Mechanisms Regulating Somatostatin Release and Somatostatin-Induced Acetylcholine Release From the Myenteric Plexus

Yuanxu Lu, John Wiley, and Chung Owyang

The present studies were performed to characterize the molecular form(s) of somatostatin present in the myenteric plexus and to examine some aspects of the regulatory mechanisms underlying somatostatin release and somatostatin-induced release of acetylcholine from this tissue. We observed the following: (1) Somatostatin-like immunoreactivity (SLI) is present in the myenteric plexus of the guinea pig ileum with somatostatin-14 being the predominant molecular form. (2) Somatostatin-like immunoreactivity is released from isolated myenteric ganglia after stimulation with veratridine or the ganglionic agonist dimethylphenylpiperazinium (DMPP). (3) Calcium entry via the N-type channel appears to play a dominant role in DMPP-induced release of SLI. (4) Somatostatin regulates its own release via a pertussis toxin-sensitive mechanism. (5) Under basal conditions somatostatin-14 stimulates release of acetylcholine in a concentration-dependent manner. (6) Calcium entry via L-type channels is associated with the release of acetylcholine evoked by somatostatin-14.

SOMATOSTATIN-LIKE immunoreactivity (SLI) is widely distributed in the enteric nervous system in a population of interneurons[1] and serves to regulate the release of neurotransmitters.[2] The molecular form(s) of somatostatin present in the myenteric plexus are unknown.

Somatostatin can stimulate or inhibit gastrointestinal motility depending on the experimental conditions.[3,4] This may be associated with its ability to stimulate acetylcholine release under basal conditions while the peptide inhibits the release of this transmitter under stimulated conditions.[5] Somatostatin appears to inhibit acetylcholine release by activating a pertussis toxin (PTX)-sensitive guanine nucleotide binding protein (Gi/Go) coupled to cyclic adenosine monophosphate (cAMP)-dependent cholinergic transmission.[6] However, neither the regulation of somatostatin release nor somatostatin-mediated release of acetylcholine is well understood.

Neurosecretion is associated with calcium entry into the cell via voltage-dependent calcium channels.[7] Three types of calcium channels have been described on vertebrate neurons, designated T, L, and N, which differ in their electrophysiological properties and response to application of pharmacological agents.[8] It has been suggested that the calcium channel subtypes may be preferentially associated with release of different neurotransmitters.[9,10]

The goals of these studies were twofold: (1) To characterize the molecular species of somatostatin present in the myenteric plexus; and (2) To examine the regulation of somatostatin release and somatostatin-mediated release of acetylcholine.

MATERIALS AND METHODS

Characterization of SLI

Isolated strips of guinea pig ileal longitudinal muscle-myenteric plexus were prepared as described previously.[11] Somatostatin-like immunoreactivity was characterized using the methods of Yamada et al.[12] Briefly, the tissue strips were initially boiled in 3% acetic acid. The extract was placed on Sephadex G50 (Pharmacia, Piscataway, NJ) superfine columns (1 × 120 cm) and eluted with 0.1 mol/L ammonium acetate, pH 5.0 to separate the molecular forms of somatostatin. Somatostatin-like immunoreactivity was measured by radioimmunoassay using antibody 1001, which is specific for the central ring portion of the molecule.

Release of SLI From Dissociated Myenteric Ganglia

Isolated ganglia from the guinea pig ileum myenteric plexus were prepared as described previously.[13] Approximately 200 ganglia were placed in each of several chambers and perfused with Krebs buffer under standard conditions at 1 mL/min. Samples were collected at 2-minute intervals and assayed for SLI. The ganglia were exposed to the depolarizing agents veratridine (100 μmol/L) or the ganglionic agonist dimethylphenylpiperazinium (DMPP, 100 μmol/L) for 4 minutes after a 30-minute basal collection period. This was followed by an additional 30-minute collection period. Calcium channel antagonists nitrendipine (L channel blocker, 10 μmol/L) and cadmium (nonselective calcium channel blocker at 500 μmol/L) were added to the perfusate 4 minutes before and during the depolarization period. Some tissue samples were pretreated with ω-conotoxin (N and L channel blocker, 10 μmol/L) for 1 hour prior to beginning the perfusion studies. For experiments designed to examine the question of whether somatostatin regulates its own release, (Leu^{8}-D-Trp^{22}-Tyr^{25}) S28, a nonimmunoreactive but biologically active analog of somatostatin (S28a, 1 μmol/L) was added to the perfusate 4 minutes before and during the depolarization period.[14] Some experiments were performed after pretreating the tissue for 3 hours with PTX (200 ng/mL). This agent inactivates the inhibitory guanine nucleotide binding proteins (Gi/Go) that are coupled to adenylate cyclase.[15]

Effect of Somatostatin on the Release of ^{3}H-Acetylcholine From Myenteric Ganglia

Approximately 200 ganglia per perfusion chamber were prepared from the guinea pig ileum myenteric plexus and labeled with ^{3}H-acetylcholine for 40 minutes as described previously.[13] The labeled ganglia were perfused at 1 mL/min with Krebs buffer maintained under standard conditions. Samples were collected as described above. Randomized concentrations of somatostatin-14 were added to the perfusate for 4 minutes following a 30-minute basal collection period. This was followed by an additional 30-minute collection period. Calcium channel blockers were added to the perfusate as described above. Tritium radioactivity released during each collection period was counted in a liquid scintillation spectrometer. Assessment of what percent of the labeled metabolites was in the form of ^{3}H-acetylcholine was performed on ion exchange columns as described previously.[16] We observed that more than 85% of the labeled metabolites of choline was in the form of ^{3}H-acetylcholine during stimulated conditions.

