in SW adaptation (7). Moreover, the intestine of SW-adapted teleosts also expresses basolateral NKCC1 and apical NKCC2 (14, 20, 35). Immunohistochemistry in the present study also reveals apical expression of CFTR-like and NKCC2-like immunoreactivity, consistent with a previous report on the Gulf toadfish (62), and basolateral NKCC1-like immunoreactivity in the Gulf toadfish intestine. However, the distribution of these transporters is not uniform and the discontinuity of transporter types coincides with the present report’s physiological findings of $\text{Cl}^{-}$ secretion. In the anterior intestine, there is clear NKCC1-like and NKCC2-like immunoreactivity in the basolateral and apical membranes, respectively (Fig. 9A). Conversely, while NKCC1-like and NKCC2-like immunoreactivity is also found in the posterior intestine, apical CFTR-like immunoreactivity is present within this tissue as well (Fig. 9, B and C). These regional differences in transporter immunoreactivity correlate well with the region-specific differences in $\text{Cl}^{-}$ and fluid secretion observed in this study. Although under normal conditions, NKCC2 acts as an important pathway for net $\text{Cl}^{-}$ absorption by the marine teleost intestine, the effects of eel RGN in this study and those of GN and UGN in a previous report (68) lead to a reversal of the $I_{sc}$ and result in net $\text{Cl}^{-}$ secretion. Staining for NKCC1 and apical CFTR in the Gulf toadfish posterior intestine and the aforementioned eel RGN-induced $\text{Cl}^{-}$ secretion suggest that NKCC1 and apical CFTR are stimulated, while NKCC2 may be inhibited, as suggested in the Japanese eel (68), to provide a conduit for $\text{Cl}^{-}$ to travel from the blood, via the enterocytes, into the intestinal lumen (Fig. 10). Although the intracellular processes that lead to the activation of NKCC1 and apical CFTR, and possible inhibition of NKCC2, are unclear in fish, it is apparent that the guanylin peptides modify intracellular mechanisms to facilitate $\text{Cl}^{-}$ and fluid secretion.

Fig. 7. $\text{HCO}_3^{-}$ secretion (A), TEP (B), and $I_{sc}$ (C) of isolated posterior intestinal tissue exposed to mucosal application of cystic fibrosis transmembrane conductance regulator (CFTR) inhibitor (CFTRinh-172; 10 $\mu$mol/l) and RGN (5 x 10^{-8} mol/l), indicated by arrows. Values are means ± SE ($n$ = 5–6).

*Significant differences ($P < 0.05$) from the control period (0–30 min).

Fig. 8. $\text{HCO}_3^{-}$ secretion rates by anterior intestinal epithelia as a function of mucosal $\text{Cl}^{-}$ concentrations of the Gulf toadfish. Values are means ± SE. An initial control flux was measured using salines described in Table 1, before replacing luminal salines with different $\text{Cl}^{-}$ levels. Data are presented as normalized to the $\text{HCO}_3^{-}$ secretion rates during the initial 1-h control period. Horizontal error lines indicate the change in $\text{Cl}^{-}$ concentration over time during the second flux period while vertical error bars indicate variation in $\text{HCO}_3^{-}$ secretion rate over time during the second flux period.
The present study also revealed an unexpected inhibition of HCO\textsubscript{3}\^-/H\textsubscript{11002}\^- secretion, as opposed to the stimulatory effect observed in mammals. It has been shown that in mammalian intestinal tissues, using Cl\^-/H\textsubscript{11002}\^- free saline inhibits HCO\textsubscript{3}\^-/H\textsubscript{11002}\^- secretion even in the presence of cAMP agonists (57), suggesting apical anion exchange. Terrestrial mammals are generally Cl\^-/H\textsubscript{11002}\^- limited, thus, an apical Cl\^-/H\textsubscript{11002}\^- secreting CFTR channel would fuel apical Cl\^-/H\textsubscript{11002}\^- by recycling Cl\^- across the apical membrane (57), which does not seem to be the case for the Gulf toadfish.

Accordingly, in mammals, HCO\textsubscript{3}\^- can be secreted into the intestine either through apical CFTR directly or by an apical anion exchanger that is secondary to Cl\^- secretion via CFTR. To examine if a similar increase in HCO\textsubscript{3}\^- secretion in the Gulf toadfish was masked by an increase in VHA activity, bafilomycin was applied to inhibit this ATPase and revealed no effect of peptides on apical VHA (Fig. 5A). Consequently, in response to the guanylin peptides, it seems that mammals and marine teleosts modify HCO\textsubscript{3}\^- secretion oppositely from one another, yet, similarly, increase both Cl\^- and fluid secretion.