From the Department of Internal Medicine, The University of Michigan Medical Center, Ann Arbor, MI.

Address reprint requests to Chung Owyang, MD, 3912 Taubman Center, The University of Michigan Medical Center, Ann Arbor, MI 48109-0362.

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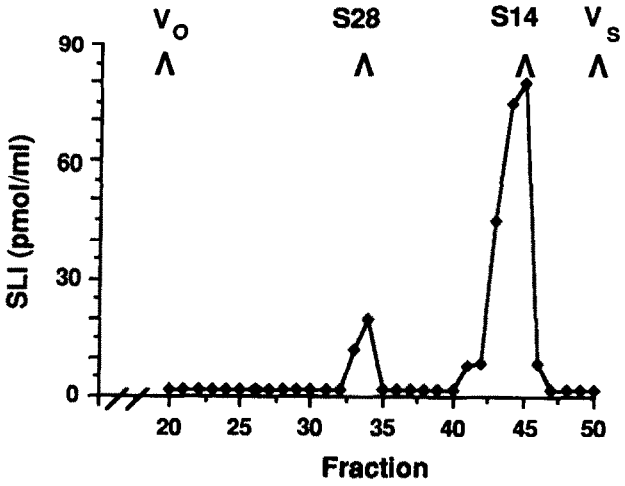

Fig 1. Representative gel filtration profile for (SLI) in the guinea pig ileum myenteric plexus. A 1-mL aliquot of tissue was applied to a Sephadex G50 superfine column (1 × 120 cm) equilibrated in 0.1 mol/L ammonium acetate, pH 5.0. Aliquots (2 mL) were collected and quantified for SLI by radioimmunoassay. Column was calibrated by noting elution fractions for bovine serum albumin (V_o), somatostatin-28 (S28), somatostatin-14 (S14), and NaCl (V_s).

RESULTS AND DISCUSSION

We observed that somatostatin-14 is the predominant molecular species of the peptide present in the guinea pig ileum myenteric plexus. The elution profile of somatostatin extracted from longitudinal muscle-myenteric plexus preparations from this region is shown in Fig 1. A smaller peak that coeluted with somatostatin-28 was also detected. These results are similar to those reported previously describing the molecular forms of somatostatin present in the gastric mucosa

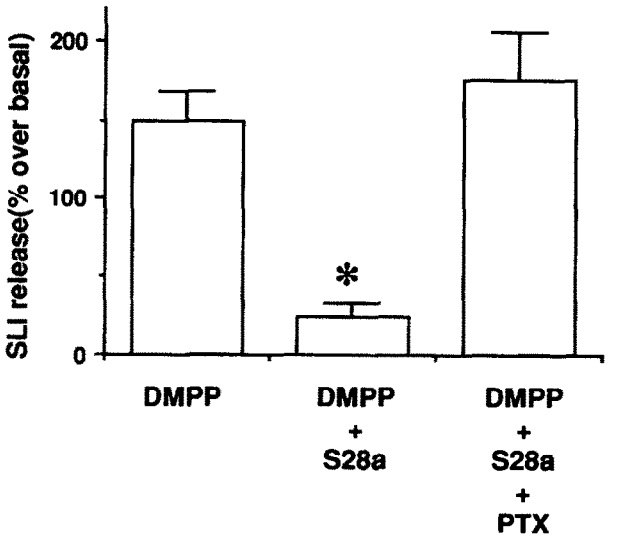

Fig 2. Effect of somatostatin analog (Leu^8-D-Trp^{22}-Tyr^{25}) S28, a nonimmunoreactive but biologically active analog of somatostatin (D S28a) on somatostatin (SLI) release evoked by DMPP. Pretreatment of the tissues with PTX reversed the inhibitory effect of S28a. Data are presented as means ± SE, n = 4. *denotes values significantly different from those observed with DMPP alone.

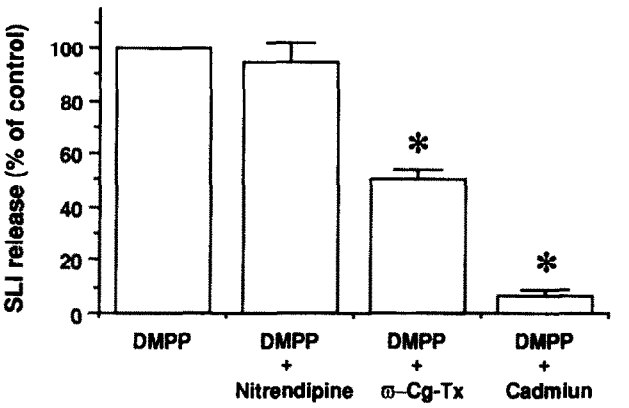

Fig 3. Effect of calcium channel antagonists on somatostatin (SLI) release evoked by DMPP. ω-Conotoxin (N and L channel antagonist) and cadmium (nonselective calcium channel antagonist) significantly inhibited release of somatostatin evoked by DMPP. No effect was observed with nitrendipine (L channel antagonist), n = 4. *$P < .05$.

and released after meal stimulation.[17] We next examined whether somatostatin regulates its own release from myenteric plexus ganglia. The results of these studies are depicted in Fig 2. DMPP-evoked release of somatostatin was significantly reduced by 85 ± 5% (n = 4, $P < .05$) after exposure of the ganglia to (Leu^8-D-Trp^{22}-Tyr^{25}) S28. Pretreatment of the samples with PTX reversed the inhibitory effect of S28a. This suggests that the release of somatostatin is autoregulated via an inhibitory feedback pathway involving a PTX-sensitive G-protein.

Pharmacological studies involving potassium-evoked transmitter release from cultured neurons suggest that N channels may be preferentially associated with norepinephrine release from sympathetic neurons while calcium entry via L channels is associated with release of substance P from dorsal root ganglion neurons.[9,10] We examined the effect of calcium channel blockers on DMPP-induced release of somatostatin and somatostatin-evoked release of acetylcholine from myenteric plexus ganglia. DMPP stimulated significant

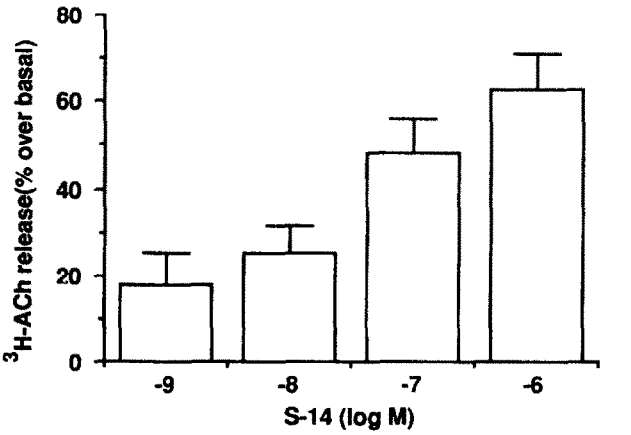

Fig 4. Dose-dependent effect of somatostatin-14 on release of ^{3}H-acetylcholine (^{3}H-ACh). Data are presented as means ± SE, n = 4.

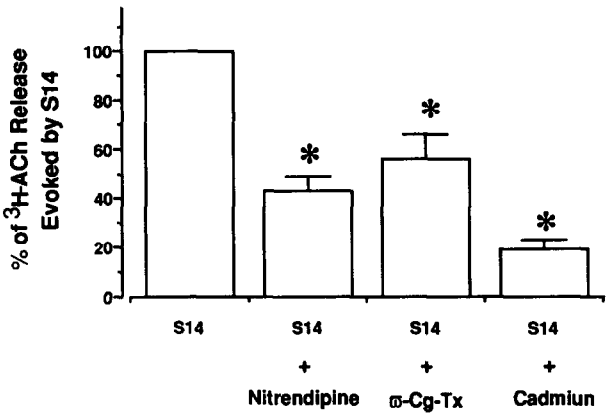

Fig 5. Effect of calcium channel antagonists on ^{3}H-acetylcholine release evoked by somatostatin. Nitrendipine (L channel antagonist), ω-conotoxin (N and L channel antagonist), and cadmium (nonselective calcium channel antagonist) significantly inhibited release of ^{3}H-acetylcholine evoked by somatostatin.

release of somatostatin that was unaffected by the L channel antagonist nitrendipine but inhibited 48% ± 4% and 88% ± 3% (n = 4, $P < .05$) by the combined N and L channel antagonist ω-conotoxin and nonselective calcium channel blocker cadmium (500 μmol/L), respectively. The results are presented in Fig 3. These observations confirm that neurosecretion of somatostatin is calcium-dependent and suggest that calcium entry via N channels is preferentially associated with release of somatostatin from myenteric plexus ganglia. Somatostatin-14 produced concentration-dependent release of ^{3}H-acetylcholine that is shown in Fig 4. Somatostatin-induced release of acetylcholine was significantly inhibited 56% ± 5%, 46 ± 8%, and 82% ± 4% (n = 4, $P < .05$) by nitrendipine, ω-conotoxin, and cadmium, respectively. The results of these studies are presented in Fig 5. Thus, in contrast to the situation with DMPP-induced somatostatin release, calcium entry via the L channel plays an important role in somatostatin-evoked release of acetylcholine from myenteric ganglia.

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