The failure of the three eel guanylin peptides to increase HCO\textsubscript{3}\^- secretion in the Gulf toadfish intestine is perhaps in part explained by the lack of Cl\^- limitation of the apical anion exchanger as demonstrated by the unaltered HCO\textsubscript{3}\^- secretion rates, even at luminal Cl\^- concentrations in the low millimoles per liter range. It appears that the SLC26a6 anion exchanger, responsible for intestinal HCO\textsubscript{3}\^- secretion in the gulf toadfish (27), displays high Cl\^- affinity. Furthermore, our observations provide evidence that Gulf toadfish apical CFTR does not transport HCO\textsubscript{3}\^- [as has been suggested in mammalian and fish tissues (37, 59)], since mucosal application of CFTRinh-172 alone had no effect on the HCO\textsubscript{3}\^- secretion rate of the posterior intestine (Fig. 7A) and since stimulation of Cl\^- secretion results in an inhibition, rather than stimulation, of HCO\textsubscript{3}\^- secretion.

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secretion. The mechanisms for the eel RGN-induced inhibition of \( \text{HCO}_3^- \) secretion in the Gulf toadfish intestine are unknown and require further investigation.

**Could inhibition of \( \text{HCO}_3^- \) enhance fluid secretion?** While the inhibition of \( \text{HCO}_3^- \) secretion may be unexpected, it may serve to enhance fluid secretion into the intestinal lumen in vivo. The majority of \( \text{HCO}_3^- \) secretion and NaCl absorption takes place in the anterior intestine, leading to a hypertonic absorbate of 650 mosmol/kg \( \text{H}_2\text{O} \) within the lateral interspace (liss) that drives fluid absorption (23, 28). Under normal conditions, intestinal tissues can produce luminal \( \text{HCO}_3^- \) concentrations as high as 100 mmol/l, and precipitation of \( \text{HCO}_3^- \) by \( \text{Ca}^{2+} \) (and to a lesser extent \( \text{Mg}^{2+} \)) can lead to luminal osmolalities being reduced by 70–100 mosmol/kg \( \text{H}_2\text{O} \) (47, 64–66). Furthermore, luminal titration of \( \text{HCO}_3^- \) by \( \text{H}^+ \) secretion across the apical membrane by VHA further decreases the osmolarity of the intestinal lumen by 20–30 mosmol/kg \( \text{H}_2\text{O} \) (25). Both these processes rely on luminal \( \text{HCO}_3^- \) and promote water absorption (47). The present study reveals that eel RGN inhibits \( \text{HCO}_3^- \) secretion, which, in vivo, would act to elevate luminal osmolality due to reduced precipitate formation and limited \( \text{HCO}_3^- \) titration. Thus, by inhibiting \( \text{HCO}_3^- \) secretion in the posterior intestine, the increased osmotic pressure generated by unbound \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \), untitrated \( \text{H}^+ \), and secreted \( \text{Cl}^- \) (due to apical CFTR activation) would draw fluid from the blood.

**Conclusion.** In both the Gulf toadfish and Japanese eel, guanylin peptide stimulation leads to a reversal of the absorptive \( I_c \) of the posterior intestine, but not the anterior intestine. In contrast, the entire length of the mammalian intestine responds to the GN and UGN. The present study clearly demonstrates that guanylin peptide stimulation in the Gulf toadfish drives net \( \text{Cl}^- \) and fluid secretion in the posterior intestine, but, in contrast to mammals, results in the inhibition of \( \text{HCO}_3^- \) secretion. The present study demonstrates inhibited \( \text{HCO}_3^- \) secretion during CFTR-stimulated \( \text{Cl}^- \) secretion and also reveals a lack of inhibition of \( \text{HCO}_3^- \) secretion following mucosal application of CFTRinh-172. These results indicate that apical CFTR is not permeable to \( \text{HCO}_3^- \), at least in the Gulf toadfish intestine. Additionally, the present study also provides immunohistological evidence for a potential \( \text{Cl}^- \)-secretory pathway via apical CFTR and basolateral NKCC1, present only in the posterior intestine, which could drive the observed \( \text{Cl}^- \) and fluid secretion. Nevertheless, the contribution of the seemingly counterproductive fluid secretion by the distal intestine in osmoregulation remains to be investigated.

**Perspectives and Significance**

The traditional view of the marine teleost intestine is that of a net absorbing epithelium, for the purposes of rehydration. However, the present and previous studies (68) demonstrate a robust secretory response in the distal intestinal segments following application of guanylin peptides. Furthermore, it is demonstrated that multiple plasma membrane transporters, such as SLC26a6 and NBCe1 (a basolateral \( \text{Na}^+/\text{HCO}_3^- \)-cotransporter), in addition to apical CFTR or NKCC1, may be acted on by the guanylin peptides, based on the observed inhibition of \( \text{HCO}_3^- \) secretion. Indeed, a recent study revealed that UGN injected into mice resulted in decreased mRNA and protein expression of renal pendrin/SLC26a4, another \( \text{Na}^+/

**REFERENCES**


